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GUIDELINES FOR THE MONITORING OF THE SAPROXYLIC BEETLES PROTECTED IN EUROPE

Edited by

Giuseppe M. Carpaneto, Paolo Audisio,
Marco A. Bologna, Pio F. Roversi, Franco Mason

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LIFE11 NAT/IT/000252
Monitoring of insects
with public participation

With the contribution of the LIFE financial instrument of the European Union

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Pio Federico Roversi, Franco Mason

Special issue published with the contribution of the LIFE financial instrument of the European Union.

Project LIFE11 NAT/IT/000252 MIPP, coordinated by the Centro Nazionale Biodiversità Forestale "Bosco Fontana", Verona/Mantova. Comando Tutela Forestale Ambientale Agroalimentare, Arma dei Carabinieri. Project Manager: Col. Franco Mason.

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Back cover photos: Participants in the European Workshop “Monitoring of saproxylic beetles and other insects protected in the European Union” organized by MIPP, 24–26 May 2017, Mantova (above); Participants to the Day for technical staff of the Natura 2000 Network, organized by MIPP, 29 May 2017, Mantova (below).

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Contents

1 **The LIFE Project "Monitoring of insects with public participation" (MIPP): aims, methods and conclusions**
Giuseppe Maria Carpaneto, Alessandro Campanaro, Sönke Hardersen, Paolo Audisio, Marco Alberto Bologna, Pio Federico Roversi, Giuseppino Sabbatini Peverieri, Franco Mason

37 **Guidelines for the monitoring of *Lucanus cervus***
Marco Bardiani, Stefano Chiari, Emanuela Maurizi, Massimiliano Tini, Ilaria Toni, Agnese Zauli, Alessandro Campanaro, Giuseppe Maria Carpaneto, Paolo Audisio

79 **Guidelines for the monitoring of *Osmoderma eremita* and closely related species**
Emanuela Maurizi, Alessandro Campanaro, Stefano Chiari, Michela Maura, Fabio Mosconi, Simone Sabatelli, Agnese Zauli, Paolo Audisio, Giuseppe Maria Carpaneto

129 **Guidelines for the monitoring of *Cerambyx cerdo***
Lara Redolfi De Zan, Marco Bardiani, Gloria Antonini, Alessandro Campanaro, Stefano Chiari, Emiliano Mancini, Michela Maura, Simone Sabatelli, Emanuela Solano, Agnese Zauli, Giuseppino Sabbatini Peverieri, Pio Federico Roversi

165 **Guidelines for the monitoring of *Rosalia alpina***
Alessandro Campanaro, Lara Redolfi De Zan, Sönke Hardersen, Gloria Antonini, Stefano Chiari, Alessandro Cini, Emiliano Mancini, Fabio Mosconi, Sarah Rossi de Gasperis, Emanuela Solano, Marco Alberto Bologna, Giuseppino Sabbatini Peverieri

205 **Guidelines for the monitoring of *Morimus asper funereus* and *Morimus asper asper***
Sönke Hardersen, Marco Bardiani, Stefano Chiari, Michela Maura, Emanuela Maurizi, Pio Federico Roversi, Franco Mason, Marco Alberto Bologna

237 **Training of a dog for the monitoring of *Osmoderma eremita***
Fabio Mosconi, Alessandro Campanaro, Giuseppe Maria Carpaneto, Stefano Chiari, Sönke Hardersen, Emiliano Mancini, Emanuela Maurizi, Simone Sabatelli, Agnese Zauli, Franco Mason, Paolo Audisio

265 **Analyses of occurrence data of protected insect species collected by citizens in Italy**
Alessandro Campanaro, Sönke Hardersen, Lara Redolfi De Zan, Gloria Antonini, Marco Bardiani, Michela Maura, Emanuela Maurizi, Fabio Mosconi, Agnese Zauli, Marco Alberto Bologna, Pio Federico Roversi, Giuseppino Sabbatini Peverieri, Franco Mason

Foreword

A fallen tree, a life that ends after having seen so many seasons go by. I am sitting in my living room and speculating on this simple almost inexistent event, while out there the world keeps quarrelling over countries borders, power, money and many other issues. I am trying to put all this in an order, but I cannot. The fallen tree keeps coming back as a more central and prominent event for the life of mankind. All that wood was once fine particles of CO_2 from the atmosphere or water and minerals from the ground. The tree fixed all these components into a majestic natural monument during hundreds of years using a single source of energy: the sun!

And now its time has come. Birds and insects are the first to take advantage, soon to be followed by fungi and bacteria, while a passing mammal will also dig in. Year after year the majestic tree turns into rotten dead wood and then into organic matter. In the same place in the meantime a young tree, one of the many offsprings of the long dead "father" is now growing and part of its supply are coming from the left over of its ancestor. Isn't it just beautiful I say to myself? Simple but almost magic.

Insects that live in dead wood, also called saproxylic beetles, are one of the building blocks of the armada that attacked the dead giant. Some of them are impressive demolition machines and they are responsible for a large share of the work. Without them, the work of decomposing the base structure of the tree tissues would simply not be possible. They are mostly elusive, living most of their existence as larvae, but when they become adults one should see the variety of shapes, colours, smells and behavioural patterns they show. It is just amazing! I could not refrain from thinking how strongly interlaced are life of mankind and that of those beetles and now everything went back into the right perspective.

This book is about these beetles. It's a book of science and knowledge, not a book for everyone. Yet, it is an essential contribution to our ability to understand what (or shall I say who) our little friends are. It's the result of years of work of passionate people that I want to congratulate and thank for what they did. We need more attention to the less visible side of biodiversity and the LIFE Program is proud to support initiatives like this.

The lyrics of a song by Louis Armstrong come to my mind and they seem a perfect closing for this short story:

"*I see trees of green, red roses too*
I see them bloom for me and you
And I think to myself what a wonderful world"

Angelo Salsi
Responsible for LIFE and CIP Eco-innovation Unit
Bruxelles, August 2017

Introduction

One action envisaged by "The EU Biodiversity Strategy to 2020" is integrating biodiversity measures by maintaining optimal levels of deadwood in forests. Currently, the discussion and scientific research on optimal levels of deadwood for conservation are still ongoing.

The concept of conservation of deadwood is today widely accepted. For example, the European Union considers deadwood a "proxy" of forest biodiversity, as is evident from technical documents. For example, research has shown that about 30% of the species present in temperate forests are dependent on deadwood; particularly important are large, old trees, because they are rich in micro-habitats and often contain cavities. It is planned to strengthen the conservation of deadwood in the reserves managed by the Comando Unità per la Tutela Forestale, Ambientale e Agroalimentare (Command Unit for the Protection of Forests, Environment and Food) of the Carabinieri. A renowned example for a forest already rich in dead wood is the reserve "Sasso Fratino", an ancient beech forests, recently recognized by the UNESCO as a World Heritage Site. Another example is the reserve Bosco Fontana, well-known to the international scientific community and to Europe's forest managers for pioneering deadwood restoration techniques.

The National Centre for Forest Biodiversity Carabinieri "Bosco Fontana" has been working on the conservation of dead wood habitats, also by carrying out concrete actions, for the last 15 years. Two Life projects are indicative for the approaches followed. “Bosco Fontana - urgent conservation actions in relict habitat” (LIFE99 NAT/IT/006245) lasted from 1999 to 2003. More recently, the project “Monitoring of Insects with Public Participation” (MIPP, 2012-2017) developed methods for the monitoring of five saproxylic beetles, also employing "Citizen Science", which meant actively engaging citizens for recording protected insects in Italy.

The results of the MIPP project, were presented during a "European Workshop", held in Mantua in May 2017 and in the “guidelines” presented in a special issue for forest managers. This work is well integrated with the manuals published by the Italian National Institute for Environmental Protection and Research (ISPRA), and serve to evaluate the conservation status of species listed in the Habitats Directive (Art. 11 and 17 HD 92/43/CEE). In the guidelines, new technologies, offered by the internet, have been employed to make this publication well known: e-books, hypertext and indexing in the major international platforms. This effort was made to reach the maximum number of managers of sites of the European Network "Natura 2000".

I hope that this manual will provide a useful tool for the work of reserve managers; it is the result of five years of work by the National Centre for Forest Biodiversity Carabinieri "Bosco Fontana" in collaboration with Sapienza – University of Rome, University Roma Tre, Italian Ministry for the Environment, Region Lombardy and the Council for Agricultural Research and Economics – Research Centre for Plant Protection and Certification.

Antonio Ricciardi
Generale di Corpo d'Armata
Comandante Unità Tutela Forestale Ambientale Agroalimentare Carabinieri
Rome, August 2017

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CONSERVATION IN PRACTICE

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The LIFE Project "Monitoring of insects with public participation" (MIPP): aims, methods and conclusions

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Abstract

The Life Project "Monitoring of insects with public participation" (LIFE11 NAT/IT/000252) had as the main objective to develop and test methods for the monitoring of five beetle species listed in the Annexes of the Habitats Directive (92/43/EEC): *Osmoderma eremita* (hermit beetle, Scarabaeidae), *Lucanus cervus* (European stag beetle, Lucanidae), *Cerambyx cerdo* (great capricorn beetle, Cerambycidae), *Rosalia alpina* (rosalia longicorn, Cerambycidae) and *Morimus asper/funereus* (morimus longicorn, Cerambycidae). The data gathered represent an important contribution to the monitoring of these target species in Italy. The methods developed for monitoring of the target species are intended for use by the local management authorities and staff of protected areas. These developed methods are the result of extensive fieldwork and ensure scientific validity, ease of execution and limited labour costs. The detailed description of methods and the results for each species are published in separate articles of this special issue of Nature Conservation. A second objective of the project was to gather faunistic data with a Citizen Science approach, using the web and a mobile application software (app) specifically built for mobile devices. The validation of the records collected by the citizens was carried out by experts, based on photographs, which were obligatory for all records. Dissemination activities represented the principal way to contact and engage citizens for the data collection and also offered the possibility of providing information on topics such as Natura 2000, the Habitats Directive, the role of monitoring in nature conservation, the importance of forest ecosystems and the ecological role of the saproxylic insects. An innovative method tested during the project was the training of a dog for searching and monitoring the elusive hermit beetle; the trained dog also added a "curiosity" factor to attract public attention towards this rare insect and the issues mentioned above.

Keywords

Saproxylic beetle monitoring, Insect conservation, Forest biodiversity, Habitats Directive, Citizen Science, LIFE Nature Project

Introduction

Monitoring biodiversity and habitats directive

The inaugural meeting of the International Union for Protection of Nature (IUPN, later renamed IUCN), held in 1948, began to set up instruments to monitor the status of animals and plants worldwide. Over subsequent years, the monitoring of habitats and species became a core activity of nature conservation across the planet and an important chapter of conservation biology (Goldsmith 1991, Elzinga et al. 2001, Marsh and Trenham 2008, Schmeller 2008). Species monitoring is the regular observation and recording of changes in status and trend of species in a certain territory (Kull et al. 2008). The major aim of monitoring is to collect information that can be used for conservation policy, to examine the outcomes of management actions and to guide future management decisions (Kull et al. 2008). It consists of collecting reliable data which in turn allow the researchers to draw conclusions that species and ecosystems are changing their status through time and space, either naturally or as a consequence of deliberate or unintentional human intervention. It is often applied to assess the status of threatened species, the spread of alien or invasive species, the health of ecosystems, the efficacy of protected areas and other conservation actions. In any case, monitoring programmes should be focused on providing precisely the information needed to make the right conservation decisions. Therefore, the formulation of clear and explicit monitoring objectives is a key first step in the planning of any wildlife monitoring programme (Elzinga et al. 2001, Yoccoz et al. 2001, Williams et al. 2002, Noon 2003, Lindenmayer and Likens 2010, Legg and Nagy 2006, Nichols and Williams 2006; Martin et al. 2007). It should be kept in mind that there is no "best" survey method that suits all purposes, and is efficient, precise, reliable, simple and cheap at the same time. The suitability of a method may depend on the local circumstances, as environmental features, budget and the number of volunteers involved.

Monitoring programmes range from small scale, local programmes, to large-scale, national and international programmes (Schmeller et al. 2012), and from simple field surveys to complex procedures which require a considerable budget to cover staff costs, as well as expensive material and equipment. Moreover, governmental and local budgets allocated to biodiversity conservation are often scarce, and gathering data exclusively by professionals may not be possible. The management authorities often turn to volunteers, such as local naturalists coordinated by a small team of professionals. In these cases, citizen science offers an additional way for the monitoring of living organisms (Chandler et al. 2017, McKinley et al. 2006, Lindenmayer and Likens 2010). In any case, every effort should be made to involve local people and organisations in monitoring, as they have a vested interest in the areas and the species concerned. Involving local volunteers in a project can bring out questions, ideas and techniques that might not otherwise surface (McKinley et al. 2006). The Council Directive 92/43/EEC, better known as the Habitats Directive and adopted by the European Community in 1992, is focused on the conservation of natural habitats, flora and fauna. Its aim is to promote the maintenance of biodiversity, taking account of economic, social, cultural and regional requirements. Together, the Habitats Directive and the Birds Directive (79/409/CEE) comprise the cornerstone of Europe's nature conservation policy and they established the EU wide Natura 2000 ecological network of protected areas which are safeguarded against potentially damaging developments (http://ec.europa.eu/environment/nature/legislation/habitatsdirective/index_en.htm). This network, in 2011 accounted for over 26,400 sites with a total surface area of about 986,000 km^2, comprising nearly 768,000 km^2 of land. The terrestrial component of the network represents 17.9 % of the surface area covered by the EU 27 countries.

The Habitats Directive, which has gone through a number of updates and corrections, mainly to the annexes, ensures the conservation of a wide range of rare, threatened or endemic animal and plant species (as listed in Annex II, IV and V), as well as some 200 rare and characteristic habitats (as listed in Annex I). The current species lists in Annexes II and IV have a dominant proportion of vertebrates and very few arthropods (of which only eleven are priority species) (Cardoso 2012). Additionally, it must be noted that many emblematic, endemic and threatened insect species of southern Europe do not figure in the Habitats Directive (e.g., the renowned Italian moth *Brahmaea* (*Acanthobrahmaea*) *europaea* Hartig, 1963 (cf. Mosconi et al. 2014). All of these observations suggest an urgent need for updating and revision of the Habitats Directive.

Monitoring of conservation status is an obligation arising from Article 11 of the Habitats Directive for all habitats and species of Community interest. Consequently, this provision is not restricted to Natura 2000 sites and data should be collected both in and outside the Natura 2000 network to achieve a full assessment of the conservation status of the species. The main results of this monitoring should be reported to the Commission every six years according to Article 17 of the Directive. In Italy, as in many other countries, population trends for most of the insect species listed in the Annexes are currently unknown as coordinated monitoring programmes are lacking (Genovesi et al. 2014, Carpaneto et al. 2015, Stoch and Genovesi 2016).

The saproxylic organisms

Saproxylic organisms depend on decaying wood of moribund or dead trees (standing or fallen), or upon the presence of other saproxylic species at least during some phases of their life-cycle (Speight 1989, Alexander 2008). On the whole, saproxylic organisms (especially saproxylic insects) account for a considerable part of forest biodiversity (Stokland et al. 2012). The percentage values of saproxylic species, with respect to other living organisms, vary in different environmental contexts, e.g., 10% of all living organisms in Scandinavia (Stokland et al. 2012), 30% of forest species in Germany (Müller et al 2008) and probably more in southern European countries (Carpaneto et al. 2015). Many saproxylic species (saproxylophagous species) depend upon decaying woody material (dead wood) as a nutrition source but others are predators and parasitoids of saproxylophagous insects and of other animals that use wood as a breeding site or refuge, as well as fungi, mosses and lichens on the surface of dead wood (Stokland 2012, Stokland et al. 2012, Carpaneto et al. 2015).

The order Coleoptera is the most species rich taxon worldwide and gives a relevant contribution to saproxylic biodiversity (Brunet and Isacsson 2009). Thus, saproxylic beetles are one of the most important components of forest ecosystems and play a key role in nutrient cycling and ecosystem functioning (Stokland 2012). Consequently, their abundance, richness and diversity depend on the availability of food resources linked to mature forest habitats (Redolfi De Zan et al. 2014a). It has been shown that forest management practices, which increase the amount and variety of dead wood, benefit saproxylic beetles as well as other indicators for the conservation status of forests such as hole-nesting birds (Hardersen 2003, Redolfi De Zan et al. 2014b). In particular, a direct correlation likely exists between diversity and abundance of saproxylic beetles and of specialised insectivore birds that feed on them, such as hole-nesting birds (Redolfi De Zan et al. 2014b).

As European forests have suffered from intensive wood exploitation by man for several centuries, most of the managed forests contain very little dead wood and low levels of biodiversity. Nevertheless, a slight increase in both deadwood and biodiversity has been observed in the last decades, probably due to the current policies of environmental protection in European countries (Vallejo 2015). For example, primeval beech forests in the Ukrainian Carpathians harbour the highest volumes of dead wood, from 147 to 181 m^3/ha (Commarmot et al. 2013), whereas managed beech forests in Italy have only 7.6 m^3/ha (Gasparini and Tabacchi 2011). Generally, the estimates for standing and lying deadwood in European forests range between 5 and 15 m^3/ha for most countries (Vallejo 2015). Restoration of functional saproxylic communities is not an easy task because the addition of dead wood to a forest does not necessarily lead to recolonisation by all saproxylic insects. In fact, saproxylic species need different quantity and quality of dead wood, characterised by a structural heterogeneity and various degrees of biodegradation (Stokland et al. 2012, Parisi et al. 2016). Moreover, many saproxylic beetles have limited dispersal abilities (Fayt et al. 2006; Buse 2012), whereas some species cannot fly at all (e.g. *Morimus*) and tend to aggregate in small areas. As a consequence, forests with a recent accumulation of dead wood are inhabited by fewer red-listed species than forests with an uninterrupted history of dead wood abundance (Nilsson and Baranowski, 1997). The continuity of deadwood availability plays a major role for many saproxylic beetles, as sensitive species may disappear from cultivated forests where traditional management has been interrupted or changed, e.g. from pollarding to coppicing (Müller et al. 2005, Dubois 2009). Thus, the conservation status of saproxylic beetles and the species richness of their communities cannot be inferred directly from the amount of dead wood currently present. The conservation of saproxylic insects is also pursued in managed forests by applying particular management methods, such as "retention forestry" (Lindenmayer and Franklin 2002), the "îlots de sénescence" [senescence islands] (Lachat and Bütler 2008), "forest biodiversity artery" (Mason and Zapponi 2015) or by artificially increasing dead wood (Cavalli and Mason 2003, Zapponi et al. 2014).

Many factors are threatening saproxylic insects, particularly the large saproxylic beetles (LSB), such as stag beetles, hermit beetles and other fruit chafers, rosalia and great capricorn, to which this special issue and the MIPP project were dedicated. These species, owing to their great body size, need a larger volume of dead wood in aggregate form, i.e. in the same tree, for successful larval development. Consequently, their body size limits the abundance of their populations and makes them easily detectable by mammal and bird predators as well as by people. The major threats to saproxylic beetles are the fragmentation and/or structural simplification of woodlands, as well as the loss of suitable microhabitats. In particular, the so called habitat trees, i.e. standing live or dead trees represent a fundamental resource for saproxylic beetles, providing ecological niches and microhabitats, such as cavities, bark pockets, large dead branches, cracks, sap runs or trunk rot (Gibbons and Lindenmayer 2002, Cavalli and Mason 2003, Bütler et al. 2013). In particular, for LSB, the possible threats suggested by some of us according to our personal experience and in order of decreasing importance, are the following: the decreasing density of old trees (especially hollow trees) from forests due to commercial management, the old-growth forest fragmentation, the eradication of old tree rows from agricultural landscapes, the changes in tree management with the abandonment of pollarding, the spread of pesticides against invasive moths or other pests and the removal of old trees used as biomass for industrial fuel production. In some European countries or areas, other factors can represent a threat such as: the increasing drought in forest soils due to intensive groundwater exploitation (especially in Mediterranean areas), the demographic escalation of predators of large insects (especially crows), beetle killing by increased car traffic in summer months on roads crossing forested areas or stretching along the forest edge, the commercial exploitation of beetles by insect dealers for collectors and the deliberate killing of adult beetles in woodlands near to human settlements (especially for stag beetle males and great capricorn). The combination of threats currently facing LSB requires immediate conservation actions at various levels from site-level initiatives, through national and regional strategies, to international conventions and action plans. Baseline density estimates and subsequent monitoring of LSB populations are essential for assessing the impacts of particular threats and measuring whether conservation programmes are succeeding. Therefore, the conservation of LSB requires a detailed understanding of their population size, spatial distribution and demographic trends. Seibold et al. (2015) showed that the saproxylic beetles which face a higher extinction risk are: large-sized species, lowland forest-dwelling species, open canopy species and species that rely on large diameter, broad-leaved trees. However, quantitative data for many species are still lacking and their conservation status is poorly known, both of which are due to their elusive nature (Bouget et al. 2008, Seibold et al. 2015), the restricted daily and seasonal activity of the adults (e.g. Drag et al. 2011, Campanaro et al. 2016), the low density of their populations (e.g. Castro and Fernández 2016) and the scarcity of researchers involved in their ecological study (Haslett 2007, Hochkirch 2016). All these factors together, combined with the current scarcity of suitable habitats, are reasons why the implementation of efficient monitoring programmes is notoriously difficult.

The first methods for monitoring some LSB in Italy were published by Campanaro et al. (2011), while Trizzino et al. (2013) were the first to propose a review of the existing standard monitoring protocols for all arthropods listed in the Habitats Directive and occurring in Italy. A recently published manual provided methods and protocols for the monitoring of all species and habitats of community interest occurring in Italy (Stoch and Genovesi 2016). However, the methods so far proposed for these insect species are not based on a comparative approach tested in different nature reserves.

The Project Life MIPP: aim and objectives

The Life Project "Monitoring of insects with public participation" (LIFE11 NAT/IT/000252) (hereafter: MIPP) started on 1/10/2012 and will end in 30/09/2017 (Mason et al. 2015). The coordinating beneficiary of the project is the Comando Unità per la Tutela Forestale, Ambientale e Agroalimentare Carabinieri (hereafter: CUTFAA) (formerly: Corpo Forestale dello Stato), in particular the National Centre for the Study and Conservation of Forest Biodiversity Carabinieri "Bosco Fontana", Marmirolo, Mantova. Associate beneficiaries of MIPP are: the Council for Research in Agriculture and Analysis of Agrarian Economy, Cascine del Riccio - Firenze; the Sapienza University of Rome, Department of Biology and Biotechnologies "Charles Darwin"; the Roma Tre University, Department of Science; the Italian Ministry of the Environment and Protection of Land and Sea, General Direction for Protection of Nature and Sea; and the Lombardy Region (DG Ambiente, energia e sviluppo sostenibile).

The MIPP Project had, as first objective, to develop and test methods for the monitoring of five species of saproxylic beetles listed in Annexes II and IV of the Habitats Directive: *Lucanus cervus* (Linnaeus, 1758), *Osmoderma eremita* (Scopoli, 1763), *Cerambyx cerdo* Linnaeus, 1758, *Rosalia alpina* (Linnaeus, 1758) and the complex *Morimus asper asper* (Sulzer, 1776) / *Morimus asper funereus* Mulsant, 1863. The guidelines presented in separate contributions of this special issue of Nature Conservation are the result of extensive fieldwork carried out in order to develop readily standardised methods which ensure scientific validity, ease of execution and limited labour costs for monitoring the above mentioned species. These methods were developed for use by local management authorities, staff of protected areas and the conservation community at large, to gather raw data for assessing the status of the species every six years. The standardised methods developed by the MIPP staff resulted in quantitative data collected from various sites, using traps and/or lures, mark-recapture techniques, transects and, in some cases, genetic analyses. For *Osmoderma eremita*, the most elusive of these species, a dog was trained to search for the larvae of this beetle. In the last few decades, conservation dogs have been used for an array of activities, including detection of a large variety of taxonomic groups (Mosconi et al. (2017). In many cases, these dogs resulted in being more efficient than other survey methods in detecting the target species (Beebe et al. 2016). Many of the large and protected saproxylic beetles are cryptic (Bouget et al. 2008, Seibold et al. 2015) but in the case of *O. eremita* detection is also difficult due to its elusive nature, i.e. its limited daily activity (Le Gouar et al. 2015). Therefore it was decided to train a dog to find this protected beetle. The conservation dog of the MIPP subproject "Osmodog" is the first to be trained for an endangered beetle species.

A second objective of the project was to apply a Citizen Science approach (Zapponi et al. 2016) to increase the current knowledge of the regional and ecological distribution of nine species of insects protected by the Habitats Directive, i.e. the five beetles selected for monitoring and four additional species belonging to other two insect orders: *Saga pedo* Pallas, 1771, *Lopinga achine* (Scopoli, 1763), *Parnassius apollo* (Linnaeus, 1758) and the complex *Zerynthia cassandra* (Geyer, 1828) / *polyxena* (Denis and Schiffermüller, 1775). The first species belongs to the Order Orthoptera, the other three to Lepidoptera. By means of the citizen science approach, data were collected using a dedicated homepage of the web-site and an application for mobile device (app), while validation of the records based on photographs was carried out by experts.

A relevant component of the project was dissemination and communication which were used to inform people on issues such as Natura 2000, the Habitats Directive, the monitoring activities and the ecological role of the saproxylic insects within the forest ecosystems. The dog trained for the monitoring of the hermit beetle, was also used as a "curiosity" factor to attract public attention towards this rare beetle and the issues mentioned above.

Methods

Target species

The target taxa selected for the MIPP Project are listed in Table 1, together with an updated taxonomic arrangement, references to the Annexes of the Habitats Directive where they are included, and the MIPP objectives in which they were involved.

As deduced from Table 1, the taxonomic situation of the genera *Zerynthia*, *Osmoderma* and *Morimus* has varied in comparison with that which occurred in the

Table 1. Target taxa (species and subspecies) of the MIPP Project, with indications on: taxonomic arrangement (valid scientific names, author and year of description, order and family to which taxa are currently assigned); Annexes of the Habitats Directive where the taxa are listed (II and/or IV); MIPP objectives where taxa were involved (CZ: Citizen Science; TM: test of monitoring methods); study areas where monitoring methods have been tested for saproxylic taxa (BF: Bosco della Fontana, BM: Bosco della Mesola, FC: Foreste Casentinesi, PA: Parco Nazionale d'Abruzzo, Lazio e Molise, PG: Parco Naturale Regionale delle Prealpi Giulie).

Taxon, author and year of description	Order	Family	Annex HD	MIPP objectives	Study areas
Saga pedo Pallas, 1771	Orthoptera	Tettigoniidae	IV	CZ	
Parnassius apollo (Linnaeus, 1758)	Lepidoptera	Papilionidae	IV	CZ	
Zerynthia cassandra (Geyer, 1828) *Zerynthia. polyxena* (Denis and Schiffermüller, 1775)	Lepidoptera	Papilionidae	IV	CZ	
Lopinga achine (Scopoli, 1763)	Lepidoptera	Nymphalidae	IV	CZ	-
Lucanus cervus (Linnaeus, 1758)	Coleoptera	Lucanidae	II	CZ, TM	BF, FC
Osmoderma eremita eremita (Scopoli, 1763) *Osmoderma eremita italicum* Sparacio, 2000 *Osmoderma cristinae* Sparacio, 1994	Coleoptera	Scarabaeidae	*II, IV	CZ, TM	PA, FC
Cerambyx cerdo Linnaeus, 1758	Coleoptera	Cerambycidae	II, IV	CZ, TM	BF, BM
Rosalia alpina (Linnaeus, 1758)	Coleoptera	Cerambycidae	*II, IV	CZ, TM	PA, FC
Morimus asper asper (Sulzer, 1776) *Morimus asper funereus* Mulsant, 1863	Coleoptera	Cerambycidae	II	CZ,TM	BF / PG

*priority species

original Annexes of the Directive, due to some changes being made to systematics in consequence of morphological and molecular studies. Either *Zerynthia polyxena* or *Osmoderma eremita* have been divided into two species, while *Morimus funereus* was downgraded to a subspecies of *Morimus asper*. Moreover, the *Osmoderma* populations of southern Italy (Campania, Basilicata and Calabria regions) were assigned to a new subspecies, *O. eremita italicum*. Therefore, the Italian populations of *Osmoderma* now include two subspecies of *O. eremita* and a valid species endemic to Sicily, *O. cristinae*.

The saproxylic beetles (*Lucanus*, *Osmoderma*, *Cerambyx*, *Rosalia* and *Morimus*) have been used for both the main objectives of the project: 1) the definition of a standardised method for the monitoring of the species in Europe, in agreement of the criteria identified in the former chapter and 2) the collection of data on their distribution, altitude and phenology in Italy, through a Citizen Science approach. On the contrary, the butterflies (*Parnassius*, *Zerynthia* and *Lopinga*) and the bush cricket (*Saga pedo*) were used only for the second objective. Details of the Citizen Science approach are given by Campanaro et al. (2017b).

The target species of saproxylic beetles were selected as they share the following characteristics: (1) are listed in the Annexes II and/or IV of the Habitats Directive, (2) have a large body size, (3) are relatively easy to identify, (4) lack well tested monitoring methods, (5) depend on dead wood for completing their life cycle and (6) live in dif-

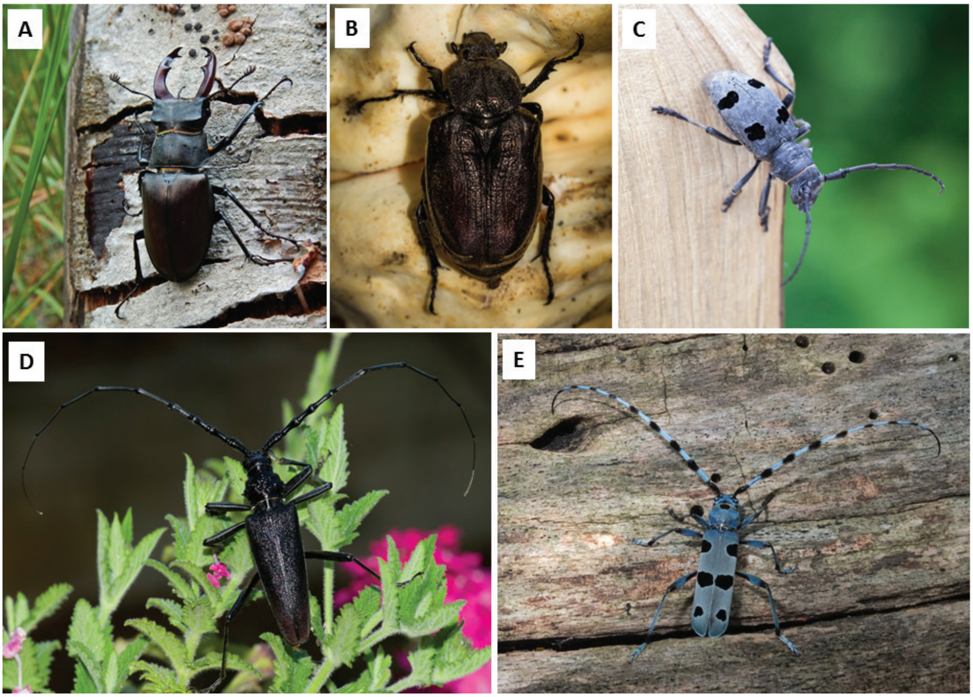

Figure 1. The target species of saproxylic beetles monitored by the Life MIPP Project. **A** *Lucanus cervus* (Photo by Fabio Garzuglia) **B** *Osmoderma eremita* (Photo by Francesco Lemma) **C** *Morimus asper funereus* (Photo by Kajetan Kravos) **D** *Cerambyx cerdo* (Photo by Elia Ferro) **E** *Rosalia alpina* (Photo by Sönke Hardersen).

ferent and representative micro-habitats of dead wood food-chains. These species are briefly introduced in the following.

Lucanus cervus (English name: European stag beetle): it lives in deciduous woodlands and flies at sunset. Larvae feed on dead wood in contact with the ground, e.g., under logs and stumps, or in senescent tree roots. Biology, ecology and monitoring methods of this species are treated in detail by Bardiani et al. (2017).

Osmoderma eremita (English name: hermit beetle) and its derived taxa: a typical inhabitant of old tree hollows. Larvae dig and feed into the walls of the cavities or in the wood mould settled at the bottom of the tree. The adult is very elusive. Biology, ecology and monitoring methods of this species are discussed in detail by Maurizi et al. (2017).

Cerambyx cerdo (English name: great capricorn beetle): nocturnal adults fly after sunset. Larvae live inside the decaying wood of large, old trees (especially oaks), often exposed to the sun. Biology, ecology and monitoring methods of this species are discussed in detail by Redolfi De Zan et al. (2017).

Rosalia alpina (English name: rosalia longicorn): diurnal adults often seen on the bark of old beech trees. Larvae typically develop in the wood of large beech trees often in other accompanying broadleaf species. Biology, ecology and monitoring methods of this species are discussed in detail by Campanaro et al. (2017a).

Table 2. Study areas where the monitoring methods were tested during the MIPP fieldwork.

Macro-areasand their acronyms	Physical geography	Administrative Region	Subareas (study sites)	Altitudem a.s.l.	Coordinates (Decimal Degrees)
Parco Naturale Regionaledelle Prealpi Giulie (PG)	Eastern Alps	Friuli-Venezia Giulia	Starmiza di Resia	750–850	46.3435-46.3414°N, 13.2994-13.3078°E
Bosco della Fontana (BF)	Po River Valley	Lombardia		19–25	45.2003°N, 10.7408°E
Bosco della Mesola (BM)	Po River Delta	Emilia-Romagna		0–2	44.8485°N, 12.2511°E
Foreste Casentinesi (FC)	Tuscan-Emilian Apennines	Emilia-Romagna	Rifugio La Lama and its surroundings	700–900	43.4312°N, 11.8381°E
		Toscana	Camaldoli	820–870	43.7874°N, 11.8208°E
Parco Nazionale d'Abruzzo, Lazio e Molise (PA)	South-central Apennines	Abruzzo	Difesa di PescasseroliVal Fondillo	1234–1352, 1090–1216	41.8461°N, 13.8600°E, 41.7841°N, 13.9563°E
			Zio Mas	ca 1030	42. 0802°N, 14.0566°E
			Feudo Intramonti e Colle di Licco	ca 1030	41.7818°N, 13.8974°E

Morimus asper funereus and *Morimus asper asper* (English name: morimus longicorn): originally only the first taxon had been included in Annex II of the Habitats Directive as a valid species (*Morimus asper*). A recent genetic study (Solano et al. 2013) suggests that all European populations of this genus should be referred to as *Morimus asper*, a genetically and morphologically variable species. Despite being unable to fly, this apterous species has unexpected dispersal ability. Larval development takes place in recently cut wood, stumps and trunks of damaged trees. Biology, ecology and monitoring methods of this species are discussed in detail by Hardersen et al. (2017).

Study areas

The methods for monitoring the target species were tested in five areas, mostly managed by CUTFAA. All areas investigated are an integral part of the Italian Natura 2000 network (SCIs and SPAs), and the data gathered represent an important contribution to the monitoring of the target species in Italy. See Table 2 for an overview of the study areas, their subdivision in subareas (study sites), with their geographic location, altitude and coordinates.

Parco naturale regionale delle Prealpi Giulie

The Parco Naturale Regionale delle Prealpi Giulie (=Julian Prealps Natural Park) covers 9,400 hectares and lies at the boundary between the Julian Alps and the Julian Prealps,

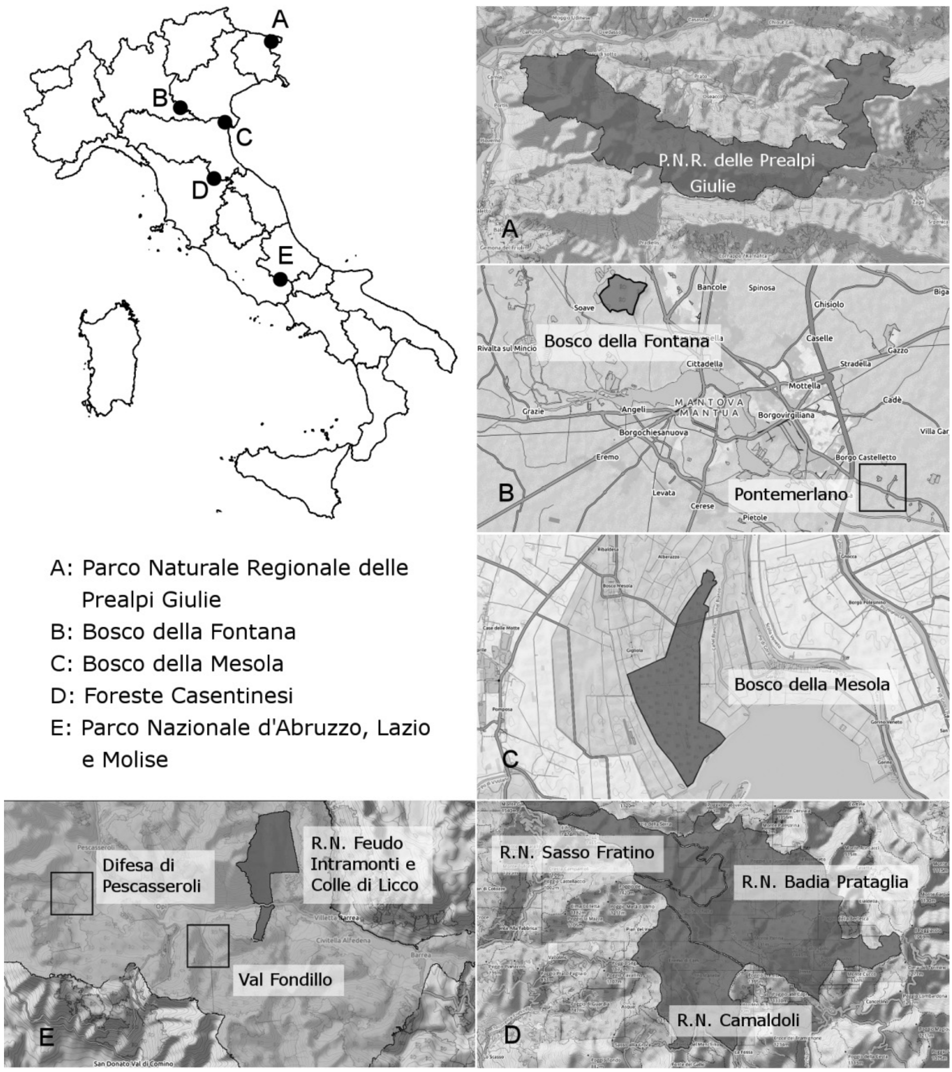

Figure 2. Location of the study areas and study sites used for monitoring the target species.

close to the Slovenian border. The park is a mountainous system which ranges from 300 to 2587 m a.s.l. and is divided into two main catchment areas. It is part of the two Special Areas of Conservation (SAC): IT3320012 (Prealpi Giulie Settentrionali) and IT3321002 (Alpi Giulie). Beech forests occur up to around 850 m a.s.l. and are replaced by *Pinus mugo* stands above 1400 m. The southern flanks of the mountains, characterised by poor soils and relatively high temperatures, are dominated by *Ostrya carpinifolia* and *Fraxinus ornus*. Other important formations present in the Park are forests of black pine (*Pinus nigra*) and Scots pine (*P. sylvestris*). The research for the MIPP project was carried out in the forest near to Starmiza di Resia (between 46.3435°N, 13.2994°E and 46.3414°N, 13.3078°E).

Figure 3. Parco Naturale Regionale delle Prealpi Giulie: young and even-aged beech trees, poor in dead wood. Wood piles were built for attracting *Morimus asper funereus* (Photo by Sönke Hardersen).

This area (750–850 m a.s.l.) is managed by shelterwood cutting and dead wood is removed. It resulted in young and even-aged beech trees and was poor in dead wood. However, owing to the local very steep morphology, dead wood occurs in small and isolated pockets of forests, favoured by limited accessibility or avalanches. Although the Park hosts populations of *Lucanus cervus*, *Rosalia alpina* and *Morimus asper funereus*, monitoring methods were only tested for the latter.

Bosco della Fontana

The Riserva Naturale Biogenetica Bosco della Fontana [=Bosco Fontana Nature Reserve] is located in the province of Mantova [Mantua], at an altitude of 19 to 25 m a.s.l. Bosco Fontana (45.200299°N, 10.740841°E) is a site of the Natura 2000 network (IT20B0011), managed by the Ufficio Territoriale Carabinieri per la Biodiversità di Verona [= Territorial Office of Carabinieri for Biodiversity of Verona]. It covers an area of 233 ha, of which 198 ha represent one of the last remnants of the ancient lowland broadleaf forests (with *Quercus robur* and *Carpinus betulus*) of the Po Valley (Mason 2002a). The reserve is an isolated forest patch, as the surrounding landscape has been significantly modified by human activities, including agricultural fields, rural settlements

Figure 4. Bosco della Fontana: large amount of dead wood due to special conservation management since 1992 (Photo by Sönke Hardersen).

and industrial buildings. The forest is dominated by pedunculate oak (*Quercus robur*) and hornbeam (*Carpinus betulus*), with a dense understory of hazel (*Corylus avellana*), hawthorn (*Crataegus laevigata*) and European spindle (*Euonymus europaeus*). In the northern part of the forest, the pedunculate oak is replaced by Turkey oak (*Quercus cerris*), while in the eastern part, southern ash (*Fraxinus angustifolia*) and black alder (*Alnus glutinosa*) are abundant. Between 1952 and 1958, allochthonous tree species (*Quercus rubra*, *Platanus* x *acerifolia*, *Juglans nigra*) were planted and the Life Project NAT/IT99/6245 initiated the elimination of the most invasive species, *Q. rubra* (Cavalli and Mason 2003, Mason 2003, Mason and Minari 2009). From the early 1990s, no wood has been removed from the forest and therefore the amount of dead wood increased: in 1995 it consisted of ca 26 m^3/ha on average (Mason 2002a) but locally reached 124 m^3/ha (Travaglini et al. 2007). Further information on management of the reserve are in Cavalli and Mason (2003), Mason et al. (2004), Campanaro et al. (2014). At Bosco della Fontana monitoring methods were tested for *L. cervus*, *C. cerdo* and *M. asper*.

Bosco della Mesola

The Riserva Naturale Bosco della Mesola [= Bosco della Mesola Nature Reserve] is managed by the Ufficio Territoriale Carabinieri per la Biodiversità di Punta Marina [= Territorial Office of Carabinieri for Biodiversity of Punta Marina] and covers an area of 1500 hectares. The Reserve, which is part of the Natura 2000 network

Figure 5. Bosco della Mesola: coastal lowland forest, where the abandonment of traditional management of the forest since the 1970s has resulted in an increase in dead wood (Photo by Gloria Antonini).

(IT 4060006) and of the Regional Park of the Po Delta, is located in the Province of Ferrara about 16 km from the Adriatic coast. This forest is a coastal lowland woodland of high importance for conservation, dominated by holm oak (*Quercus ilex*) and pedunculate oak (*Quercus robur*). Other important tree species occurring in the area are hornbeams (*Carpinus betulus* and *C. orientalis*), ashes (*Fraxinus angustifolia* and *F. ornus*), poplars (*Populus alba*, *P. tremula*, *P. nigra*) and field elm (*Ulmus minor*). The understory is scarce and the most abundant species are common hawthorn (*Crategus monogyna*) and wild privet (*Ligustrum vulgare*). Between 1945 and 1971, non-native trees were planted, such as stone pine (*Pinus pinea*) and maritime pine (*Pinus pinaster*). The abandonment of traditional management of the forest since the 1970s has resulted in an increase in dead wood which is now very abundant. The research conducted during the MIPP project in the Bosco della Mesola (44.8485°N, 12.2511°E) was focused on testing the monitoring methods for *C. cerdo* and *M. asper asper*.

Foreste Casentinesi

The Foreste Casentinesi [Casentine Forests] are natural and historical forest areas located in the Tuscan-Emilian Apennines, within the Parco Nazionale delle Foreste

Figure 6. Foreste Casentinesi: old chestnut orchards where a population of *Osmoderma eremita* was discovered for the first time by the MIPP researchers (Photo by Sönke Hardersen).

Casentinesi, Monte Falterona e Campigna [= Foreste Casentinesi, Monte Falterona and Campigna National Park]. The research was carried out in two areas managed by the Ufficio Territoriale Carabinieri per la Biodiversità di Pratovecchio [= Territorial Office of Carabinieri for Biodiversity of Pratovecchio]: the Lama Forest and adjacent zones which are part of the Natura 2000 network (IT 4080001) and the chestnut stand Foreste di Camaldoli, Badia Prataglia (IT 5180018). The core area of the Casentine forests is the area of Sasso Fratino (764 ha), where cuts have been abandoned for more than 100 years. This forest is amongst the most natural woodlands of western Europe and consist of beech woods, mixed stands of silver fir and beech, and mixed deciduous broadleaf forests. Beech forests are widespread in the Park from 600–700 m up to 1,700–1,800 m a.s.l. Although their composition varies with altitude, they are mostly dominated by beech (*Fagus sylvatica*) and harbour a number of associated trees such as sycamore maple (*Acer pseudoplatanus*), European ash (*Fraxinus excelsior*), whitebeam (*Sorbus aria*), alpine laburnum (*Laburnum alpinum*) and rowan (*Sorbus aucuparia*). The mixed deciduous broadleaf forests are widespread throughout sub-montane areas and represent the most diverse tree association of the Apennines, with various combinations of manna ash (*Fraxinus ornus*), Turkey oak (*Quercus cerris*), downy oak (*Quercus pubescens*), Italian maple (*Acer opalus*), Norway maple (*Acer platanoides*), European hop-hornbeam (*Ostrya carpinifolia*), large-leaved lime (*Tilia platyphyllos*), com-

Figure 7. Foreste Casentinesi: natural beech forest where three target species were monitored, *Lucanus cervus*, *Osmoderma eremita* and *Rosalia alpina* (Photo by Sönke Hardersen).

mon laburnum (*Laburnum anagyroides*), wild cherry (*Prunus avium*) etc. Finally, near to human settlements, e.g. near the Monastery of Camaldoli, there are some very old stands of *Castanea sativa* which are managed as coppice or chestnut orchards. The

Figure 8. Parco Nazionale d'Abruzzo, Lazio e Molise: old growth beech forests where monitoring methods were tested for both *Osmoderma eremita* and *Rosalia alpina* (Photo by Emilia Capogna).

Forests of the Park are home to at least four species of saproxylic beetles investigated by the project: *L. cervus*, *O. eremita*, *R. alpina* and *M. asper asper*. Monitoring methods were tested only for the first three species in the transitional belt between beech forest and mixed deciduous broadleaf forest, at an altitude of 700 to 900 m a.s.l., in several sites around the Lama Forest refuge (43.4312°N, 11.8381°E), i.e. Poggio Ghiaccione, Poggio Piano, La Vetreria, and the road to Badia. Only *O. eremita* was also monitored in the old chestnut orchard near to Camaldoli (43.7874°N, 11.8208°E) at an altitude of 820–870 m a.s.l.

Parco Nazionale d'Abruzzo, Lazio e Molise

Within the Parco Nazionale d'Abruzzo, Lazio e Molise [=Abruzzi, Lazio and Molise National Park], centred in the southern part of the Abrutian Apennines, surveys for the MIPP project have been carried out at four sites: "Difesa di Pescasseroli" (41.8461°N, 13.8600°E), "Val Fondillo" (41.7841°N, 13.9563°E), "Riserva Naturale Orientata Feudo Intramonti e Colle di Licco" (41.7818°N, 13.8974°E) and "Zio Mas" (42. 0802°N, 14.0566°E). The first two areas (Difesa di Pescasseroli and Val Fondillo) are managed by the Park, while the other two (Zio Mas and Feudo Intramonti) are run by the Ufficio Territoriale Carabinieri per la Biodiversità di Castel di Sangro [= Territorial Office of Carabinieri for Biodiversity of Castel di Sangro] (province of L'Aquila). Difesa di Pescasseroli and Val Fondillo are located near Pescasseroli (province of L'Aquila), at an altitude of 1234–1352m and 1090–1216m respectively and consist of a mosaic of mature and old-growth forests dominated by beech (*Fagus sylvatica*) and its associated tree species, such as sycamore maple (*Acer pseudoplatanus*), European ash (*Fraxinus excelsior*), whitebeam (*Sorbus aria*), hornbeam (*Carpinus betulus*), yew (*Taxus baccata*) etc. Beech forests cover more than 60% of the Park surface and harbour very old trees, due to the limited accessibility of some steep mountainsides or to the presence of nature reserves where all exploitation of resources is strictly forbidden. Zio Mas and Feudo Intramonti e Colle di Licco are located at an altitude around 1030 m, near to Casone Crugnale, in mesophilous deciduous forests. Monitoring methods were tested only for *O. eremita* and *R. alpina*.

Developing the methods for monitoring

In order to develop and test the most appropriate monitoring methods, all relevant literature was reviewed in order to select the most suitable approaches to be tested for the monitoring of the target species. In Action A1 of the project MIPP, these methods were critically reviewed and the reviews were sent to 15 experts from other European countries for critical examination. Comments were received from renowned specialists and their advice considerably improved the reviews. Based on these documents, a work plan was developed for the years 2013–2017 in collaboration with the Region Lombardy (Action A2) and the appropriate statistical approaches were selected. It became obvious that the suitability of the statistical approach was influenced by many variables, e.g. local population structure. Whereas an abundant and evenly distributed population of *M. asper* at Bosco della Fontana allowed the adoption of an approach based on randomisation, a clustered population structure, as observed in the Julian Prealps, called for a randomised block design. The target parameters to be monitored were always the number of individuals detected in relation to a given effort. It has been shown that such counts are often highly correlated with estimates of population sizes in insects (e.g. Dolek and Geyer 2000, Collier et al. 2008). The correlations between the number of individuals observed and environmental parameters were also considered, with the aim of providing practical indications for the monitoring (i.e. temperature, tree-diameters, time of the year). Generally, the methods chosen were based on the means by which the target species could be attracted or situations where/when individuals naturally occur at higher densities. When monitoring rare or cryptic organisms, the use of attractants can help to increase detection rates (e.g., Larsson and Svensson 2011, Ray et al. 2009). For example, adults of *O. eremita* are attracted by the pheromone (R)-(C)-γ -decalactone (Larsson et al. 2003) and adults of *M. asper* by freshly cut wood (Chiari et al. 2013a). An example for one of the target species naturally occurring at higher densities is the 30 minute period around sunset between 18 June and 8 July for *L. cervus* (Campanaro et al. 2016). A further important point was that the methods to be tested did not kill or harm the insects. Therefore, traps were intended to catch live adults of the target species and were checked daily; the insects caught were carefully handled and quickly released. The methods selected for each species were applied in at least two study areas and were carried out for a minimum of three seasons.

The large amount of field work carried out for the development of the monitoring methods was only possible because many people helped the project staff. Many field assistants from various countries joined the team during the field work. A total of 10 Theses for Bachelor, Master and PhD degrees (see names in Acknowledgements) were written in collaboration with the project MIPP, the contribution by some of these students being crucial for the field work as well. The final aim of the fieldwork was to develop methods which ensure scientific validity, ease of execution and low cost. Obviously, the methods published are compromises as, particularly for small populations, it is difficult to balance limited labour costs and high detection probability. The results of the field work were analysed statistically, with a large variety of approaches, always with the aim of comparing the different methods tested and defining the best possible monitoring method.

A special permit was obtained from the Italian Ministry of Environment for handling and capturing individuals of the target species (collection permit: Ministero dell'ambiente e della Tutela del Territorio e del Mare – DG Protezione della Natura e del Mare, U.prot PNM 2012-0010890 del 28/05/2012).

Methods tested for the MIPP project

Different methods have been used for either detecting the target species (to assess their presence in the areas) or monitoring their populations (to develop a suitable technique for non-expert operators).

L. cervus was counted along 500m linear transects at dusk (15min before and after sunset), using both sightings and net captures in two study sites, Bosco Fontana and Foresta della Lama. At Bosco Fontana, stag beetle remains from bird predation were collected by day along transects, while aerial traps, baited with wine and/or beer with sugar or banana juice, were set in trees at different heights from the ground. Details on methods are reported by Bardiani et al. (2017a, 2017b).

Osmoderma eremita was lured by pheromone traps (BCWT) and unbaited pitfall traps (PT) in three study sites of the Abruzzi, Lazio and Molise National Park (Difesa di Pescasseroli, Val Fondillo and Feudo Intramonti- Colle di Licco) and in two sites of the Casentine Forests (Lama Forest and Monastery of Camaldoli). Larvae were searched for with the aid of the trained dog (Osmodog subproject) in several sites of all macro-areas. Details on methodologies are reported by Maurizi et al. (2017).

Cerambyx cerdo was monitored in Bosco della Fontana and Bosco della Mesola, by four methods: using attractant for adults i.e. ash sap in feeding stations on trees; setting aerial traps baited with fermented mixtures of wine, beer, banana or sugar; detecting the adults during Visual Encounter Surveys (VES); and collecting remains along transects. Details on methods are reported by Redolfi De Zan et al. (2017).

Rosalia alpina was monitored in several beech forest sites of the Foreste Casentinesi and of Abruzzo, Lazio and Molise National Park. Methods consisted of detecting adults during visual surveying of single logs or groups of logs; and searching on artificial tripods made from beech woods. Trees were considered suitable for these beetles when characterised by the presence of dead wood on the trunk and exposed to direct sun light for at least 1–2 hours. Logs consisted of beech trunks with diameters of 28–75cm. Tripods consisted of 3 beech logs (diameters 20–25cm) and were placed in open areas with easy access (along forest roads or clearings) at a minimum distance of 30m from each other. Details on methodologies are reported by Campanaro et al. (2017a).

Morimus asper was monitored in the Julian Prealps (*M. asper funereus*) and Bosco della Fontana (*M. asper asper*) by using freshly cut log piles and pitfall traps baited with chemical compounds. Between 2014 and 2016, the influence of the characteristics of logs (e.g. diameter, age of wood, tree species) on the number of adults observed has been evaluated. Additionally, the attraction of chemical compounds for the species was assessed. Details on methods are reported by Hardersen et al. (2017).

Faunistic data were collected with the Citizen Science action developed within the LIFE MIPP Project using a website and an app for mobile devices, with an attempt being made to involve the highest number of persons possible. All data were validated by experts, based on pictures taken by citizens.

Osmodog: training a dog for searching *Osmoderma eremita*

The dog, a Golden Retriever named "Teseo", was chosen from a stock selected for the CITES Service of the former CFS (today: CUTFAA) and trained under the supervision of experienced staff. Originally, it was hypothesised to exploit the fact that males of *O. eremita* release large quantities of a sex pheromone which even humans can smell. However, after consulting with the Department of Forest Protection of the Austrian Research Centre for Forests, which has for many years trained scent detection dogs for the longhorn beetle *Anoplophora glabripennis*, it was decided to focus the training on the larvae of the hermit beetle. The reasons for this decision were: 1) Larvae of saproxylic beetles have a species-specific scent (Hoyer-Tomiczek et al. 2016), even though it cannot be smelt by humans; 2) Larvae of *O. eremita* are present all year round whereas the adults only occur from June to September; 3) Only larvae reliably indicate trees in which the species reproduces, as adults can fly up to 1.5km (Chiari et al. 2013b). Teseo started working with its trainer at the age of 6 months and actual fieldwork was carried out once the dog had reached adulthood (24 months). To imprint the target odour on the dog, a hermit beetle larva was kept in a box filled with wood mould collected from a tree. During the initial trials, the larva was washed with water and kept in a perforated box. When the dog had learned to recognise the target odour, the training continued by hiding the boxes (full and empty) inside basal cavities of trees and other microhabitats within a fenced training area. This training phase ended when Teseo had

Figure 9. The trained dog searching for hermit beetles with his handler (Fabio Mosconi) in the old growth forest of Difesa di Pescasseroli (Photo by Emanuela Maurizi).

successfully learned to locate and signal the target to the trainer. The accuracy of Teseo was measured in eight wooded areas by two different approaches. The first set of tests was carried out in six sites without populations of hermit beetle which did however contain hollow or fractured trees suitable for its larvae. Further tests were based on filters soaked with the larval odour, hidden in randomly chosen trees by a field assistant unbeknown to the dog and to the handler. During the search sessions, the handler reported on the behaviour of the dog to a field assistant by assigning a score to the dog response: no reaction, partial signalling and complete signalling. Details on the methods are giving by Mosconi et al. (2017).

Results and discussion

Monitoring the target species

For each target species, the MIPP research staff identified a monitoring method judged to be the "best method" for detecting changes in occurrence and abundance of a population over time, according to the following criteria: scientific validity, high number of detected or captured individuals, ease of execution, low level of invasiveness and low cost.

For *Lucanus cervus*, the selected method consisted of walking at dusk, along a standard length transect (500m long and 10m width) and counting all adults of stag beetles seen flying or walking on the ground. This transect walk was carried out by one operator, from 15 minutes before sunset to 15 minutes after sunset. Overall, a transect walk lasted 30 minutes. The transect was divided into 5 sectors of 100 m; each sector had to be walked in 6 minutes. Transects were chosen along forest paths, tracks or roads with acceptable light conditions at dusk and with a suitable canopy openness. Details on the method are reported by Bardiani et al. (2017).

For *O. eremita*, the selected method for monitoring was based on black cross window traps (BCWT) baited with the sex pheromone of the species and hung on tree branches. Traps had to be checked every second day to prevent the animal from dying. In order to minimise disturbance of reproductive activity and behaviour, this monitoring was undertaken no more frequently than every three years. As an alternative method, a high number (at least 150) of pitfall traps (PT) could be placed into the same number of tree cavities. Although this method is cheaper and less invasive (as no pheromones interfere with the breeding activities), it may be used only in areas where tree hollows are very abundant and rich in wood mould. Additionally, the team needed to be composed of several people in order to ensure the checking of so many traps every two days. Details on traps and problems related to the sampling methods of this elusive species are reported by Maurizi et al. (2017).

For *C. cerdo*, the selected method is the use of purpose-made aerial traps composed of two stacked plastic containers embedded together one above the other with a wire insect net between them. Traps should be positioned on tree branches (especially oaks with a diameter larger than 50cm), at a height of 10m above ground and baited with red wine, white wine and sugar. Ten traps were positioned in each sampling area and needed to be checked every day. Details on traps and protocol are reported by Redolfi De Zan et al. (2017).

For *R. alpina*, the selected method consisted of surveying 15 beech trees with a large diameter (over 30cm), dead or rich in deadwood and exposed to the sun, these being the natural breeding habitat of the species. The results showed that the use of artificial wood baits (tripods) or logs specifically placed for monitoring did not represent practical alternatives. Details on results are reported by Campanaro et al. (2017a).

For *M. asper*, selected method consisted of building and checking freshly cut wood piles which functioned as an attractant for the adults of this species. Each pile was built from single tree species, using wood with a diameter larger than 12cm. The tree species used for building the piles needed to be chosen carefully and the preferred trees seemed to be hardwood species such as beech and oaks. Details on results are reported by Hardersen et al. (2017).

Citizen science

A total number of 2,308 records were transmitted to the project database by a total of 695 citizens. The high rate of correct validations (73%) confirmed that the majority of the data collected by volunteers was correct. The number of annual records constantly grew from 2014 to 2016 as did the number of participants. The species most commonly recorded was *L. cervus*, followed by *M. asper* and *R. alpina*. The records, collected by the citizen science approach, allowed detailed analysis of altitudinal distribution and phenology of the target species, particularly for those with the highest numbers of records. These data were in line with the phenology and altitudinal distribution published by authors of scientific publications on the subject. For four species it was also possible to analyse how phenology changed with increasing altitude and it was found that peak activity was delayed by 10 days on average when moving 400m upwards.

Osmodog

For each field session, the dog was able to work for about 50 minutes, after which it needed to rest for 15 to 60 minutes before restarting its work. High temperatures caused a general decrease in the dog's working ability. At the end of the training, Teseo learnt to signal exclusively for the larvae of *O. eremita* even though it sometimes showed some faint reactions to the larvae of other species such as *Oryctes nasicornis* and *Gnorimus variabilis* (e.g., by sitting adjacent to the source of the odour or barking weakly). The results of the tests showed that the use of a trained dog to find hermit beetle larvae is better than the traditional wood mould sampling. In fact, the dog showed a higher degree of success in detecting colonised trees (over 70%) in two areas formerly checked with wood mould sampling (up to 50%) (Chiari et al. 2014). Furthermore, the use of a dog was much less time consuming than wood mould sampling: Teseo, during a day with good weather conditions, was able to detect larvae of *O. eremita* with high accuracy and employed less than one tenth of the time needed for wood mould sampling. In addition, the use of a dog eliminated the risk of harming larvae and the fragile equilibrium of its microhabitat where a whole biocoenosis of saproxylic organisms occurs.

Dissemination of knowledge and communication

Participation by children in environmental education programmes seems to have a great impact on their attitude and behaviour. Some studies have shown that children who participate in such programmes are more concerned about nature, want to learn more about environmental issues and are more prone to follow pro-environmental behaviour (e.g. waste recycling) than children who did not participate (see Wells and Lekies 2012, for a review). The MIPP actions focused on Dissemination and Communication, to inform the public on issues such as Natura 2000, the Habitats Directive, monitoring activities, the forest ecosystem and the ecological role of the saproxylic insects. These actions involved a large variety of media and approaches: radio-television programmes, press releases, interviews, articles on nature magazines, stories in newspapers, school lessons, presence at fairs, public lectures, creation of comic strips, leaflets, posters, etc. The dog trained to monitor the hermit beetle, was also used as a "curiosity" factor to attract public attention toward the rare beetle and the issues mentioned above. For example, the dog was used as the principal character in the brochure "Osmodog and the small forest dwellers" [Italian title: Osmodog e i piccoli abitanti delle foreste), intended for school children (it can be downloaded free at: http://lifemipp.eu/mipp/data/download/Osmodog.pdf).

In the MIPP project, a specific education activity, named "MIPP-iacciono gli insetti" [literal translation: I like insects] for young people from primary to high school, was developed and carried out in several regions of Italy. One of the objectives of the project was to perform 60 activities (lessons) per year and involve 3000 students. The aims of the activity were to disseminate information related to the protection and conservation of old forest and dead wood and to allow children to learn about saproxylic insects. "MIPP-iacciono gli insetti" started with an interactive discussion stimulated by a variety of pictures of old and artificial forests, dead wood and saproxylic insects. After this, children were encouraged to learn how to identify the target insects of the MIPP project, by using their sense of hearing, sight and smell. In particular, during the game of "smell" they impersonated Teseo, the dog, by sniffing a series of smells and guessing that of *Osmoderma eremita*.

Other educational activities for young people from primary to high school performed during the MIPP project included guided tours in natural reserves, where particular attention was paid to old forests, dead wood and saproxylic insects.

Figure 10. Dissemination activities with children: digging into dead wood and handling beetles (Photo by Michela Maura).

Concluding remarks and recommendations

The Life Project MIPP was a challenge from many points of view: collaborating between different institutions, coordinating a large amount of field work in five study

Figure 11. Dissemination activities in primary school: a field trip together with MIPP researchers (Photo by Emanuela Maurizi).

areas, developing monitoring methods for cryptic and elusive species, organising a citizen science project to increase the knowledge on the distribution of rare and protected insects, educating people on biodiversity and Natura 2000 and on the importance of veteran trees in forest ecosystems, etc. Due to the goodwill and hard work of many people, obstacles and hurdles were overcome and the project managed to complete all planned actions successfully. The articles in this special issue provide the scientific results on testing monitoring methods and the detailed presentation of the method which resulted in being the most appropriate in terms of costs and accuracy for monitoring the conservation status of the five saproxylic beetles targeted by the project. Two of these articles have been dedicated respectively to the successful results of two subprojects: the citizen science and the training of a dog for detecting *O. eremita* in the field.

The results on monitoring methods showed that the management authorities can be able to provide the six-year report requested by the Habitats Directive on the five saproxylic species listed in the Annexes, by means of simple, efficient and low cost methods and procedures. Moreover, they can easily obtain the help of Master's students and PhD scholars from several universities where saproxylic insect ecology is the subject of local research groups. A 'symbiotic relationship' or simply mutual support can be developed between research institutions and conservation authorities with reciprocal benefits. Many students and scholars are available to work for free in monitoring threatened species in order to get data for their theses, vocational training, internship at a protected area or research project. The management authorities can provide these helpers with logistical support, e.g., transportation inside the protected area on off-road vehicles (if necessary), rangers as guides, accommodation in refuges or local guest-rooms. Simple and basic cottages with bunk beds for scholars and volunteers should be one of the first actions to be made for improving scientific research and monitoring in a protected area. With regards to the use of the data collected every year on the populations of the target species (i.e. number of specimens recorded in each area by each method per day/week/month/total), each country should delegate a ministerial office or other governmental agency to gather this precious information in a simple database and this should be published on line, in an official site, with access reserved for the scientific community.

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References

Alexander KNA (2008) Tree biology and saproxylic Coleoptera: issues of definitions and conservation language. Revue d'Ecologie (Terre et Vie) 63: 1–6.

Bardiani M, Chiari S, Maurizi E, Tini M, Toni I, Zauli A, Campanaro A, Carpaneto GM, Audisio P (2017) Guidelines for the monitoring of *Lucanus cervus*. In: Carpaneto GM, Audisio P, Bologna MA, Roversi PF, Mason F (Eds) Guidelines for the Monitoring of the Saproxylic Beetles protected in Europe. Nature Conservation 20: 37–78. https://doi.org/10.3897/natureconservation.20.12687

Beebe SC, Howell TJ, Bennett PC (2016) Using scent detection dogs in conservation settings: a review of scientific literature regarding their selection. Frontiers in Veterinary Science 3: 1–13. https://doi.org/10.3389/fvets.2016.00096

Bonn A, Richter A, Vohland K, Pettibone L, Brandt M, Feldmann R, Goebel C, Grefe C, Hecker S, Hennen L, Hofer H, Kiefer S, Klotz S, Kluttig T, Krause J, Küsel K, Liedtke C, Mahla A, Neumeier V, Premke-Kraus M, Rillig MC, Röller O, Schäffler L, Schmalzbauer B, Schneidewind U, Schumann A, Settele J, Tochtermann K, Tockner K, Vogel J, Volkmann W, von Unger H, Walter D, Weisskopf M, Wirth C, Witt T, Wolst D, Ziegler D (2016): Green paper citizen science strategy 2020 for Germany. Helmholtz Centre for Environmental Research (UFZ), German Centre for integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Leipzig, Museum für Naturkunde Berlin, Leibniz Institute for Evolution and Biodiversity Science (MfN), Berlin-Brandenburg Institute of Advanced Biodiversity Research, Berlin.

Bouget C, Brustel H, Brin A, Noblecourt T (2008) Sampling saproxylic beetles with window flight traps: methodological insights. Revue d'Ecologie (Terre et Vie) 63: 13–24.

Brunet J, Isacsson G (2009) Restoration of beech forest for saproxylic beetles: effects of habitat fragmentation and substrate density on species diversity and distribution. Biodiversity and Conservation 18: 2387–2404. https://doi.org/10.1007/s10531-009-9595-5

Buse J (2012) "Ghosts of the past": flightless saproxylic weevils (Coleoptera: Curculionidae) are relict species in ancient woodlands. Journal of Insect Conservation 16: 93–102. https://doi.org/10.1007/s10841-011-9396-5

Bütler R, Lachat T, Larrieu L, Paillet Y (2013) Habitat trees: key elements for forest biodiversity. In: Kraus D, Krumm F (Eds) Integrative approaches as an opportunity for the conservation of forest biodiversity, European Forest Institute, Freiburg, 84–91.

Campanaro A, Bardiani M, Spada L, Carnevali L, Montalto F, Antonini G, Mason F, Audisio P (Eds) (2011) Linee guida per il monitoraggio e la conservazione dell'entomofauna saproxilica. Quaderni Conservazione Habitat, 6. Cierre Grafica, Verona, 1–8 + CD-ROM

Campanaro A, Hardersen S, Minari E, Toni I, Mason F (2014) Piano di gestione della Riserva Naturale Statale e Sito Natura 2000 "Bosco della Fontana" – Aggiornamento 2014–2020. Quaderni Conservazione Habitat, 8, Cierre edizioni, Verona, 1–265.

Campanaro A, Zapponi L, Hardersen S, Méndez M, Al Fulaij N, Audisio P, Bardiani M, Carpaneto GM, Corezzola S, Della Rocca F, Harvey D, Hawes C, Kadej M, Karg J, Rink M, Smolis A, Sprecher E, Thomaes A, Toni I, Vrezec A, Zauli A, Zilioli M, Chiari S (2016) European monitoring protocol for the stag beetle, a saproxylic flagship species. Insect Conservation and Diversity 9: 574–584. https://doi.org/10.1111/icad.12194

Campanaro A, Redolfi De Zan L, Hardersen S, Antonini G, Chiari S, Cini A, Mancini E, Mosconi F, Rossi de Gasperis S, Solano E, Bologna MA, Sabbatini Peverieri G (2017a) Guidelines for the monitoring of *Rosalia alpina*. In: Carpaneto GM, Audisio P, Bologna MA, Roversi PF, Mason F (Eds) Guidelines for the Monitoring of the Saproxylic Beetles protected in Europe. Nature Conservation 20: 165–203. https://doi.org/10.3897/natureconservation.20.12728

Campanaro A, Hardersen S, Redolfi De Zan L, Antonini G, Bardiani M, Maura M, Maurizi E, Mosconi F, Zauli A, Bologna MA, Roversi PF, Sabbatini Peverieri G, Mason F (2017b) Analyses of occurrence data of protected insect species collected by citizens in Italy. In: Carpaneto GM, Audisio P, Bologna MA, Roversi PF, Mason F (Eds) Guidelines for the Monitoring of the Saproxylic Beetles protected in Europe. Nature Conservation 20: 265–297. https://doi.org/10.3897/natureconservation.20.12704

Cardoso P (2012) Habitats Directive species lists: urgent need of revision. Insect Conservation and Diversity 5: 169–174. https://doi.org/10.1111/j.1752-4598.2011.00140.x

Carpaneto GM, Baviera C, Biscaccianti AB, Brandmayr P, Mazzei A, Mason F, Battistoni A, Teofili C, Rondinini C, Fattorini S, Audisio P (2015) A Red List of Italian Saproxylic Beetles: taxonomic review, ecological features and conservation issues (Coleoptera). Fragmenta entomologica 47(2): 53–126. https://doi.org/10.4081/fe.2015.138

Castro A, Fernández J (2016) Tree selection by the endangered beetle *Rosalia alpina* in a lapsed pollard beech forest. Journal of Insect Conservation, 20: 201–214. https://doi.org/10.1007/s10841-016-9854-1

Cavalli R, Mason F (2003) Techniques for reestablishment of dead wood for saproxylic fauna conservation. LIFE Nature project NAT/IT/99/ 6245 "Bosco della Fontana" (Mantova, Italy). Scientific Reports 2, Centro Nazionale per lo Studio e la Conservazione della Biodiversità Forestale di Verona - Bosco della Fontana. Gianluigi Arcari Editore, Mantova, 1–112.

Chandler M, See L, Copas K, Bonde AMZ, López BC, Danielsen F, Legind JK, Masinde S, Miller-Rushing AJ, Newman G, Rosemartin A, Turak E (2017) Contribution of citizen science towards international biodiversity monitoring. Biological Conservation. https://doi.org/10.1016/j.biocon.2016.09.004

Chiari S, Bardiani M, Zauli A, Hardersen S, Mason F, Spada L, Campanaro A (2013a) Monitoring of the saproxylic beetle *Morimus asper* (Sulzer, 1776) (Coleoptera: Cerambycidae) with freshly cut log piles. Journal of Insect Conservation, 17: 1255–1265. https://doi.org/10.1007/s10841-013-9606-4

Chiari S, Carpaneto GM, Zauli A, Zirpoli GM, Audisio P, Ranius T (2013b) Dispersal patterns of a saproxylic beetle, *Osmoderma eremita*, in Mediterranean woodlands. Insect Conservation and Diversity, 6: 309–318. https://doi.org/10.1111/j.1752-4598.2012.00215.x

Collier N, Mackay DA, Benkendorff K (2008) Is relative abundance a good indicator of population size? Evidence from a fragmented population of a specialist butterfly (Lepidoptera: Lycaenidae). Population Ecology, 50: 17–23. https://doi.org/10.1007/s10144-007-0056-2

Commarmot B, Brändli U-B, Hamor F, Lavnyy V (Eds) (2013) Inventory of the largest primeval beech forest in Europe. A Swiss-Ukrainian scientific adventure. Birmensdorf, Swiss Federal Research Institute WSL; L'viv, Ukrainian National Forestry University; Rakhiv, Carpathian Biosphere Reserve, 1–69.

Dickinson JL, Zuckerberg B, Bonter DN (2010) Citizen science as an ecological research tool: challenges and benefits. Annual Review of Ecology, Evolution and Systematics 41: 149–172. https://doi.org/10.1146/annurev-ecolsys-102209-144636

Dolek M, Geyer A (2000) Anwendung im Naturschutz: Fang-Wiederfang-Studien in Kombination mit anderen Methoden an Apollofaltern (*Parnassius apollo* L.). Beiträge zur Ökologie, 42: 145–155.

Drag L, Hauck D, Pokluda P, Zimmermann K, Cizek L (2011) Demography and dispersal ability of a threatened saproxylic beetle: a mark-recapture study of the Rosalia Longicorn (*Rosalia alpina*). PLoS ONE 6(6): e21345. https://doi.org/10.1371/journal.pone.0021345

Dubois G (2009) Écologie des coléoptères saproxyliques: Biologie des populations et conservation d'*Osmoderma eremita* (Coleoptera: Cetoniidae). Thèse PhD, Université de Rennes 1, École doctorale Vie-Agro-Santé (Paimpont): 1–262.

Elzinga CL, Salzer DW, Willoughby JW, Gibbs DP (2001) Monitoring Plant and Animal Populations. Blackwell Scientific Publications, Abingdon, UK.

Fayt P, Dufrêne M, Branquart E, Hastir P, Pontégnie C, Henin JM, Versteirt V (2006) Contrasting responses of saproxylic insects to focal habitat resources: the example of longhorn beetles and hoverflies in Belgian deciduous forests. Journal of Insect Conservation 10: 129–150. https://doi.org/10.1007/s10841-006-6289-0

Gasparini P, Tabacchi G (Eds.) (2011) L'Inventario nazionale delle foreste e dei serbatoi forestali di carbonio (INFC 2005). Secondo inventario forestale nazionale italiano. Metodi e risultati. Ministero delle Politiche Agricole, Alimentari e Forestali; Corpo Forestale dello Stato. Consiglio per la Ricerca e la Sperimentazione in Agricoltura, Unità di ricerca per il Monitoraggio e la Pianificazione Forestale. Edagricole-Il Sole 24 ore, Bologna, 1–653.

Genovesi P, Angelini P, Bianchi E, Dupré E, Ercole S, Giacanelli V, Ronchi F, Stoch F (2014). Specie e habitat di interesse comunitario in Italia: distribuzione, stato di conservazione e trend. ISPRA, Serie Rapporti, 194/2014.

Gibbons P, Lindenmayer DB (2002) Tree hollows and wildlife conservation in Australia. CSIRO, Collingwood, Australia, 1–211.

Goldsmith FB [Ed.] (1991) Monitoring for Conservation and Ecology. Chapman and Hall, London.

Hardersen S (2003) Two lowland beech-oak forest areas abandoned for more than 30 years: what do bird and beetle communities tell us? In: Mason F, Nardi G, Tisato M (Eds) Proceedings of the International Symposium "Dead wood: a key to biodiversity", Mantova (Italy), May 29th- 31st 2003. Sherwood 95 (Suppl. 2): 33–36.

Hardersen S, Bardiani M, Chiari S, Maura M, Maurizi E, Roversi PF, Mason F, Bologna MA (2017) Guidelines for the monitoring of *Morimus asper funereus* and *Morimus asper asper*. In: Carpaneto GM, Audisio P, Bologna MA, Roversi PF, Mason F (Eds) Guidelines for the Monitoring of the Saproxylic Beetles protected in Europe. Nature Conservation 20: 205–236. https://doi.org/10.3897/natureconservation.20.12676

Haslett JR (2007) European Strategy for the Conservation of Invertebrates. Council of Europe: 1–91.

Hochkirch A (2016) The insect crisis we can't ignore. Nature 539: 141. https://doi.org/10.1038/539141a

Hoyer-Tomiczek U, Sauseng G, Hoch G (2016) Scent detection dogs for the Asian longhorn beetle, *Anoplophora glabripennis*. EPPO Bulletin 46: 148–155. https://doi.org/10.1111/epp.12282

Kobori H, Dickinson JL, Washitani I, Sakurai R, Amano T, Komatsu N, Kitamura W, Takagawa S, Koyama K, Ogawara T, Miller-Rushing AJ (2016) Citizen science: a new approach to advance ecology, education, and conservation. Ecological Research 31: 1–19. https://doi.org/10.1007/s11284-015-1314-y

Kull T, Sammul M, Kull K, Lanno K, Tali K, Gruber B, Schmeller D, Henle K (2008) Necessity and reality of monitoring threatened European vascular plants. Biodiversity and Conservation 17: 3383–3402.

Lachat T, Bütler R (2008) Îlots de sénescence et arbres-habitat pour augmenter la biodiversité en forêt. La Forêt 6: 20–21. https://doi.org/10.1007/s10531-008-9432-2

Larsson MC, Hedin J, Svensson GP, Tolasch T, Francke W (2003) Characteristic odor of *Osmoderma eremita* identified as a male released pheromone. Journal of Chemical Ecology 29: 575–587. https://doi.org/10.1023/A:1022850704500

Larsson MC, Svensson GP (2011) Monitoring spatiotemporal variation in abundance and dispersal by a pheromone- kairomone system in the threatened saproxylic beetles *Osmoderma eremita* and *Elater ferrugineus*. Journal of Insect Conservation 6: 891–902. https://doi.org/10.1007/s10841-011-9388-5

Le Gouar PJ, Dubois GF, Vignon V, Brustel H, Vernon P (2015) Demographic parameters of sexes in an elusive insect: implications for monitoring methods. Population. Ecology 57: 227–236. https://doi.org/10.1007/s10144-014-0453-2

Legg C, Nagy L (2006) Why most conservation monitoring is, but need not be, a waste of time. Journal of Environmental Management 78: 194–199. https://doi.org/10.1016/j.jenvman.2005.04.016

Lindenmayer DB, Likens GE (2010) Effective Ecological Monitoring. Csiro Publishing, Earthscan, London, Washington DC, 1–170.

Lindenmayer DB, Franklin JF (2002). Conserving forest biodiversity: a comprehensive multiscaled approach. Island Press, Washington DC, 1–351.

Lindenmayer DB, Likens GE (2010) Effective Ecological Monitoring. CSIRO Publishing, Collingwood (Australia) and Earthscan, London, 1–169.

Longo L (2003) Habitat trees: instructions for creating and monitoring nest holes. In: Cavalli R, Mason F (Eds) Techniques for reestablishment of dead wood for saproxylic fauna conservation. Life nature project NAT/IT/99/6245 "Bosco della Fontana" (Mantova, Italy). Gianluigi Arcari Editore, Mantova, 63–68.

Marsh DM, Trenham PC (2008) Tracking current trends in plant and animal population monitoring. Conservation Biology 22: 647–655. https://doi.org/10.1111/j.1523-1739.2008.00927.x

Martin J, Kichens WM, Hines JE (2007) Importance of Well-Designed Monitoring Programs for the Conservation of Endangered Species: Case Study of the Snail Kite. Conservation Biology 21(2): 472–481. https://doi.org/10.1111/j.1523-1739.2006.00613.x

Mason F (2002a) Dinamica di una foresta della Pianura Padana. Bosco della Fontana - Primo contributo. Centro Nazionale Biodiversità Forestale Verona Bosco Fontana. Rapporti scientifici 1. Monitoraggio 1995. Arcari Editore, Mantova, 1–208.

Mason F (2002b) Problems of conservation and management. In: Ruffo S (Ed.) Woodlands of the Po Plain. Italian Ministry of the Environment and Territory Protection, Friuli Museum of Natural History, Udine, 91–137.

Mason F (2003) Guidelines and aims of the project Life NAT/IT/99/006245 "Bosco della Fontana: urgent actions on relict habitat", p. 41–43. In: Mason F, Nardi G, Tisato M (Eds) Proceedings of the International Symposium "Dead wood: a key to biodiversity", Mantova, May 29th–31th 2003. Sherwood, 95 (Suppl. 2): 41–43.

Mason F (2004) Dinamica di una foresta della Pianura Padana. Bosco della Fontana. Primo contributo, monitoraggio 1995. Seconda edizione con linee di gestione forestale. Rapporti scientifici 1. Centro Nazionale Biodiversità Forestale Verona - Bosco della Fontana. Arcari Editore, Mantova, 1 224 pp.

Mason F, Minari E (2009) La teoria silvigenetica di Oldeman nella gestione delle aree protette. Atti del Terzo Convegno Nazionale di Selvicoltura, Volume 3, 16–19 ottobre 2008, Taormina.

Mason F, Roversi PF, Audisio P, Bologna MA, Carpaneto GM, Antonini G, Mancini E, Sabbatini Peverieri G, Mosconi F, Solano E, Maurizi E, Maura M, Chiari S, Sabatelli S, Bardiani M, Toni I, Redolfi De Zan L, Rossi de Gasperis S, Tini M, Cini A, Zauli A, Nigro G, Bottacci A, Hardersen S, Campanaro A (2015) Monitoring of insects with public participation (MIPP; EU LIFE project 11 NAT/IT/000252): overview on a citizen science initiative and a monitoring programme (Insecta: Coleoptera; Lepidoptera; Orthoptera). Fragmenta entomologica 47: 51–52. https://doi.org/10.4081/fe.2015.134

Mason F, Zapponi L (2005) The forest biodiversity artery: towards forest management for saproxylic conservation. iForest - Biogeosciences and Forestry 9: 2015–216.

Maurizi E, Campanaro A, Chiari S, Maura M, Mosconi F, Sabatelli S, Zauli A, Audisio P, Carpaneto GM (2017) Guidelines for the monitoring of *Osmoderma eremita* and closely related species. In: Carpaneto GM, Audisio P, Bologna MA, Roversi PF, Mason F (Eds) Guidelines for the Monitoring of the Saproxylic Beetles protected in Europe. Nature Conservation 20: 79–128. https://doi.org/10.3897/natureconservation.20.12658

McKinley DC, Miller-Rushing AJ, Ballard HL, Bonney R, Brown H, Cook-Patton SC, Evans DM, French RA, Parrish JK, Phillips TB, Ryan SF, Shanley LA, Shirk JL, Stepenuck KF, Weltzin JF, Wiggins A, Boyle OD, Briggs RD, Chapin III SF, Hewitt DA, Preuss PW, Soukup MA (2017) Citizen science can improve conservation science, natural resource management, and environmental protection. Biological Conservation. https://doi.org/10.1016/j.biocon.2016.05.015

Mosconi F, Zilli A, Spicciarelli R, Maurizi E, Vigna Taglianti A, Audisio P (2014) An overview on the most outstanding Italian endemic moth, *Brahmaea* (*Acanthobrahmaea*) *europaea* (Lepidoptera: Brahmaeidae). Fragmenta entomologica 46(1-2): 1–9. https://doi.org/10.4081/fe.2014.70

Mosconi F, Campanaro A, Carpaneto GM, Chiari S, Hardersen S, Mancini E, Maurizi E, Sabatelli S, Zauli A, Mason F, Audisio P (2017) Training of a dog for the monitoring of *Osmoderma eremita*. In: Carpaneto GM, Audisio P, Bologna MA, Roversi PF, Mason F (Eds) Guidelines for the Monitoring of the Saproxylic Beetles protected in Europe. Nature Conservation 20: 237–264. https://doi.org/10.3897/natureconservation.20.12688

Müller J, Bussler H, Bense U, Brustel H, Flechtner G, Fowles A, Kahlen M, Möller G, Mühle H, Schmidl J, Zabransky P (2005) Urwald relict species - Saproxylic beetles indicating structural qualities and habitat tradition. Waldökologie online 2: 106–113.

Müller J, Bußler H, Kneib T, (2008) Saproxylic beetle assemblages related to silvicultural management intensity and stand structures in a beech forest in Southern Germany. Journal of Insect Conservation 12: 107–124. https://doi.org/10.1007/s10841-006-9065-2

Nichols JD, Williams BK (2006) Monitoring for conservation. Trends in Ecology and Evolution 21: 668–673. https://doi.org/10.1016/j.tree.2006.08.007

Nieto A, Alexander KNA (2010) European Red List of Saproxylic Beetles. Publications Office of the European Union, Luxembourg, 1–46.

Nilsson SG, Baranowski R (1997) Habitat predictability and the occurrence of wood beetles in old-growth beech forests. Ecography 20: 491–498. https://doi.org/10.1111/j.1600-0587.1997.tb00417.x

Noon BR (2003) Conceptual issues in monitoring ecological resources. In: Busch DE, Trexler JC (Eds) Monitoring Ecosystems: Interdisciplinary Approaches for Evaluating Ecoregional Initiatives, Island Press, Washington, DC, 27–72.

Parisi F, Lombardi F, Sciarretta A, Tognetti R, Campanaro A, Marchetti M, Trematerra P (2016) Spatial patterns of saproxylic beetles in a relic silver fir forest (Central Italy), relationships with forest structure and biodiversity indicators. Forest Ecology and Management 381: 217–234. https://doi.org/10.1016/j.foreco.2016.09.041

Pocock MJO, Chapman DS, Sheppard LJ, Roy HE (2014) Choosing and Using Citizen Science: a guide to when and how to use citizen science to monitor biodiversity and the environment. Centre for Ecology and Hydrology, Wallingford, Oxfordshire, 1–26.

Ray AM, Millar JG, McElfresh JS, Swift IP, Barbour JD, Hanks LM (2009) Male-produced aggregation pheromone of the cerambycid beetle *Rosalia funebris*. Journal of Chemical Ecology, 35: 96–103. https://doi.org/10.1007/s10886-008-9576-2

Redolfi De Zan L, Bellotti F, D'Amato D, Carpaneto GM (2014a) Saproxylic beetles in three relict beech forests of central Italy: analysis of environmental parameters and implications for forest management. Forest Ecology and Management 328(2014) 229–244 Https://doi.org/10.1016/j.foreco.2014.05.040

Redolfi De Zan L, Battisti C, Carpaneto GM (2014b) Bird and beetle assemblages in relict beech forests of central Italy: a multi-taxa approach to assess the importance of dead wood in biodiversity conservation. Community Ecology 15: 235–245. https://doi.org/10.1556/ComEc.15.2014.2.12

Redolfi De Zan L, Bardiani M, Antonini G, Campanaro A, Chiari S, Mancini E, Maura M, Sabatelli S, Solano E, Zauli A, Sabbatini Peverieri G, Roversi PF (2017) Guidelines for the monitoring of *Cerambyx cerdo*. In: Carpaneto GM, Audisio P, Bologna MA, Roversi PF, Mason F (Eds) Guidelines for the Monitoring of the Saproxylic Beetles protected in Europe. Nature Conservation 20: 129–164. https://doi.org/10.3897/natureconservation.20.12703

Roy HE, Pocock MJO, Preston, CD, Roy DB, Savage J, Tweddle JC, Robinson LD (2012) Understanding Citizen Science & Environmental Monitoring. Final Report on behalf of UK-EOF. NERC Centre for Ecology & Hydrology and Natural History Museum, London, 1–174.

Schmeller DS (2008) European species and habitat monitoring: Where are we now? Biodiversity and Conservation 17: 3321–3326. https://doi.org/10.1007/s10531-008-9514-1

Science Communication Unit, University of the West of England, Bristol (2013) Science for Environment Policy In-depth Report: Environmental Citizen Science. Report produced for EC DG Environment. http://ec.europa.eu/environment/integration/research/newsalert/pdf/IR9_en.pdf

Seibold S, Brandl R, Buse J, Hothorn T, Schmidl J, Thorn S, Müller J (2015) Association of extinction risk of saproxylic beetles with ecological degradation of forests in Europe. Conservation Biology 29: 382–390. https://doi.org/10.1111/cobi.12427

Silvertown J (2009) A new dawn for citizen science. Trends in Ecology and Evolution 24: 467–471. https://doi.org/10.1016/j.tree.2009.03.017

Solano E, Mancini M, Ciucci P, Mason F, Audisio P, Antonini G (2013) The EU protected taxon *Morimus funereus* Mulsant, 1862 (Coleoptera: Cerambycidae) and its western Palaearctic allies: systematics and conservation outcomes. Conservation Genetics 14: 683–69. https://doi.org/10.1007/s10592-013-0461-3

Speight MCD (1989) Saproxylic Invertebrates and their Conservation. Council of Europe, Strasbourg, 1–79.

Stoch F, Genovesi P (Eds) (2016) Manuali per il monitoraggio di specie e habitat di interesse comunitario (Direttiva 92/43/CEE) in Italia: specie animali. ISPRA, Serie Manuali e linee guida, 141/2016 (Roma): 1–364.

Stokland JN (2012) The saproxylic food web. In: Stokland JN, Siitonen J, Jonsson BG (Eds) Biodiversity in dead wood. Cambridge University Press (New York): 29–54. https://doi.org/10.1017/cbo9781139025843.004

Stokland JN, Siitonen J, Jonsson BG (2012) Biodiversity in dead wood. Cambridge University Press, New York. https://doi.org/10.1017/CBO9781139025843

Travaglini D, Bottalico F, Brundu P, Chirici G, Minari E (2007) Sampling deadwood within Bosco della Fontana. In: Gianelle D, Travaglini D, Mason F, Minari E, Chirici G, Chemini

C (Eds) Canopy Analysis and Dynamics of a Floodplain Forest. Rapporti Scientifici 3, Centro Nazionale per lo Studio e la Conservazione della Biodiversità Forestale "Bosco della Fontana", Cierre Grafica Editore, Verona, 59–68.

Trizzino M, Audisio P, Bisi F, Bottacci A, Campanaro A, Carpaneto GM, Chiari S, Hardersen S, Mason F, Nardi G, Preatoni DG, Vigna Taglianti A, Zauli A, Zilli A, Cerretti P (2013) Gli artropodi italiani in Direttiva Habitat: biologia, ecologia, riconoscimento e monitoraggio. Quaderni Conservazione Habitat, 7. CFS-CNBFVR, Centro Nazionale Biodiversità Forestale. Cierre Grafica, Sommacampagna, Verona, 1–256.

Tulloch AIT, Possingham HP, Joseph LN, Szabo J, Martin TG (2013) Realising the full potential of citizen science monitoring programs. Biological Conservation 165: 128–138. https://doi.org/10.1016/j.biocon.2013.05.025

Vallejo MM (2015) State of Europe's Forests 2015, Ministerial Conference on the Protection of Forests in Europe, Forest Europe Liaison Unit Madrid, 1–312.

Viterbi R, Cerrato C, Bassano B, Bionda R, Hardenberg A, Provenzale A, Bogliani G (2013) Patterns of biodiversity in the northwestern Italian Alps: a multi-taxa approach. Community Ecology 14: 18–30. https://doi.org/10.1556/ComEc.14.2013.1.3

Wells NM, Lekies KS (2012) Children and Nature. Following the Trail to Environmental Attitudes and Behaviour. In: Dickinson JL, Bonney R (Eds) Citizen science: Public participation in environmental research. Cornell University Press, Ithaca, NY, 1–304. https://doi.org/10.7591/cornell/9780801449116.003.0015

Williams BK, Nichols JD, Conroy MJ (2002) Analysis and Management of Animal Populations: Modeling, Estimation and Decision Making, Academic Press, San Diego, California, USA.

Yoccoz NG, Nichols JD, Boulinier T (2001) Monitoring of biological diversity in space and time. Trends in Ecology and Evolution 16: 446–453. https://doi.org/10.1016/S0169-5347(01)02205-4

Zapponi L, Minari E, Longo L, Toni I, Mason F, Campanaro A (2014) The Habitat-Trees experiment: using exotic tree species as new microhabitats for the native fauna. iForest 8: 464–470. [online 2014-10-22]

Zapponi L, Cini A, Bardiani M, Hardersen S, Maura M, Maurizi E, Redolfi De Zan L, Audisio P, Bologna MA, Carpaneto GM, Roversi PF, Sabbatini Peverieri G, Mason F, Campanaro A (2016) Citizen science data as an efficient tool for mapping protected saproxylic beetles. Biological Conservation. https://doi.org/10.1016/j.biocon.2016.04.035

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Guidelines for the monitoring of *Lucanus cervus*

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Abstract

Lucanus cervus is one of the most charismatic saproxylic beetles, widely distributed in Europe. The species is typical of mature deciduous forests, especially oak woodlands. Loss and fragmentation of suitable habitats is one of the major threats for this species which is included in Annex II of the Habitats Directive. Despite several studies carried out in the last years for the monitoring methods of the species, an analytical comparison between them is still lacking.

The aims of this paper are (i) to review the current knowledge about systematics, ecology and conservation practices on *L. cervus* and (ii) to present the research carried out during the Life MIPP project, in order to define a standard monitoring method with a suitable protocol to be used for addressing the obligations of the Habitats Directive. Overall, five methods were tested during three years in two different study areas. Based on these results, a suitable standard method for *L. cervus* is proposed in this paper and, in order to assess the conservation status of populations and to compare them over time, a simple method for the calculation of a reference value is provided.

Keywords
Habitats Directive, saproxylic beetles, Coleoptera, Lucanidae, monitoring methods, forest biodiversity, sightings along transect

Introduction

Lucanus cervus (Linnaeus, 1758), belonging to the family Lucanidae, is the largest saproxylic beetle in Europe. Populations of this species inhabit mature deciduous forests, especially the lowland and medium-altitude oak woodlands having rotten dead wood at ground level. *Lucanus cervus* is considered a flagship species and is included in Annex II of the Habitats Directive (Council Directive 92/43/EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora). The Habitats Directive provides that Member States prepare, every six years, a report on the conservation status of the species listed in the Annexes. In order to address this obligation, the Life Project "Monitoring of insects with public participation" (LIFE11 NAT/IT/000252) (Mason et al. 2015) (hereafter, MIPP) conducted experimental fieldwork to develop a standardised method for the monitoring of the saproxylic beetle species of the project: *Osmoderma eremita* (hermit beetle, Scarabaeidae), *Lucanus cervus* (European stag beetle, Lucanidae), *Cerambyx cerdo* (great capricorn beetle, Cerambycidae), *Rosalia alpina* (rosalia longicorn, Cerambycidae), *Morimus asper/funereus* (morimus longicorn, Cerambycidae).

The present paper is part of a special issue on the monitoring of saproxylic beetles protected in Europe and is dedicated to *L. cervus*. Therefore, it starts with an indepth revision of the current knowledge on systematics, distribution, ecology, ethology and conservation. The review is followed by a detailed account of the fieldwork carried out during the project and concludes with the description of the proposed monitoring method.

Systematics and distribution

Lucanus Scopoli, 1763 is a genus of scarabaeoid beetles of the family Lucanidae (stag beetles), subfamily Lucaniinae. The family includes about 1,700 species distributed worldwide (Fujita 2010, Bartolozzi et al. 2014). The holarctic genus *Lucanus* accounts for some 70 species, mostly in Eurasia, from the Iberian Peninsula to Japan and Indochina. Taxonomy and phylogeny of the genus are far from being fully resolved and several species are difficult to separate from closely related taxa.

In Europe, following the most recent taxonomic reviews (Bartolozzi et al. 2016a, 2016b), five different species have so far been recorded (Figure 1): *Lucanus barbarossa* Fabricius, 1801 (previously in the discussed subgenus *Pseudolucanus* Hope & Westwood, 1845; Iberian Peninsula and northwestern Africa, reaching northwards to the extreme SW France); *L. cervus* (Linnaeus, 1758) (most of Europe); *L. pontbrianti* Mul-

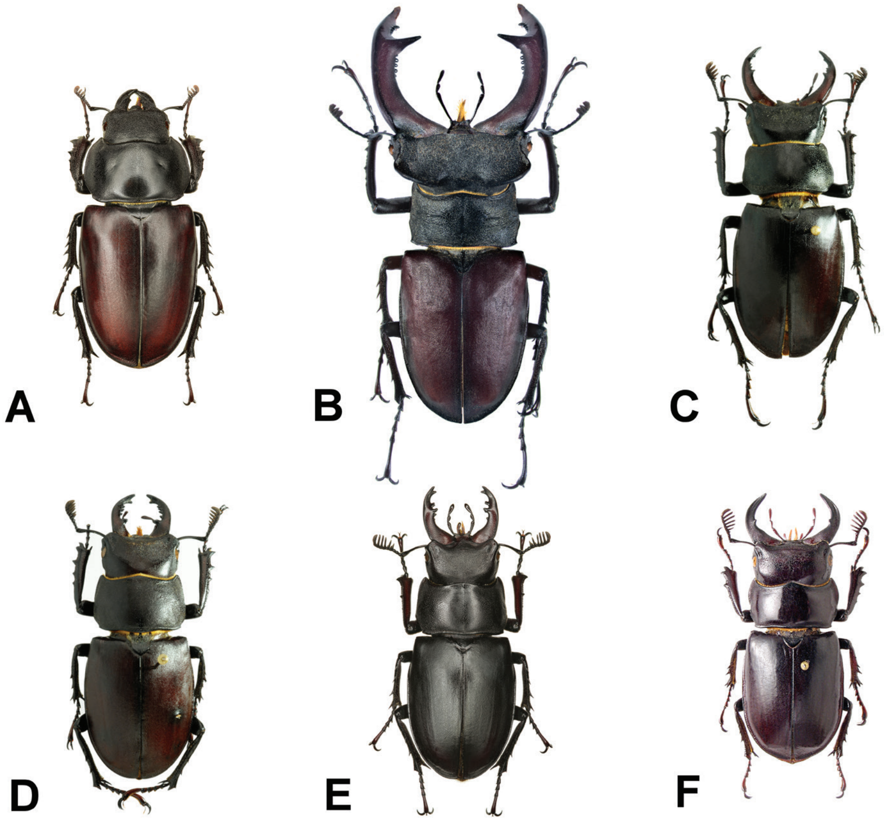

Figure 1. The five European species of *Lucanus*: **A** *L. cervus* female (Dresden, Germany; photo by I. Belousov) **B** *L. cervus* male (Schweinfurt, Germany; photo by U. Schmidt) **C** *L. pontbrianti* male (La Cadière, France; photo by S. Bambi and L. Bartolozzi) **D** *L. tetraodon* male (Cosenza province, Italy; photo by S. Bambi and L. Bartolozzi) **E** *L. ibericus* male (Dagestan, Russia; photo by I. Belousov) **F** *L. barbarossa* male (N Spain; photo by M. Zilioli).

sant, 1839 (central-southern France and N Spain; only recently considered a distinct species, clearly separated from the related *L. cervus*: see Tronquet 2014, Fernández de Gamboa and Garzón 2009); *L. tetraodon* Thunberg, 1806 (Peninsular Italy, Corsica and Sicily, with old records from Sardinia and relict populations in NE Spain (Atarés) and S France (Janas Forest); also mentioned from Algeria and even the Balkans, probably confused with the next species); *L. ibericus* Motschulsky, 1845 (Albania, Greece, Turkey, Caucasus, eastwards to Iran).

In particular, *L. cervus*, is widely spread throughout Europe, reaching northwards to the southernmost counties of Great Britain and Sweden, and southwards to the northern parts of the Iberian Peninsula, the central regions of the Italian peninsula, the Greek mainland and Anatolia (Franciscolo 1997, Bartolozzi and Sprecher-Uebersax 2006, Harvey et al. 2011a). The eastern and southeastern borders of its range are not well known as the taxonomic status of the populations of Near and Middle East has still to be defined. The easternmost populations seem to extend to the Caucasus mountain slopes while the southernmost populations find their last refuges in some elevations of the Levant (Franciscolo 1997, Bartolozzi and Sprecher-Uebersax 2006, Harvey et al. 2011a, Cox et al. 2013, Bartolozzi et al. 2016a, 2016b). Further molecular analyses are certainly needed on these eastern populations to better understand their actual taxonomic position. As for taxonomic problems involving the correct identification of certain populations of *L. cervus* and *L. tetraodon* from Central Italy, refer to Solano et al. (2016), and to the discussion in the paragraph "Identification and comparison with similar taxa". In fact, *L. tetraodon* replaces *L. cervus* in southern Italy, Corsica and Sicily (Miksic 1959a, 1959b, 1961, Franciscolo 1997, Lapiana and Sparacio 2006, Santoro et al. 2009) but there is a well-documented area of overlapping in central Italy, where the two species often live in syntopy (Bartolozzi 1986, Bartolozzi and Maggini 2007, Cortellessa et al. 2014, Solano et al. 2016) and many specimens (purported to be hybrid) exhibit intermediate morphological characters between the two species (Santoro et al. 2009). In the last dozen years, some records of *L. tetraodon* in Emilia-Romagna (Fabbri 2010), Liguria (Bartolozzi and Maggini 2007) and Lombardy (Zilioli and Pittino 2004) have extended the Italian distribution range northwards, while in Sardinia, the species has not been found recently (Carpaneto et al. 2011b).

A summary of the Italian localities reported for both *L. cervus* and *L. tetraodon* is presented in Figure 2.

Morphology

The larval instars of the stag beetles do not differ substantially in shape but show a marked increase in size: from 5 mm of a new born larva, up to 10–11 cm in length of the last larval instar (Franciscolo 1997, Percy et al. 2000). The larva hatches from a subspherical (4 mm in diameter) and ivory coloured egg (Figure 3). The typical Lucanid larva (Figure 3) is whitish or creamy, soft, oligopod, roughly "C-shaped" in lateral view, without transverse folds in the abdominal segments and with a longitudinal anal slit. Head capsule is large, from reddish-brown to orange, more sclerified than body. Ocelli and ommata are absent, antennae rather elongated and pubescence only just developed in the anterior portion of body. The nine pairs of spiracles are moderately developed (the first one is larger and wider than the others), roughly "C-shaped" (Figure 3). Larvae have a typical stridulatory apparatus, formed by two sclerified organs on the second and third leg pairs respectively. A pair of soft, gibbose and translucid oval protuberances, occurs at the abdominal apex in dorsal-posterior view (Figure 3), these being larger and closer to each other in their inner portions, in *Lucanus*, when compared with those present in the much smaller larvae of the lucanid genus *Dorcus* MacLeay, 1819. The ventral side of the distal abdominal portion of larvae exhibits a large group of short brownish bristles (widely convergent to form a roughly "U-shaped" figure),

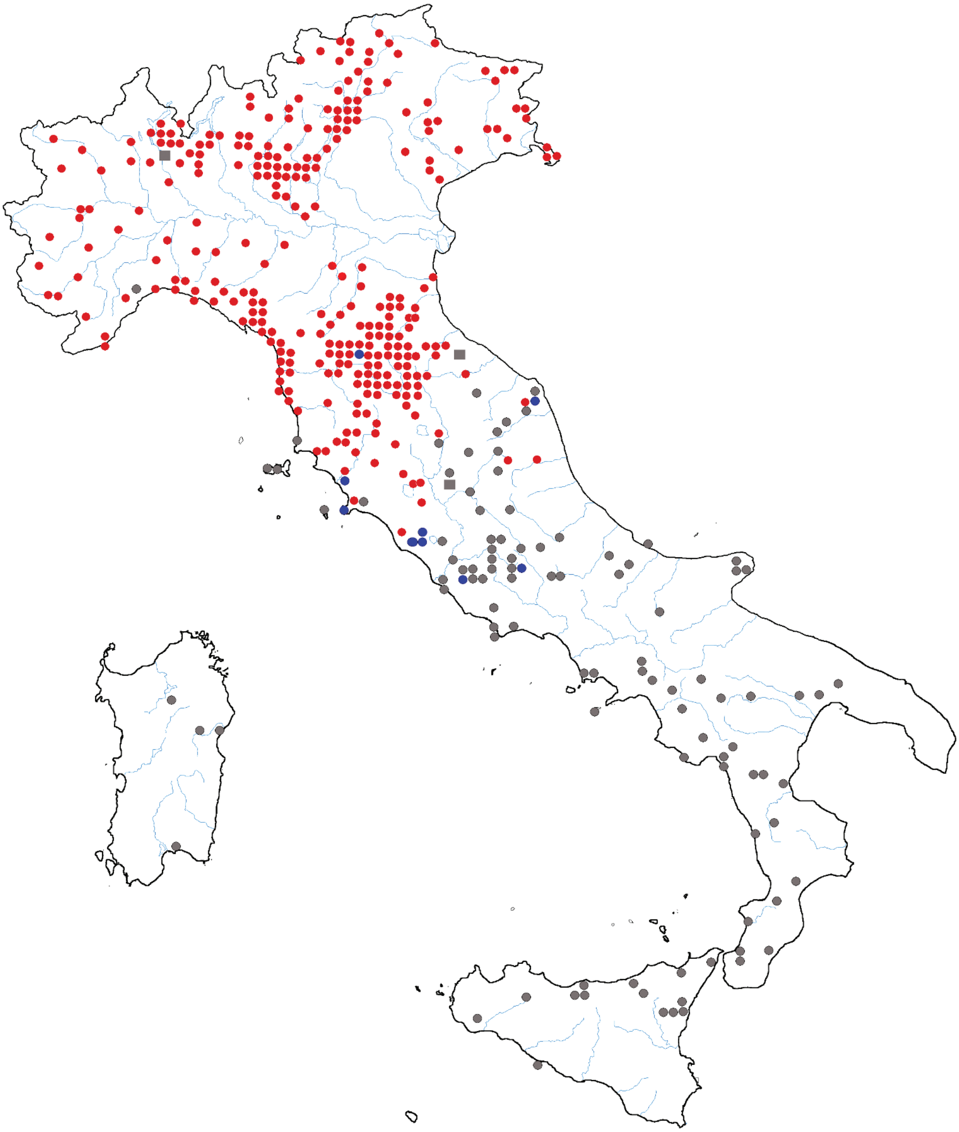

Figure 2. Distribution in Italy of the *Lucanus* species (red dots: *L. cervus*; grey dots: *L. tetraodon*; blue dots: both species; grey squares: recent data of *L. tetraodon* by Zilioli and Pittino 2004, Fabbri 2010). (Bartolozzi and Maggini 2007, modified by the Authors).

much denser, stronger and numerous than in *Dorcus*. Additionally, the mandibles of *Lucanus* larvae are larger and more curved distally, while those of *Dorcus* are smaller and almost parallel-sided (Figure 4).

The pupa is exarate, i.e. showing free appendices, including the large mandibles of the adult males (Figure 3).

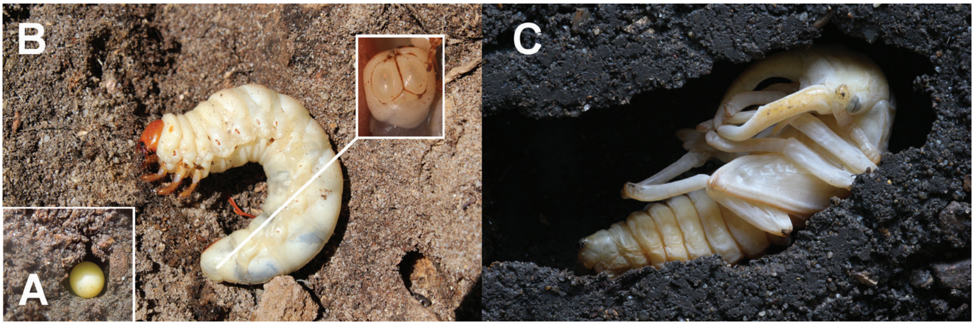

Figure 3. Pre-imaginal stages of *Lucanus cervus*: **A** egg (photo by C. Molls) **B** mature larva in lateral view (photo by M. Przewoźny) and detail of the apex of the abdomen in posterior view (photo by M. Fremlin) **C** Pupa of a male in lateral view (photo by M. Fremlin).

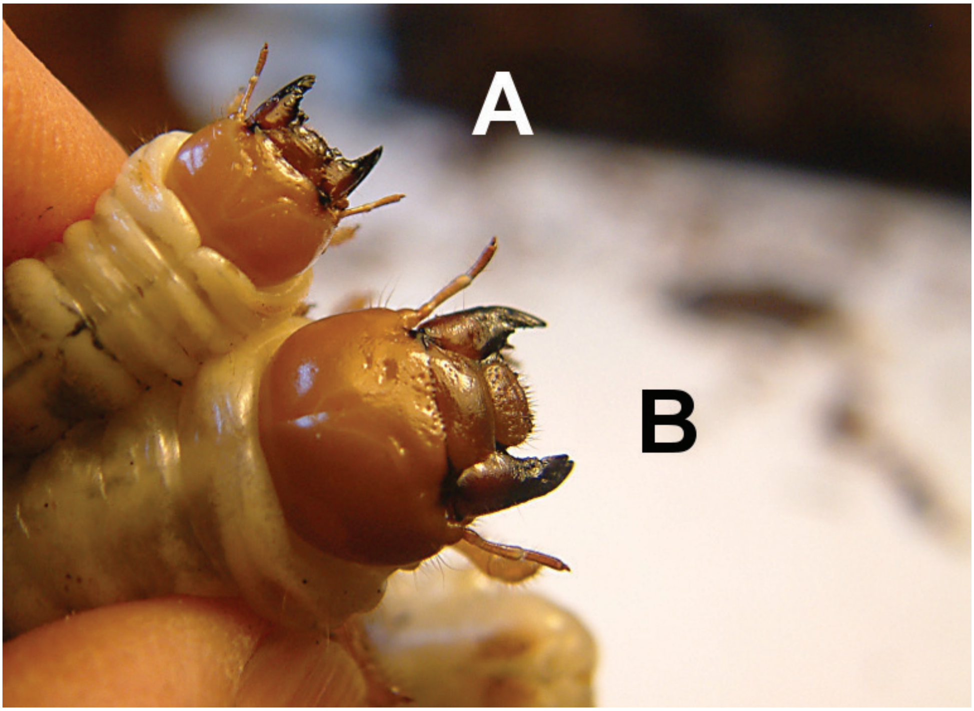

Figure 4. Head capsule, mandibles and relative size of lucanid larvae: **A** *Dorcus parallelipipedus* **B** *Lucanus cervus* (photo by M. Fremlin).

Adults of *L. cervus*, chiefly males, exhibit a strong morphological variability in shape and size of several characters, more or less uniformly expressed throughout the whole geographic range of the species, as shown in Figure 5.

These adults are 25–89 mm long (ca. 25–49 mm in females; ca. 30–89 mm in males), including the mandible length. The colour varies from reddish-brown to very

Figure 5. Polymorphism in *Lucanus cervus* males (all specimens from northern Italy; photo by M. Zilioli).

dark brown, almost black. Frequently, males exhibit reddish elytra and mandibles contrasting with the dark colour of the other body parts, even though the smallest specimens are more uniformly and blackish coloured. The antennal club exhibits four (or more rarely five) antennomeres, usually more abruptly enlarged when compared to the last antennomere just before the club. The species exhibits a strong sexual dimorphism: the male has large mandibles, longer than its head, while the female has much shorter mandibles, not longer than its head.

In males, the mandibles of *L. cervus* are characterised by the position of the largest inner tooth which lies in the distal half of the mandible or close to the middle of the mandible. Using a traditional morphometric approach, Romiti et al. (2015) highlighted the presence of two morphological classes: large males (which invest significantly more in mandibles and head size) and minor males. Furthermore, a geometric morphometric approach revealed that smaller males show highly variable mandibles (usually less curve dorsoventrally, thickset, with barely outlined teeth and a head without prominent basal edges) whereas larger males exhibit less variable mandibles (slender and equipped with fully developed median teeth, and deep, divaricate apical forks, supported by wide head basal edges) (Romiti et al. 2016).

Identification and comparison with similar taxa

The genus *Lucanus* is easily distinguishable from all other genera of European Lucanidae, while the specific distinction amongst closely related congeneric species is often problematic (Figure 1). Hereinafter a brief comparison of the five *Lucanus* species is presented, mainly focused on male diagnostic features.

Lucanus tetraodon (Figure 1), is on average smaller (26–52 mm in length) than *L. cervus*, as well as the size of the mandibles in males. The position of the largest inner tooth lies in the proximal half of the mandible, sometimes very near to the base. The antennal club is usually made up of the last 5 or 6 (rarely 7) antennomeres, gradually enlarged, when compared to the last antennomere just before the club. As discussed above, these characters are not always diagnostic (see Figure 6) in central Italy where *L. cervus* and *L. tetraodon* frequently overlap and probably exhibit phenomena of past hybridisation (Solano et al. 2016). This phenomenon occurs mostly in areas between the provinces of Viterbo and Rome in Latium, as well as between the provinces of Perugia and Terni in Umbria. In this geographic area, there are very large intermediate individuals with huge mandibles (such as in larger *L. cervus* specimens) but with five or six antennomeres in the club and with the last inner tooth near to the mandible base (as is usual in *L. tetraodon*). In addition, the shape of the male genitalia is not very useful for distinguishing the two species, although they always appear markedly separated by molecular analyses, even when apparently "intermediate" sympatric forms are analysed. In fact, most "intermediate" specimens from central Italy univocally resulted in being *L. cervus* from a molecular point of view (Solano et al. 2016; Figure 6).

Lucanus pontbrianti (Figure 1) is 25–55 mm in length. The mandibles of the males are smaller than males of *L. cervus*, as well as the inner tooth, positioned between the middle and the distal third of the mandible. Additionally, the distal portion of the mandibles is much more slender, with apex almost simple or only minutely and obtusely forked than *L. cervus*. Finally, in the males of *L. pontbrianti*, the antennal club is proportionally markedly larger and longer than in *L. cervus* and made up of the last 5–7 antennomeres.

Lucanus ibericus (Figure 1) is 27–51 mm in length. The mandibles of the males have usually smaller and more slender distad than *L. cervus*, with apex not markedly forked. The inner tooth is positioned near to the middle of the mandible. The most relevant diagnostic character between the two species is perhaps represented by the relative size of the antennal club, its length being ca. 1/4–1/5 of the fore tibiae in males in *L. cervus* (where it is made up of the last 4 or 5 antennomeres), while it is usually much larger (made up of the last 6 antennomeres) and a little shorter than 1/2 of the fore tibiae in males of *L. ibericus*.

Lucanus barbarossa (Figure 1) is on average smaller (28–48 mm in length) than *L. cervus*. The mandibles are almost without teeth along their inner edge and their distal portion is much more slender and almost simple, arcuately pointed. *L. barbarossa* exhibits a larger relative size of the antennal club (coupled with distinctly shorter antennal scape); its dorsal body surface is much shinier, the pronotum is more arcuately shaped at sides and the anterior portion of the head between the eyes and the base of the mandibles is proportionally longer and much more regularly and arcuately shaped than in *L. cervus.*

Figure 6. *L. cervus* from a contact area with *L. tetraodon* in Central Italy (Northern Latium, northern Rome province, Manziana; photo by M. Zilioli).

Ecology

Lucanus cervus inhabits mature deciduous forests, especially the lowland and medium-altitude oak woodlands (Campanaro et al. 2011a) but can also occur in urban habitats (e.g. city parks, private gardens) (Franciscolo 1997, Hawes 2008, Harvey et al. 2011a). The altitudinal range varies from sea level to 1,000 m a.s.l. (Campanaro et al. 2011a), it has been reported up to 1,700 m in Bulgaria (Harvey et al. 2011a).

The larvae are xylophagous, feeding on rotten dead wood at ground level (e.g. under stumps and fallen logs or amongst the roots of standing dead trees) (Franciscolo 1997, Campanaro et al. 2011a). In urban habitats, the larvae can also be found in small wood sources (e.g. bark chippings, fence posts, railway sleepers) (Harvey et al. 2011a). A massive suitable habitat can be exploited by several generations of larvae for several years, until the complete disintegration of it (Franciscolo 1997).

The host plants of the larvae belong to the genera *Quercus*, *Fagus*, *Salix*, *Populus*, *Tilia*, *Aesculus*, *Ulmus*, *Pirus*, *Prunus* and *Fraxinus* (Franciscolo 1997) but also *Castanea*, *Alnus* and *Pinus* (Harvey et al. 2011a). Amongst these, oaks and in particular, the English oak (*Quercus robur*), seem to be their favourites (Harvey et al. 2011a) and

Tini et al. (2017a) reported that *L. cervus* can also develop within suitable substrates of the allochthonous northern red oak (*Quercus rubra*).

Two species belonging to Diptera Phoridae, *Megaselia rufipes* (Meigen, 1804) and *Aphiocheta rufipes* (Meigen, 1830), were reported by Franciscolo (1997) as parasitoids of the stag beetle larvae. Although some mites can attack larvae and pupae, they tend to pursue adult stag beetles when they are still within their emergence galleries (Franciscolo 1997).

The main predators of larvae and pupae of stag beetle are the wild boar (*Sus scrofa*) and the badger (*Meles meles*) (Harvey et al. 2011a, Chiari et al. 2014).

The imagines of *L. cervus* usually emerge from the ground in May and males about a week before females (Harvey et al. 2011a). Emergence is influenced by local climate and can occur early (late April) or later, e.g. in cooler countries such as Sweden or regions with wet springs such as Switzerland (Harvey et al. 2011a). The phenology of the species has been described in several studies carried out in Europe, mainly related to specific monitoring methods (e.g. collection of remains of predation or sightings along a transect). In Belgium, adults have been sighted from the end of June to the second half of August (Campanaro et al. 2016). In Germany, Rhineland-Palatinate region, adults have been found from mid-May to mid-June (Rink and Sinsch 2011). Data from Italy showed a restricted or expanded phenology in 3 distinct areas: western Alps (329–622 m a.s.l.) from mid-June to mid-July (Chiari et al. 2014), northern Apennines (about 700 m a.s.l.) from the last week of June to the first week of August (present work) and central Po plain (about 25 m a.s.l.) from mid-May to the second half of August (Campanaro et al. 2011b, Corezzola et al. 2012). Data gathered by citizens (Campanaro et al. 2017), showed an overall phenology of the species for the whole of Italy from the beginning of May to the beginning of September. In Poland, Campanaro et al. (2016) reported data from mid-June to mid-August. In Slovenia, Vrezec (2008) analysed data from the first half of May to the second half of August (but only few and isolated data between the end of March and the first half of April and for the second half of September have also been reported). In Spain, Asturias region, Álvarez Laó and Álvarez Laó (1995) reported data from the second half of June to the first half of September. Campanaro et al. (2016), for the central Iberian region (Guadarrama Range), reported data from the beginning of June to the second part of July. In Switzerland, Sprecher-Uebersax and Durrer (1998), reported data from May to July. In the United Kingdom, Harvey et al. (2011b) reported data from the last week of May to the first week of August.

The adults are mainly active at dusk and there is a seasonal peak of activity, related to the night swarming of males which are seeking females for mating (Franciscolo 1997). This peak can vary from the end of May (beginning of June) to the end of June (Campanaro et al. 2016). In Italy, data from northern Apennines show a later peak of activity, close to mid-July (present work).

The flight arrangement is characteristic: the males (and mainly the "majors", with large mandibles) keep the body close to the vertical position to balance head weight and fly slowly along straight lines (few corners and wide-ranging) with several height variations; females, characterised by a small head armed with a short mandible, keep the body much less vertical (Franciscolo 1997). Take off requires a vertical position above ground, such as from the trunk and branches of trees or shrubs (Rink and Sinsch 2007). Flights will be avoided during heavy rain or strong winds (Campanaro et al. 2011a). Concerning temperature, Rink and Sinsch (2007) observed that stag beetles are able to fly within a range of 11°C to 26°C and inferred that their flight was hindered over 27°C. Harvey et al. (2011a) reported a mean threshold temperature for flight of 14.32°C (temperature range: 11–18°C). A recent radiotelemetric study at Bosco della Fontana, reported flying events within a temperature range of 18–28.7°C and a humidity range of 49–92.9% (Tini et al. in press).

About vertical use of space, females usually stay at ground level while males are more often sighted in flight, up to the canopy layer (Franciscolo 1997, Harvey and Gange 2006, Campanaro et al. 2016, Bardiani et al. 2017). Nevertheless, capture data for females found in canopy have also been reported (Bardiani et al. 2017). Both sexes feed on sap runs on tree trunks or on sugary substances (Franciscolo 1997, Fremlin and Hendriks 2011, Harvey et al. 2011a, Jansson 2011, Trizzino et al. 2013, Bardiani et al. 2017).

When more than one male detect a female, they fight amongst themselves (Franciscolo 1997). If body size of males is an advantage in a fight, it is not always the case in mating success (Franciscolo 1997, Harvey and Gange 2006). In fact, mating success depends on the “male:female” body size ratio (optimum value: 1.4) as the larger males are often unable to breed with females (Harvey and Gange 2006). Many vertebrates are predators of adult stag beetles, such as the shrew (*Sorex araneus*), fox (*Vulpes vulpes*), magpie (*Pica pica*), hooded crow (*Corvus corone cornix*), woodpeckers, kestrel (*Falco tinnunculus*) and owls (Franciscolo 1997, Campanaro et al. 2011b, Harvey et al. 2011a). Predation by the Eurasian jay *Garrulus glandarius* was observed in two localities of central Italy (Audisio and Carpaneto, unpublished data). Recently, Fremlin (http://maria.fremlin.de/stagbeetles/spiders.html, 2015, accessed 27 September 2016) reported predation of *L. cervus* by the false widow spider (*Steatoda nobilis*).

After mating, the female digs a deep gallery (up to 70–100 cm) into the soil, close to suitable substrates for larval development (e.g. tree roots, rotten wood) (Franciscolo 1997, Harvey et al. 2011a). The clutch size is about twenty eggs (Franciscolo 1997) with a range of 15–36 eggs (Harvey et al. 2011a). Females can carry out more rounds of oviposition, producing a total of 50–100 eggs (cf. Franciscolo 1997) and they can choose additional nest sites (Tini et al. 2017a, 2017b). Lucanid females release a secretion (housed in a structure called mycangium) near the laid eggs (Tanahashi et al. 2010). The secretion contains yeast that will be eaten by the larvae during their first meal: in this way, the larvae acquire yeast for the digestion of wood. After the last oviposition, the female does not re-emerge from the ground but she dies *in situ* (see Franciscolo 1997).

Data about larval and pupal stages (e.g. number and duration of instars) are difficult to obtain in nature or related to incidental occasions (Harvey et al. 2011a). The majority of available data come from captive beetles whose breeding is obtained by simulating their natural habitat conditions.

The incubation time for eggs varies from 14 (cfr. Franciscolo 1997) to 45 days (Harvey et al. 2011a). After this time, a small larva hatches using its mandible to break the eggshell (Franciscolo 1997). Larva usually develops in four years (range between 3 and 6 years) (Harvey et al. 2011a), through 3 instars (Fremlin and Hendriks 2014). Franciscolo (1997) also reports a higher duration for larval development, in particular for specimens of larger dimensions, whereas Fremlin and Hendriks (2014) reported some cases of a shorter duration (2 years). At the end of its development, the larva moves from the wood to the soil (at 20 cm depth) where it builds a cocoon (of soil and sand) for its pupation. During the preparation of the cocoon, the larva lines the wall with secretion from its gut (Hendriks and Fremlin 2012) and spreads the symbiotic yeasts (Fremlin and Tanahashi 2015). After hatching, the female everts the mycangium and sweeps the abdomen: this movement allows the mycangium to touch the cocoon wall and take up symbiotic yeasts (Fremlin and Tanahashi 2015). The pupal stage lasts on average 44 days (range from 28 to 60 days) at the end of summer: from the end of July by Harvey et al. (2011a), from the end of August by Franciscolo (1997). The adult is completely formed in autumn but it overwinters inside the cocoon and emerges from the ground in spring (Franciscolo 1997). The duration of the adult active period varies from a few weeks up to three months: Harvey et al. (2011a) reports an average period of 8 weeks for the males (range of 6–10 weeks; data from 9 countries) and 12 weeks for females (range of 8–14 weeks; data from 9 countries). Males of adult stag beetles, breeding in captivity, survive up to 200 days and female even more (cfr. Franciscolo 1997).

Threats

Lucanus cervus is protected by the Bern Convention (Annex III) and by the Habitats Directive (Annex II) (*L. pontbrianti*, recently reconsidered as a valid species by the splitting of *L. cervus,* should also be protected). The species is listed in the Red List of the European saproxylic beetles under the category Near Threatened (Nieto and Alexander 2010). In the Red List of Italian saproxylic beetles (Audisio et al. 2014, Carpaneto et al. 2015), it appears under the category Least Concern, because, on the basis of actual knowledge of the species in Italian territory and following IUCN criteria (such as population abundance and distribution area), the species should not be considered in a critical state. Following Harvey et al. (2011a) the species is rare if the entire range in Europe is considered where it seems to have a negative trend, mainly as a consequence of habitat loss: from comparison of data, taken in different periods, the species appears to be decreasing not for the area of occupancy (number of detection sites and population abundance) but for the extent of occurrence (range size).

According to Nieto and Alexander (2010), *L. cervus* is threatened principally by the decrease in the mean tree age in forests which is mainly due to intensive management and over-exploitation especially in eastern Europe. The consequence of this is the loss of the forests' dead wood and of old trees which are the required habitats for the species to complete its life cycle.

A not insignificant threat is due to the increase in predation rate by opportunistic species of birds (magpie, jay and crow) which show a marked demographic growth in anthropogenic environments (Carpaneto et al. 2015).

From the studies of Rink and Sinsch (2011), the rise in temperature could represent another threat to the continuation of stag beetle populations in Europe: hot temperatures above 27°C hinder the flight activity of these beetles during the mating season and drought could hamper the emergence of the adults in spring.

Review of monitoring experience in European countries

During the last years, different methods for the monitoring of *L. cervus* have been tested in many European countries.

The method based on counting the remains of adults killed by vehicles has been used in Spain by Álvarez Laó and Álvarez Laó (1995) and by Proyecto Ciervo Volante (1995). The count of roadkills has also been tested in Belgium (Thomaes 2008) and in the United Kingdom by Hawes (2005) and by Harvey et al. (2011b), with the aim of obtaining presence/absence data for the area and for assessing the local abundance of the species.

Counts of predation remains have been tested in Belgium by Kervyn (2006) (searching on the ground or inside owl pellets) in Germany by Malchau (2006) and in Italy by Campanaro et al. (2011b).

The use of transects for sighting of adults in the evening has been tested in Spain (Proyecto Ciervo Volante 1995), in Bulgaria (Anonymous 2005), in Belgium (Kervyn 2006), in Slovenia (Vrezec et al. 2012), in Italy (Campanaro and Bardiani 2012, Chiari et al. 2014) and in Romania (Fusu et al. 2015).

The counts of living adults have been tested in Germany by Malchau (2006) and in Italy by Chiari et al. (2014) (visual encounter surveys). Another method, consisting of surveys of trunks during the night by counting living adults, has been tested in Slovenia by Vrezec et al. (2012).

Interception traps have been tested in Belgium (Kervyn 2006) and pitfall traps (on the ground and in trees) have been tested in Slovenia by Vrezec et al. (2012).

The use of attractive baits has been associated with different traps. In Spain, GTLI (2005) used interception window traps, pitfall traps and aerial (bottle) traps baited with fruit, slices of ginger or liquids such as wine or beer (for bottle traps). In Germany, Malchau (2006) used traps baited with a mixture of fruit and alcohol. In Slovenia (Vrezec and Kapla 2007) and in Belgium (Thomaes 2008), pitfall traps, baited with fruit and ginger respectively, have been used. In the United Kingdom, Harvey et al. (2011b) tested many different baits (alcoholic and sugary liquids, chemical compounds, fruit, roots, vegetables) using laboratory and field tests (by interception window traps and pitfall traps). In Sweden, different compounds, made from sugar, yeast and enzymes combined with oak leaves or bark, have been tested in the field by Jansson (2011). In Italy, a subset of baits tested by Harvey et al (2011b) has been tested by Chiari et al. (2014) with the same traps, whereas Bardiani et al. (2017) tested two different alcoholic-sugary mixtures with aerial (bottle) traps.

Experiments, using acoustic detection of larvae, have been carried out in the United Kingdom by Harvey et al. (2011b) using a technique that requires sophisticated equipment and has not yet been tested in the field.

As the exaggerated mandibles and the large body size of *L. cervus* males enable it to attract people's attention, the stag beetle could be a good subject for educational campaigns and citizen science projects for the conservation of forests and organisms which live inside these habitats (Carpaneto et al. 2015). Since 1994, distribution data for the species have been gathered with the help of citizens in Spain (Proyecto Ciervo Volante 1995) and since 1998, in the United Kingdom (Percy et al. 2000, Smith 2003, 2011). In France, the ONG *Office pour les insectes et leur environnement (OPIE)* launched another citizen science project for *L. cervus* and gathered the sightings made by people through a web portal. In Switzerland, gathering data during the reproductive season of 2003 confirmed the previous knowledge about the distribution of the species in the Ticino and its phenology in the north-western Alps (Moretti and Sprecher-Uebersax 2004). In the Netherlands, Smit and Krekels (2006), made a public announcement with the aim of collecting observations from people: they provided a distribution map and images of the stag beetle to the public in order that they learn to recognise the species. In Italy, Zapponi et al. (2017) reported the preliminary results about MIPP citizen science, to show the efficacy of citizens' contribution in obtaining reliable data on the distribution of the stag beetle in the country.

Despite the large numbers of national monitoring studies, up to 2010 there have been few collaborative initiatives at international level. A revision of bionomics and distribution of *L. cervus* was carried out by Harvey et al. (2011a), gathering information from more than forty European and Asian countries and opening the way for the collaborative study published more recently by Campanaro et al. (2016). In this study, carried out by a team of researchers from eight European countries (Belgium, Germany, Italy, Poland, Slovenia, Spain, Switzerland and United Kingdom), a standard monitoring method was used to compare some aspects of the population ecology of the species by using the evening transect method. The study of Campanaro et al. (2016) represented an essential step in the choice of a standard method applicable at European level.

Methods

At the beginning of the project (see Carpaneto et al. 2017), a selection was made from the known monitoring methods and four methods were selected: Sighting individuals along transects at dusk, Collecting remains of predation along transects, Capturing individuals in selected points at dusk (changed to Capturing individuals along transects at dusk, after the first year) and Baited traps. In order to find the most appropriate standard monitoring method (quick, cheap, replicable protocol over the years and in different sites, easy and high detection of the species, high number of contacts in order to assess population abundance), a comparison of the selected methods has been carried out in two different study areas for three years. The methods and the sampling plan are detailed below.

Sighting individuals along transects at dusk

The method (hereafter: Sightings) is based on transect walks as described by Campanaro et al. (2016). Each transect is 500 m long and, from the centre of the path, must allow sighting up to 5 m on both sides (on the whole 10 m wide). The transect should follow landscape features such as roads, paths or forest edges, where the tree canopy over the whole transect (or most of it) should be sufficiently open to allow acceptable light conditions at dusk (Figure 7). The walk starts 15 minutes before the sunset and lasts 30 minutes on the whole. The 500 m length is divided into 5 sectors of 100 m, so that each one is walked in 6 minutes. Each "hundred metres' point" is marked with barrier tape on both sides of the transect, so that, during the walk, the operator has a reference point and can check that he/she is on schedule. All specimens observed within the transect are recorded, taking note of the sex of each animal (using the "unknown" option when it is impossible to recognise the sex of a flying uncaptured individual), the sector in which it is sighted, the height of flight (<2 m, >2 m) and the relative position on the track (left, centre or right) for both flying and walking individuals. The transects are carried out by one operator and only on days with suitable weather, i.e. without rain (only drizzle is acceptable), without strong wind and with a temperature above 13°C. For each survey, the transect direction is inverted to avoid the same end of a transect always being visited at the same hour.

Collecting remains of predation along transects

This method (hereafter: Remains) is based on the search and collection of remains of stag beetles predated by birds or mammals (Campanaro et al. 2011b) and it is similar to the collection of remains of dead specimens, killed by vehicles along road transects (Harvey et al. 2011b). The remains are mainly found after the predation of adult beetles by corvids (jays, magpies or crows) which discard the hardest parts of the exoskeleton. The collection of remains is undertaken along transects such as those previously described for sightings of live beetles. If grasses grow on the path, their height should be low enough to allow easy sightings of the remains (Figure 7). The collection of remains is carried out by one operator during daytime. All remains are collected and preserved dry in paper bags. Each paper bag reports date, identification code of the bag, transect and sector of collection, number of specimens (counting is performed by

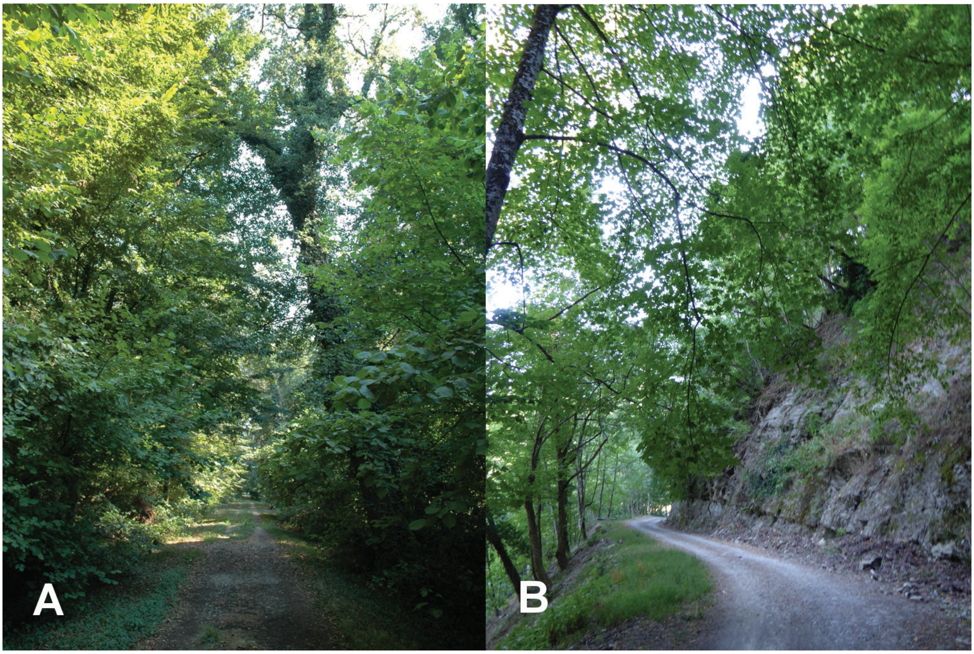

Figure 7. Examples of transect paths: **A** Bosco della Fontana (photo by I. Toni) **B** Foresta della Lama (photo by M. Bardiani).

considering all the body parts, reasonably belonging together, as one specimen) and type of remains collected (e.g. elytra, pronotum, head, whole specimens). Date, number of transect, operator, start and end time of transect and position reference of the paper bag along the transect are also reported on the field-sheet.

Capturing individuals along transects at dusk

The method (hereafter: Net-transect) is based on the live capture of the highest possible number of individuals sighted at dusk along transects. The aim of the capture is to obtain a reliable taxonomic identification for each individual sighted and avoid confusion of *L. cervus* with other related species where they live together (e.g. *L. tetraodon* in central Italy). The method is undertaken along transects with the same technical specifications (length, duration, time of the day, direction of the walk) of Sightings. Before starting the walk, containers for temporarily storing the beetles captured in each sector are placed every 100 m along the transect (at the five ends of each 100 m run including the final point). Each individual sighted or captured is noted in the same way as described for Sightings. Flying beetles are captured with a net (circular frame diameter: 50 cm; telescopic handle: up to about 2 m) while walking beetles are seized by hand. At the end of the whole transect, the operator walks back to take photographs of individuals with doubtful identification and then releases all the beetles into their sector.

Capturing individuals in selected points at dusk

The method (hereafter: Net-point) is based on the capture of all individuals sighted, when they rush to swarm in a wide clearing that may represent a place preferred by stag beetles for mating at dusk (Chiari et al. 2014). All individuals are temporarily captured during the collecting survey and kept into separate vials as described for Net-transect method. The equipment of the operator (net and containers) is the same as the previous method Net-transect.

Baited traps

The method (Bardiani et al. 2017, Redolfi De Zan et al. 2017) is based on baited and aerial traps, previously used by several authors (Allemand and Aberlenc 1991, Mason et al. 2002, Vrezec and Kapla 2007), but modified into non-lethal traps (Campanaro et al. 2011a, Corezzola et al. 2012). Traps (Figure 8) are handmade and consist of two stacked HD polyethylene jars (1,000 cm^3 each). The upper jar is the capture chamber and has the lid modified with a plastic funnel (diameter: 10 cm; stem: cut out to create an opening of 4 cm diameter). The bottom of this upper jar is cut out and connects with the lower jar which contains the bait as a liquid mixture. A 2 × 2mm mesh wire net separates both jars. The wire net prevents specimens entering the trap and falling into the liquid bait. Two mixtures were tested as baits: (i) red wine, beer and mashed banana ($R_wB_eB_a$) and (ii) red wine, white wine and sugar (R_wW_wS). The mixtures were prepared a week before trap setting. For each litre of mixture (made up of 50% of each of the two liquids), 220–330 g of banana or 220 g of sugar were added. The final volume of bait per trap was about 500 cm^3. Traps can be hung on trunks (1.5–2 m above ground) but also set up at canopy level (10–20 m) using a tree-climb slingshot (BigShot by Sherrill tree) for the launch of the "lift-up" and "pull-down" rope. Traps were checked every morning from 08:00h to 13:00h to avoid the death of individuals. Traps were activated on Monday, remained active for 4 days (i.e. surveys) and deactivated on Friday. This method is the same used for monitoring of *Cerambyx cerdo* (Redolfi De Zan et al. 2017).

Sampling plan

The methods were tested in two areas: Bosco della Fontana (hereafter: BF; Mantova, Lombardy; coordinates: 45.200299°N, 10.740841°E) (Figure 9) and Foresta della Lama (hereafter, FL; Bagno di Romagna, Emilia-Romagna; coordinates: 43.8311°N, 11.8379°E) (Figure 10). BF is a small fragment of lowland hornbeam-oak forest, at 25 m a.s.l. FL is part of a wide system of mountain deciduous forest, dominated by beech and other broadleaf trees, ranging from 600 m to 1,800 m a.s.l. (transects were located about 700 m a.s.l. whereas baited traps were set from 600 m to 900 m a.s.l.). Further details about the study areas are described by Carpaneto et al. (2017).

Figure 8. Baited trap for stag beetles (see construction details in the text) (photo by A. Cini).

The methods were tested from 2014 to 2016. Sightings and Remains were repeated in both study areas during the 3 years, while the method Net-transect was tested only in the last two years (Table 1). These methods were tested along the same 4 transects (Figures 9 and 10) and the transects remained the same from year to year. Each transect was monitored once a week and, in 2014, all transects were undertaken on the same day at BF. In 2015 and 2016, in both areas, monitoring methods at dusk (Sightings and Net-transect) were undertaken on the same day, along two different transects. The two methods were never perfomed at the same time on the same transect. At the end of each week, both methods were carried out for all 4 transects.

The method Net-point was undertaken only in 2014 at BF (Table 1). Four clearings (approximately circular; mean diameter: 46 m) were checked in the same day, once a week, from 20:00h to 21:20h. Each clearing was checked for 15min. At each survey, the order of sampling of clearings changed.

Baited traps were tested in different years in the two study areas (Tables 1 and 2). In 2014, at BF, to test the attraction of the bait and trap height, 48 traps were set, arranged in eight replicates. Each replicate consisted of six traps, set in pairs on three trees. On each tree, a trap was set at canopy level on tree branches (10–20 m height; high) and another trap on the lower part of the trunk (1.5–2 m height; low). Each pair

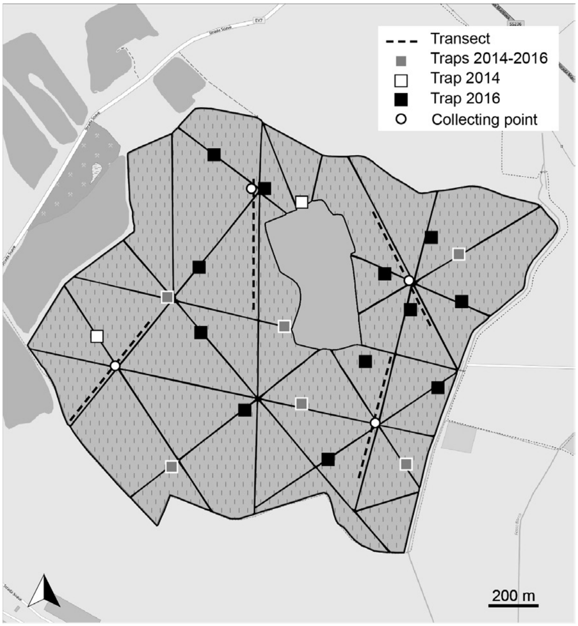

Figure 9. Map of Bosco della Fontana with the sampling sites (black dotted lines: transects for Sightings, Remains and Net-transect; white dots: clearings for Net-point; grey squares: trap sites in 2014 and 2016; black squares: trap sites in 2016; white squares: trap sites in 2014).

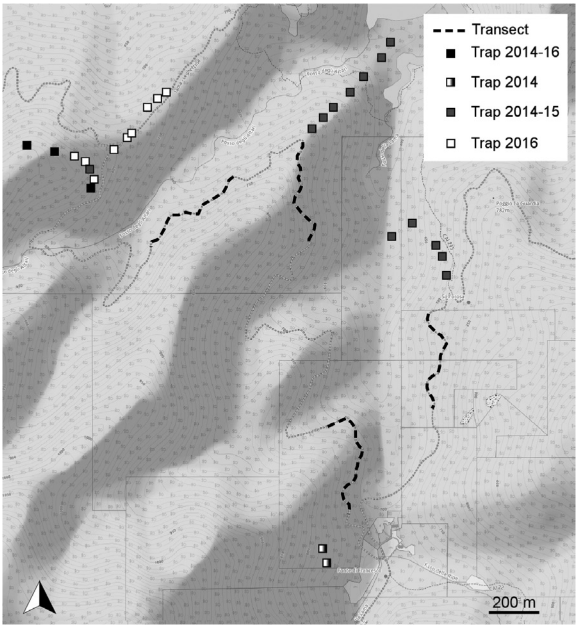

Figure 10. Map of Foresta della Lama with the sampling sites (black dotted lines: transects for Sightings, Remains and Net-transect; black squares: trap sites in 2014, 2015 and 2016; grey squares: trap sites in 2014 and 2015; shaded squares: trap sites in 2014; white squares: trap sites in 2016).

Table 1. Sampling plan at Bosco della Fontana (BF) and Foresta della Lama (FL) during the three years of monitoring. N = number of transects or traps; S = number of surveys; * indicates the number of surveys per transect.

Site	Method	2014			2015			2016		
		N	S	Dates	N	S	Dates	N	S	Dates
BF	**Sightings**	4	10*	20.5–22.7	4	8*	25.5–16.7	4	6*	6.6–14.7
	Remains	4	10*	20.5–22.7	4	10*	20.5–22.7	4	7*	1.6–14.7
	Net–transect	–	–	–	4	8*	25.5–16.7	4	6*	6.6–14.7
	Net–point	4	9	22.5–17.7	–	–	–	–	–	–
	Baited traps	48	32	27.5–18.7	–	–	–	54	24	31.5–8.7
FL	**Sightings**	4	7*	7.7–5.8	4	7*	22.6–7.8	4	5*	27.6–28.7
	Remains	4	2*	6–11.7; 3–8.8	4	7*	22.6–7.8	4	5*	29.6–27.7
	Net–transect	–	–	–	4	7*	22.6–7.8	4	5*	27.6–28.7
	Baited traps	36	8	6–11.7; 3–8.8	32	16	23.6–7.8	24	20	28.6–29.7

Table 2. Overview of the trap sample and number of traps set in the two study areas, Bosco della Fontana (BF) and Foresta della Lama (FL), during the three years of monitoring. Baits: $R_wB_eB_a$ (Red Wine, Beer, Banana); R_wW_wS (Red Wine, White Wine, Sugar); Control (empty traps used as control). Height at which traps were set: High (above 10 m), Low (1.5–2 m). Variation in ageing of the mixture: Never (mixture never changed during the season); 3 weeks (mixture changed once during the season: after three weeks); 2 weeks (mixture changed twice during the season: every 2 weeks).

Bait	Height	Ageing	2014		2015		2016	
			BF	FL	BF	FL	BF	FL
$R_wB_eB_a$	High	Never	8	9	–	–	–	–
	Low	Never	8	9	–	–	–	–
R_wW_wS	High	Never	8	9	–	16	18	12
		3 weeks	–	–	–	–	18	–
		2 weeks	–	–	–	–	18	–
	Low	Never	8	9	–	16	–	–
		3 weeks	–	–	–	–	–	–
		2 weeks	–	–	–	–	–	12
Control	High	–	8	–	–	–	–	–
	Low	–	8	–	–	–	–	–

of traps (high and low height) was baited with a different mixture: $R_wB_eB_a$, R_wW_wS, or empty as a control. Traps were checked for eight weeks (Table 1). In 2014, at FL, 36 traps were set on 18 trees (two traps per tree) and hung at two heights as described for BF (Table 2). The baits used were the same as those set at BF but no empty traps were set (Table 2). Traps were checked for two weeks. In 2015, traps were set only at FL. The overall number of traps set was 32 on 16 trees, half of them at high height and the other half at low height. Traps were checked for four weeks. The only bait used was R_wW_wS (Table 2). In 2016 at BF, 54 traps were set, arranged in 18 replicates, each one consisting of three traps, all baited with R_wW_wS but with three different fermentation classes (ageing): (i) the mixture was never changed during the study season, (ii) the mixture was replaced every third week and (iii) the mixture was replaced every second week (Table 2). In 2016, at FL, the overall number of traps set was 24 on 12 trees, half of the traps at high height and half at low height. Traps were checked for five weeks. The only bait used was R_wW_wS (Table 2).

Statistical analysis

The Chi-Square test was used to detect differences between the number of females and males for each monitoring method adopted. Individuals without sex identification (i.e. Unknown) recorded during the method Sightings were not taken into account.

To compare the number of Remains with the number of Sightings, the former were considered in two ways: (i) each head found (including whole specimens) was counted as one individual (hereinafter: head counting) and (ii) the remains likely belonging to the same individual were counted as one (total counting). In addition, for the method Net-transect, the percentage of individuals collected (captures) was compared to the sum of both sighted and captured individuals (sightings+captures) along the same transect during a given survey.

Occupancy models were applied to estimate the detection probability ($\hat{p}$) of the different methods tested (Nichols et al. 2008). In particular, "single species, single season occupancy models" were used for the methods Sightings, Net-transect and Remains, whereas "single species, single season, multi-method occupancy models" were used for Baited traps. In the first case, detection probability was modelled as a constant over time (p) or time-dependent (p_t). In the second case, detection probability was modelled as constant over time and detection methods (p), as time-independent but different amongst methods (p^s), as time-dependent but constant amongst methods (p_t) or with methods as an additive effect with time-period (p_{s+t}). Small-scale occupancy (θ), which corresponds to the presence of the species at the sample station, was modelled as either time-independent (θ) or time-dependent (θ_t). Models were ranked in decreasing order of their values of AIC (Akaike Information Criterion) and models with ΔAIC ≤ 2 from the top model were selected (Burnham and Anderson 2002). Analyses were carried out using the programme PRESENCE (Hines and MacKenzie 2004).

A Kruskal–Wallis test was used to compare the number of contacts between transects of the same study area, within a given sampling method: Sightings, Remains (total counting) or Net-transect (captures). These analyses were performed using STATISTICA 7.0 (StatSoft Inc.), with a significance level of 0.05 to reject the null hypothesis.

Regarding the capture performed by traps, only the datasets with sufficient captures were considered (i.e. BF 2014 and 2016; Table 3). The dataset BF 2014 was used to test the degree of attraction of the two different mixtures ($R_wB_eB_a$ and R_wW_wS) and the two heights (high and low) (the dataset BF 2014 is the same used in Bardiani et al. 2017). The dataset BF 2016 was used to test the degree of attraction of the mixture ageing (three different fermentation classes, as defined above).

Results

Capture data

At BF, Sightings and Remains (total counting) methods provided the highest number of contacts with *L. cervus* individuals for all three years (Table 3). Sightings provided the highest mean values of contacts per transect and survey (Table 3). At FL, Sightings provided the highest number of contacts during 2014–2015 (53 and 100 respectively) while, in 2016, the highest number of contacts was provided by the sum of captures and sightings carried out by the Net-transect method (Table 3). These results were confirmed by mean values (Table 3). Remains and Baited traps methods carried out at FL provided the lowest number of contacts with *L. cervus* (Table 3).

Head counting was 25% to 86% of the total counting, indicating that there was no constant proportionality between both ways of estimating individuals based on remains.

The percentage of individuals captured with respect to the sum of individuals sighted plus captured during the Net-transect method, varied from 0% to 100% and it was not dependent on the number of individuals sighted. The percentage of times in which the operator was able to collect all the individuals, per area and year, varied from 12% to 41%.

The Chi-Square test showed a general and significant higher number of males, with the exception of Remains at FL ($\chi^2 = 0.266$, $P = 0.606$) (Table 4).

No significant differences in number of contacts were found amongst transects for BF ($P = 0.051$ or higher) or for FL ($P = 0.077$ or higher) in any of the three sampling methods tested (Table 5).

Sightings data from BF (Figure 11) showed a peak of activity (as maximum mean value of contacts for transect and survey) at the 26th week of the year for all three years. Compared with Sightings data, Remains data provided slightly later peaks during 2014 and 2015 (Figure 11). Compared with Sightings data, Net-transect data showed a double peak at the 25th and 27th week in 2015 and an earlier peak at the 25th week in 2016.

At FL, the peak of activity shifted between the 27th and 29th week (Figure 12). Sightings data showed two peaks in 2014 (27th and 29th week), one peak in 2015 (28th week) and two peaks in 2016 (28th and 29th week) (Figure 12). Net-transect data provided a similar trend, with a peak at the 27th week in both 2015 and 2016 (Figure 12). The sum of captures and sightings, both undertaken by Net-transect, showed a peak at the 28th week (Figure 12). In all three years, Remains data (both head and total counting) never produced mean values higher than 1.0.

Table 3. Summary of number and mean values (in brackets) of contacts (c = captures; s = sightings; sp = specimens; Tot = total counting; head = head counting) for each method, in the two study areas Bosco della Fontana (BF) and Foresta della Lama (FL) during the three years of monitoring.

Method	Contact type	BF			FL		
		2014	2015	2016	2014	2015	2016
Sightings	s	156 (3.9)	143 (4.9)	195 (8.1)	53 (2.4)	100 (3.6)	151 (7.6)
Remains (Tot)	sp	152 (3.8)	150 (3.8)	202 (7.2)	4 (0.5)	14 (0.5)	7 (0.4)
Remains (head)	sp	87 (2.2)	81 (2.0)	146 (5.2)	1 (0.1)	12 (0.4)	4 (0.2)
Net-transect	c	–	80 (2.6)	91 (4.0)	–	47 (1.7)	110 (5.5)
Net-transect	c+s	–	134 (4.3)	153 (6.7)	–	–	210 (10.5)
Net-point	c	41	–	–	–	–	–
Baited traps	c	33	–	103	1	4	9

Table 4. Number of males (M), females (F) and unidentified (U) individuals contacted with different methods at Bosco della Fontana (BF) and Foresta della Lama (FL).

Site	Method	M	F	U	DF	chi-square	P
BF	**Sightings**	380	40	74	1	164.58	0.001
	Remains (head)	281	33	–	1	116.03	0.001
	Net transect (captures)	166	5	–	1	91.38	0.001
	Net points (captures)	40	1	–	1	23.99	0.001
	Baited traps	117	19	–	1	40.58	0.001
FL	**Sighting**	259	5	38	1	159.98	0.001
	Remains (head)	11	8	–	1	0.27	0.606
	Net transect (captures)	151	28	–	1	47.92	0.001
	Baited traps	14	1	–	1	6.98	0.01

Detection probability

The detection probability, for all methods based on transects, was more or less dependent on time as a function of the study area and year. Overall, the detection probability for Sightings and Net-transect seemed to be more influenced by time than for Remains (Table 6).

The detection probability for Sightings and Net-transect methods was higher than 0.50 for both study areas in all three years (Figure 13). The highest detection probability for Sightings was in 2016, reaching 0.83 (± 0.07) in BF and 0.85 (± 0.08) in FL (Figure 13). For the method Net-transect, the highest value of detection probability was registered in 2015 at BF (0.81 ± 0.03) and the lowest in 2015 at FL (0.54 ± 0.09) (Figure 13). For the method Remains, the detection probability was in general lower

Table 5. Results of the Kruskal-Wallis test comparing the total number of contacts recorded during the 3 years (2014–2016) amongst the four transects at Bosco della Fontana (BF) and Foresta della Lama (FL), for three sampling methods (Tot = total counting).

Method	BF				FL			
	DF	N	H	P	DF	N	H	P
Sightings	3	12	5.974	0.113	3	12	6.843	0.077
Remains (Tot)	3	12	7.758	0.051	3	12	2.947	0.400
Net-transect (captures)	3	8	6.452	0.092	3	8	4.849	0.183

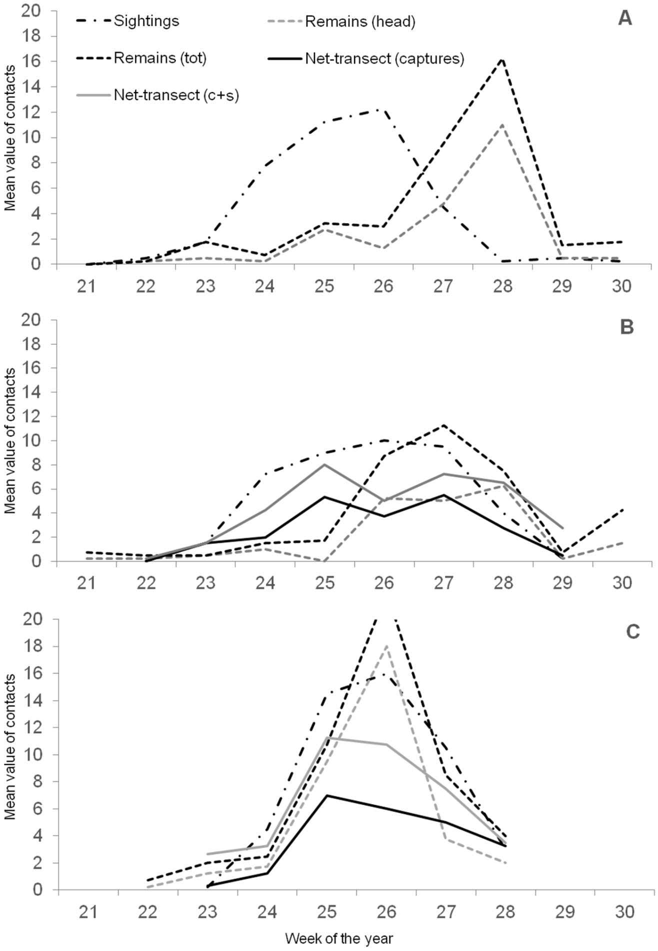

Figure 11. Phenology of the stag beetle *L. cervus* at Bosco della Fontana during **A** 2014 **B** 2015 **C** 2016. Black dashed-pointed line is the mean value of Sightings; Black dashed line is the mean value of Remains collected along transects; Grey dashed line is the mean value of Remains (heads). Black line is the mean value of captures by Net-transect; Grey line is the mean value of the sum of capture specimens and sightings performed by Net-transect. Weeks are expressed as the corresponding week of the year. The mean value of Remains at 26th week of 2016 is 22.

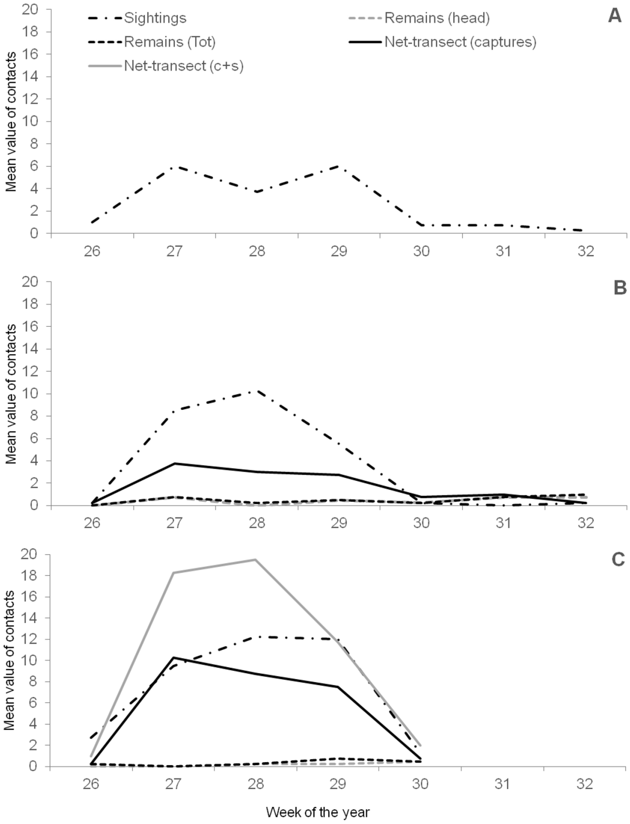

Figure 12. Phenology of the stag beetle *L. cervus* at Foresta della Lama during **A** 2014 **B** 2015 **C** 2016. Black dashed-pointed line is the mean value of Sightings; Black dashed line is the mean value of Remains collected along transects; Grey dashed line is the mean value of Remains (heads). Black line is the mean value of captures by Net-transect; Grey line is the mean value of the sum of capture specimens and sightings performed by Net-transect. Weeks are expressed as the corresponding week of the year.

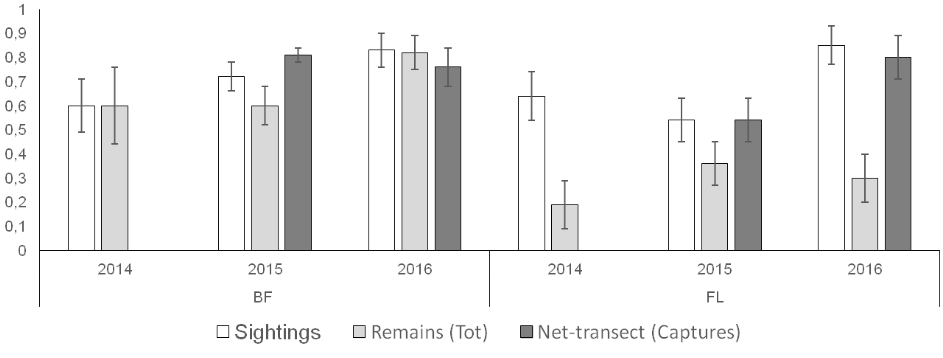

Figure 13. Detection probability values and relative standard error (SE) for the methods Sightings, Remains and Net-transect, in the two study areas Bosco della Fontana (BF) and Foresta della Lama (FL) in three years 2014–2016.

Table 6. Summary of model selection statistics for three methods, during the seasons 2014–2016 at Bosco della Fontana (BF) and Foresta della Lama (FL). *K* represents the number of parameters in the model and -2Log (*L*) is twice the negative log-likelihood value. Akaike Information Criteria (AIC) and Akaike weight (*w*) were calculated for each model. ΔAIC represents the difference in AIC value relative to the top model. Detection probability (*p*) may be constant (.) or vary amongst sampling occasions (t).

Site	Method	Year	Model	*K*	-2Log (L)	AIC	ΔAIC	*w*
BF	**Sightings**	**2014**	psi(.),*p*(t)	11	23.54	45.54	0.00	1.00
		2015	psi(.),*p*(t)	9	10.04	28.04	0.00	1.00
		2016	psi(.),*p*(t)	7	9.00	23.00	0.00	0.79
	Remains (Tot)	**2014**	psi(.),*p*(t)	11	34.63	56.63	0.00	0.65
			psi(.),*p*(.)	2	53.84	57.84	1.21	0.35
		2015	psi(.),*p*(.)	2	53.84	57.84	0.00	0.98
		2016	psi(.),*p*(.)	2	26.28	30.28	0.00	0.92
	Net-transect (Captures)	**2015**	psi(.),*p*(t)	9	5.55	23.55	0.00	1.00
		2016	psi(.),*p*(t)	7	8.32	22.32	0.00	0.95
FL	**Sightings**	**2014**	psi(.),*p*(.)	2	28.84	32.84	0.00	0.84
		2015	psi(.),*p*(t)	8	13.50	29.50	0.00	1.00
		2016	psi(.),*p*(.)	2	16.91	20.91	0.00	0.64
			psi(.),*p*(t)	6	10.04	22.04	1.13	0.36
	Remains (Tot)	**2014**	psi(.),*p*(.)	2	15.44	19.44	0.00	0.88
		2015	psi(.),*p*(.)	2	36.50	40.50	0.00	0.91
	Net-transect (Captures)	**2015**	psi(.),*p*(.)	2	38.67	42.67	0.00	0.66
			psi(.),*p*(t)	8	28.04	44.04	1.37	0.34
		2016	psi(.),*p*(t)	6	9.00	21.00	0.00	0.82

Table 7. Summary of the number of captures (N) by baited traps and relative detection probability (*p*) (Standard Error in brackets), for setting position of the trap (High: above 10 m; Low: 1.5–2 m) and for the type of bait ($R_wB_eB_a$: Red Wine, Beer, Banana; R_wW_wS: Red Wine, White Wine, Sugar; Control: empty traps used as control). Change of the mixture is indicated in brackets when expected (3 weeks: changed once during the season; 2 weeks: changed twice during the season; never: never changed during the season).

Setting	Bait	2014		2016	
		N	*p* (SE)	N	*p* (SE)
High	**Control**	0	–	–	–
	$R_wB_eB_a$ (never)	12	0.04 (0.02)	–	–
	R_wW_wS (never)	11	0.06 (0.03)	48	0.09 (0.04)
	R_wW_wS (3 weeks)	–	–	36	0.07 (0.03)
	R_wW_wS (2 weeks)	–	–	19	0.02 (0.01)
Low	**Control**	0	–	–	–
	$R_wB_eB_a$	5	–	–	–
	R_wW_wS	5	–	–	–

and more variable than for the other two methods based on transects. The lowest value for this method was registered at FL in 2014 (0.19 ± 0.10) and the highest value at BF in 2016 (0.82 ± 0.07) (Figure 13).

For traps, in 2014 the model showing more support was the one in which the detection probability was different amongst the methods (i.e. different mixtures) but not dependent on time (Bardiani et al. 2017). In 2016, the more supported model was the one with the detection probability depending on both method (i.e. different ageing) and time period with an additive effect.

In 2014, traps set at high height, performed better than traps set at low height at capturing individuals and also allowed the evaluation of the detection probability (Table 7) (Bardiani et al. 2017). In addition, traps baited with R_wW_wS showed a detection probability a little higher than those baited with $R_wB_eB_a$ (Table 7).

In 2016, only traps baited with R_wW_wS were used. Traps set at the beginning of the study period with no substitution of the bait, provided the highest detection probability (Table 7).

Discussion

The standard method for the monitoring of *Lucanus cervus*

At BF, the methods Sightings and Remains (total counting) were those which provided higher values for the total and mean number of contacts per transect and survey. Net-transect captures were lower than Sightings contacts (and the earlier Net-point method provided even less contacts). This result is expected because it was impossible to collect all the individuals sighted with a net (e.g. due to the height of flight of many individuals, to the expertise of the operator or even to the topography and characteristics of the transect). In fact, if sighted individuals (i.e. not captured but sighted) are counted with the captures (Net-transect captures+sighting), the number of contacts was close to the one provided by the Sightings method. The same patterns were found at FL, except that the Remains method provided a very low number of contacts. The Baited traps method provided the lowest number of captures compared with methods undertaken by Net, Remains or Sightings.

Sighting and Net-transect methods had sufficient time resolution to detect the peak of the activity for the species in both study areas, while Remains provided a peak only for BF but not for FL. The peak of activity was earlier at BF in the lowlands, than at FL in a mountainous area. This difference in phenology is supported by a citizen science approach, through the three years' study on the five saproxylic beetles of the MIPP (Campanaro et al. 2017). Both approaches also showed a longer adult activity period in the lowlands, compared with the mountainous areas.

All five methods tested showed a detection bias towards males. For transects at dusk, this is in line with others studies (Campanaro et al. 2016) and it was expected due to the less elusive character of the males and their propensity to fly at dusk, as supported by a recent radiotelemetry study carried out at BF (Tini et al. in press). Only the Remains method undertaken at FL showed no differences in sex detection, but this may be due to the very low number of contacts provided.

For Sightings and Net-transect methods, no significant difference was found in the number of contacts performed between the transects of a study area. This means that all transects were equally representative of the population and four transects were sufficient to carry out monitoring for the species in contrasting study areas: an isolated fragment of lowland forest (e.g. BF) and a large montane forest (e.g. FL). On the contrary, the statistical results obtained by the Remains method at BF, should be considered significant, if supported by inhomogeneous distribution of the remains in the study area reported by Campanaro et al. (2011b). This means that the number of remains collected was affected by the choice of the transects, thus not all transects were really representative of the population. The result obtained by Remains at FL might seem to be in contrast with the previous consideration. In reality, the number of remains collected was too low to emerge as a real difference for the representation of the population for each transect.

At BF, all three transect-based methods provided high detection probability values. By contrast, baited traps showed a much lower detection probability even with the best bait and the high sampling effort. At FL, Sighting and Net-transect provided the highest values of detection probability while the Remains method performed much worse. This could be due to the fact that FL is a much more extended area than BF and the populations of *L. cervus*, as well as the populations of birds preying on it, can be spread in a more extended area. Data from Baited traps did not provide a suitable detection probability value because captures were extremely low. Furthermore, in this study area, Baited traps presented an additional problem: the capture chamber was often occupied by dormice (*Glis glis*) (Figure 14). In both study areas, the detection probability values obtained for transect methods were highest in 2016. This increase is explained by the fact that, in 2016, the sampling effort was concentrated around the peak of activity and only few surveys without sightings were recorded (almost all surveys without data occurred in 2014 and 2015 at the beginning of the season).

In conclusion, Sighting individuals along transects at dusk allows a large amount of contacts and higher detection probability values in different natural areas. The method is very cheap in terms of cost and time but certainly it needs skilled operators able to recognise the stag beetle without capture; furthermore it should be applied in an area with a definite *L. cervus* population. The use of a net to perform these transects, solves the identification problem but it reduces the number of contacts (captures) which depends on different factors (operator abilty, height of beetle flight). The captures+sightings counting should increase the number of contacts but the identification problem partially remains and, in comparison, the Sighting method is cheaper and faster than Net-transect. Collecting remains along transects is also cheaper and faster and does not need to be undertaken in the evening but, in the authors' opinion, there are several problems related to the influence of the predation rate on the final result. The use of Baited traps, which provided useful ecological data (e.g. vertical use of space), seems to be the less suitable method as the advantage of being performed during daytime does not compensate for the low number of contacts provided, the

Figure 14. Dormouse captured in a baited trap at Foresta della Lama (2 July 2015). The photographs show that the animal survived in the trap due to the metallic net fixed between the bait chamber and the capture chamber (Photos by M. Bardiani and J. Röder).

high frequency of monitoring required (daily instead of weekly) and the time spent for each session (about 4 hours per day for at least 2 operators, instead of 1 hour for a transect survey). Furthermore, the low detection probability values suggested a massive sampling effort which is difficult to maintain.

Without doubt, the method Sighting individuals along transects at dusk results is the most suitable standard monitoring method for *L. cervus*, in terms of cost and results obtained. Collecting remains of predation along transects and Capturing individuals along transects at dusk should be taken into account during preliminary surveys of areas where information about the presence of the stag beetle is not available or in overlapping areas between two or more *Lucanus* species. Only when other species, like flower-chafer (Bardiani et al. 2017) or Longhorn-beetles (Redolfi De Zan et al. 2017), have to be monitored in addition to the stag beetle, the use of baited traps could also be considered for *L. cervus* (Bardiani et al. 2017).

Description of the method

The monitoring method consists of walking at dusk, along a standard length transect (500 m long and 10 m width) and counting all the adults of stag beetle seen flying or walking on the ground. This transect is carried out by one operator, from 15 minutes

Figure 15. Canopy cover along a transect at Bosco della Fontana (photo by M. Bardiani, APS-C camera, 8 mm lens).

before sunset to 15 minutes after sunset. On the whole, a transect walk lasts 30 minutes. The transect is divided into 5 sectors of 100 metres each and each sector should be walked in 6 minutes. Transects must be chosen along forest paths, tracks or roads with acceptable light conditions at dusk and with a suitable canopy openness (Figure 15). It is important to choose the transects only after the trees are turning green in spring and not before this period (to evaluate the real light conditions of the monitoring period). The monitoring method has to be carried out by trained operators or personnel able to recognise the stag beetle in flight.

Protocol, materials and equipment

The first step is to select the transects within the study area (up to 4 transects for a single study area), to measure the standard length of 500 m and to take the coordinates of the Start and End points of each one. Then, the positions of every hundred metres along the transect need to be marked with barrier tape (or other indicator: e.g. a numbered plate) on the right and left sides of the transect. The second step is to choose the weekly monitoring day for performing the surveys: (i) a single day if all the transects are checked on the same day (in this case, it is necessary to provide more than one operator) and (ii) up to 4 days if only one transect is checked per day. Setting the days is necessary to schedule every survey, as well as identifying the sunset time of each week (many websites, smartphone application or GPS function provide this information for specific localities or for any coordinates) and defining the walk direction of each survey. The walking direction of the transect should be inverted at every survey to reduce the possibility of sightings related to the space structure of the transect.

In total, for each transect, six surveys (i.e. six weeks) for lowland areas up to 400 m a.s.l. and five surveys (i.e. five weeks) for hilly and mountainous areas over 400 m a.s.l. are proposed. Surveys have to be chosen around the activity peak. The suggested monitoring period is between the 23rd and 28th week of the year for lowland areas and between the 26th and 30th week for hilly and mountainous areas. However these periods should be adjusted according to previous knowledge about stag beetle populations at the local level. If no direct information is available on the flying activity of stag beetles at local level, a preliminary study should be conducted in the year previous to starting the survey to obtain data on climatic conditions, extended sampling period and direct observation of stag beetle activities.

Just before starting the survey, the first part of the field-sheet (modified from Campanaro et al. 2016; see Suppl. material 1: field-sheet) must be completed (day, month, year, weather conditions, initial temperature and humidity recorded by a thermohygrometer). Fifteen minutes before the sunset time, the surveyor starts to walk. It is important to maintain a steady pace along the entire length of the transect (walking speed of 0.28 m/s), adjusting the walking speed by observing the hundred-metre point marks in relation to the time (to perform 100 metres every 6 minutes). For the same reason, the use of a chronometer or a wristwatch which can be easily checked during the work without distracting the observer's attention from visual hunting, is required. Every sighting is registered on the field-sheet with a letter and a number as a code indicating the sex of the beetle (using the "unknown" option when it is impossible to recognise the sex of a flying individual) and the behaviour (i.e. height of flight <2 m, >2 m or walking individuals). With the advancing time, the light will tend to weaken and it is important to also pay attention to sounds from the vegetation at the sides of the transect to detect beetles which are flying or walking by listening for humming or rustling (on dry leaves on the ground) respectively. It is important to pay attention to individuals which fly in circles and/or back and forth from the observer. Although a certain number of 'double counted' stag beetles with a consequent over-estimation of their number in every session is already expected for this method, individuals should be counted only once.

During the transect, it is possible to encounter other species of beetles. In this case, the operator should note their presence in the field-sheet note box (while attempting to be as accurate as possible). At the end of the survey, the operator completes the field sheet with time, temperature and humidity.

In order to provide data for the National Report, which each Member State must produce in the aims of article 17 of the Habitats Directive, the monitoring should be carried out at least twice during the 6-years period. In Table 8, the main aspects of the method and protocol (including materials and equipment) have been summarised.

Table 8. Summary of the main aspects of the monitoring method Sighting individuals along transect at dusk.

Number of transects	from 1 to 4
Distance between transects	at least 200 m
Length transect	500 m
Transect subdivision	100 m
Monitoring period	June–July
Number of repeats and survey weeks of the year suggested (for areas up to 400 m a.s.l.)	6 (23rd–28th)
Number of repeats and survey weeks of the year suggested (for areas over 400 m a.s.l.)	5 (26th–30th)
Survey frequency (for each transect)	Once a week
Survey-time of the day	Dusk time
Survey period	30min. (from 15min before to 15min after sunset)
Number of operators	1 per transect
Equipment for transect design	Measuring tape, barrier tape (or numbered plates), GPS
Survey equipment	A clipboard, a field sheet, a head torch, a pencil, a clock, thermohygrometer

Constraints and possible interferences

The surveys should be carried out when the temperature is above 13°C. The method is feasible in case of light rain and cloudy weather, but not with extremely bad weather conditions (heavy rain, strong wind). In these cases, it is better to postpone the procedure to the next evening (or at the first ‘free’ day, if other transects are expected during the week). If the survey cannot be postponeed, it will be cancelled.

Another issue is the lack of visibility inside the forest, especially after sunset or in the presence of a leaden sky, making it difficult to distinguish a flying stag beetle from other species with similar flight (e.g. *Oryctes nasicornis*, *Prionus coriarius*). A failure in sighting (individual not seen or misunderstood) means an under- or over-estimation of sightings. For this reason, it is highly recommended to prefer transects with an open canopy above the path and no dense undergrowth (as stag beetles tend to fly along corridors within the forests, with scarce vegetation) and to find good light conditions at the time of dusk.

In overlapping areas (where *L. cervus* co-exist with *L. tetraodon* or there are intermediate forms: see the paragraph Systematics and Distribution), the monitoring method Sighting individuals along transects at dusk should be undertaken after preliminary surveys (possibly in the former year) using other methods (i.e. Capturing individuals along transects at dusk and Collecting remains of predation along transects).

Despite the poor attraction provided by baited traps, it is highly recommended to space the transect at least 100 m from the nearest trap (e.g. used for monitoring of *Cerambyx cerdo*).

Furthermore, mist-nets for the monitoring of birds or bats should not be set in the surrounding areas because beetles are easily entangled by nets and releasing them is very difficult and time-consuming (Carpaneto et al. 2011a).

Spatial validity

A telemetric study, conducted at BF focusing on calculating the individual home range size of the stag beetle (Tini et al. 2017b), can provide assistance in estimating the spatial validity of the selected method by understanding the dispersal ability of the species. The home range was calculated by two methods: the minimum convex polygon (MCP) and the Fixed Kernel Density Estimation (KDE). The KDE method provided measures of probability for finding an individual in a particular location (Worton 1989). The home range size did not result in being statistically different between the sexes. In particular, the MCP mean values were about 7,600 m^2 for males and about 3,500 m^2 for females. The median values of KDE with 95% probability (KDE 95%) were about 14,500 m^2 for males and 9,500 m^2 for females. The KDE 50% gives information about the size of the individual core area (i.e. intensive use area) and the median values were about 3,850 m^2 for males and 3,400 m^2 for females. In the same study area, the median of the maximum distances (MMD) covered in a single relocation by males was 88 m (Tini et al. 2017b). Therefore, in homogeneous habitat conditions with high density of woody necromass and potential larval development sites (e.g. BF), the maximum survey area of the standard transect walk is: $(MMD*2)*TL + MMD^{2}*\pi$ (Figure 16). With a standard transect length (500 m long), the maximum survey area is about 11.2 ha ($88*2*500 + 88^{2}*\pi = 112{,}328$ m^2).

Counting, quantification and data sharing

Quantitative information on population size, structure and dynamics is needed for assessing species extinction risk. The most common approach for obtaining detailed information on population size is to use the capture-mark-recapture method and to treat the data gathered in such a way, with parameterised mathematical models which allow the estimation of population abundance. Other recent developed modelling approaches permit: (i) an estimate of the population size of a species across the study area by only recording the presence-absence data over multiple surveys (Royle-Nichols Abundance Induced Heterogeneity model - RNAIH) (Royle and Nichols 2003) and (ii) an estimate of the population size across the study area by recording the abundance of the species over multiple surveys, without marking individuals (Royle Repeated Count model - RRC) (Royle 2004). These approaches include the use of dedicated statistical software (e.g. PRESENCE) based on complex mathematical modelling and, in some cases, have limitations that are still and continuously under development. The reviewing of specialised tools to obtain population size estimates from data gathered in

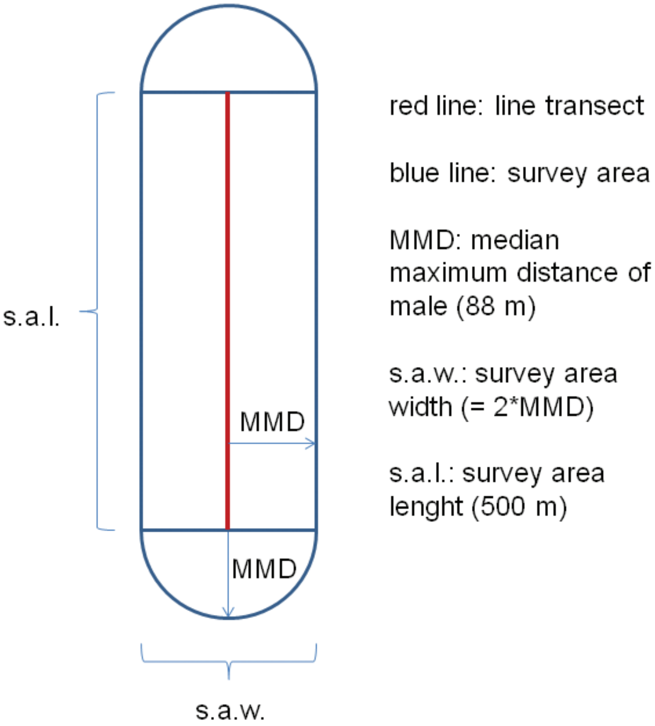

Figure 16. Graphic layout of a transect through the survey areas to show the spatial validity of the observations. MMD is the median of the maximum distance covered by a male in a single flight from the transect, as observed during the radiotelemetric fieldwork.

Table 9. Example of data summary and analysis, using the method of sightings along transect at dusk in 2016 at Bosco della Fontana. Number of sightings (Nc) for each transect (A-D) and for each Survey (1-6), total sightings and mean value for survey (Ts, Ms), total sightings and mean value for transect (Tt, Mt) and mean value of sightings for survey and transect (K) are reported.

Week of the year	Survey	Nc				Ts	Ms
		A	B	C	D		
23	**1**	0	0	0	1	1	0.25
24	**2**	13	2	1	2	18	4.50
25	**3**	21	18	6	13	58	14.50
26	**4**	18	18	18	10	64	16.00
27	**5**	10	11	14	7	42	10.50
28	**6**	3	2	0	7	12	3.00
Tt		65	51	39	40		
Mt		10.83	8.50	6.50	6.67		
K		8.13					

different ways, would greatly lengthen this paragraph, whose aim is instead to provide simple indications for homogeneously gathering and reporting ecological data on stag beetles obtained by the monitoring method, in data sheets shared with management authorities of protected areas. Therefore, at first it is suggested that the number of sightings (males, females, unknown sex, total) are maintained in a register for each field session, separated for each transect. Then, to obtain a value to be compared through the following years or with other study areas, the use of the mean number of sightings (K) is encouraged. This "Key number" is the mean of sightings for each transect and for a single survey. Table 9 provides an example of stag beetle counting to obtain the K number.

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References

Allemand R, Aberlenc H-P (1991) Une méthode efficace d'echantillonage de l'entomofaune des frondaisons: le piège attractif aérien. Bulletin de la Société Entomologique Suisse 64: 293–305.

Álvarez Laó CM, Álvarez Laó DJ (1995) Análisis de la mortalidad de ciervos volantes *Lucanus cervus* en carreteras asturianas. Boletín de Ciencias de la Naturaleza Real Instituto de Estudios Asturianos 43: 15–25.

Anonymous (2015) Operativna Programa "OKOLNA Sreda 2007–2013 G." Proekt "Terenni prouchvaniya na razprostranenie na vidove / otsenka na sŭstoyanieto na vidove i khabitati na teritoriyata na tsyalata strana – I faza", 1–14.

Audisio P, Baviera C, Carpaneto GM, Biscaccianti AB, Battistoni A, Teofili C, Rondinini C (Eds) (2014) Lista Rossa IUCN dei Coleotteri saproxilici Italiani. Comitato Italiano IUCN e Ministero dell'Ambiente e della Tutela del Territorio e del Mare, Roma, 1–132.

Bardiani M, Tini M, Carpaneto GM, Audisio P, Bussola E, Campanaro A, Cini A, Maurizi E, Mason F, Sabbatini Peverieri G, Roversi PF, Toni I, Chiari S (2017) Effects of trap baits and height on stag beetle and flower chafer monitoring: ecological and conservation implications. Journal of Insect Conservation 21: 157–168. https://doi.org/10.1007/s10841-017-9965-3

Bartolozzi L (1986) Note corologiche e morfologiche sui Lucanidae in Toscana (Coleoptera). - Atti Museo civico di Storia naturale di Grosseto 7/8: 11–26.

Bartolozzi L, Ghahari H, Sprecher-Uebersax E, Zilioli M (2014) A checklist of stag beetles (Coleoptera: Scarabaeoidea: Lucanidae) from Iran. Zootaxa 3887(3): 422–436. https://doi.org/10.11646/zootaxa.3887.4.2

Bartolozzi L, Maggini L (2007) Insecta Coleoptera Lucanidae. In: Ruffo S, Stoch F (Eds) Checklist and distribution of the Italian fauna. Memorie del Museo Civico di Storia Naturale di Verona (2nd serie, Sezione Scienze della Vita) 17(2006), 191–192. [+ CD ROM]

Bartolozzi L, Norbiato M, Cianferoni F (2016b) A review of geographical distribution of the stag beetles in Mediterranean countries (Coleoptera: Lucanidae). Fragmenta entomologica 48(2): 153–168.

Bartolozzi L, Sprecher-Uebersax E (2006) Lucanidae. In: Löbl I, Smetana A (Eds) Catalogue of Palearctic Coleoptera 3. Apollo Books, Stenstrup, 63–76.

Bartolozzi L, Sprecher-Uebersax E, Bezděk A (2016a) Family Lucanidae Latreille, 1804. In: Löbl I, Löbl D (Eds) Catalogue of Palaearctic Coleoptera 3. Scarabaeoidea – Scirtoidea – Dascilloidea – Buprestoidea – Byrrhoidea. Revised and updated Edition. Brill, Leiden, Boston, 58–84.

Burnham KP, Anderson DR (2002) Model Selection and Multimodel Inference: A Practical Information–Theoretic Approach. Springer, 1–488.

Campanaro A, Bardiani M (2012) Walk transects for monitoring of *Lucanus cervus* in an Italian lowland forest. Studia Forestalia Slovenica 137: 17–22.

Campanaro A, Bardiani M, Spada L, Carnevali L, Montalto F, Antonini G, Mason F, Audisio P (2011a) Linee guida per il monitoraggio e la conservazione dell'entomofauna saproxilica. Quaderni Conservazione Habitat 6, Cierre Grafica, Verona, 1–8. [+ CD-ROM]

Campanaro A, Hardersen S, Redolfi De Zan L, Antonini G, Bardiani M, Maura M, Maurizi E, Mosconi F, Zauli A, Bologna MA, Roversi PF, Sabbatini Peverieri G, Mason F (2017) Analyses of occurrence data of protected insect species collected by citizens in Italy. In: Carpaneto GM, Audisio P, Bologna MA, Roversi PF, Mason F (Eds) Guidelines for the Monitoring of the Saproxylic Beetles protected in Europe. Nature Conservation 20: 265–297. https://doi.org/10.3897/natureconservation.20.12704

Campanaro A, Toni I, Hardersen S, Grasso DA (2011b) Monitoring of *Lucanus cervus* by means of Remains of Predation (Coleoptera: Lucanidae). Entomologia generalis 33 (1/2): 79–89. https://doi.org/10.1127/entom.gen/33/2011/79

Campanaro A, Zapponi L, Hardersen S, Méndez M, Al Fulaij N, Audisio P, Bardiani M, Carpaneto GM, Corezzola S, Della Rocca F, Harvey D, Hawes C, Kadej M, Karg J, Rink M, Smolis A, Sprecher E, Thomaes A, Toni I, Vrezec A, Zauli A, Zilioli M, Chiari S (2016) A European monitoring protocol for the stag beetle, a saproxylic flagship species. Insect Conservation and Diversity 9: 574–584. https://doi.org/10.1111/icad.12194

Carpaneto GM, Baviera C, Biscaccianti AB, Brandmayr P, Mazzei A, Mason F, Battistoni A, Teofili C, Rondinini C, Fattorini S, Audisio P (2015) A Red List of Italian Saproxylic Beetles: taxonomic review, ecological features and conservation isssues (Coleoptera). Fragmenta entomologica 47(2): 53–126. https://doi.org/10.4081/fe.2015.138

Carpaneto GM, Campanaro A, Hardersen S, Audisio P, Bologna MA, Roversi PF, Sabbatini Peverieri G, Mason F (2017) The LIFE Project "Monitoring of insects with public participation" (MIPP): aims, methods and conclusions. In: Carpaneto GM, Audisio P, Bologna MA, Roversi PF, Mason F (Eds) Guidelines for the Monitoring of the Saproxylic Beetles protected in Europe. Nature Conservation 20: 1–35. https://doi.org/10.3897/natureconservation.35.12761

Carpaneto GM, Mazziotta A, Maltzeff P (2011a) Are mist nets a menace for threatened insects? A first attempt to gather quantitative data. Journal of Insect Conservation 15: 833–837. https://doi.org/10.1007/s10841-011-9381-z

Carpaneto GM, Piattella E, Dellacasa G, Dellacasa M, Pittino R, Mazziotta A (2011b) The lamellicorn beetles of southern Sardinia (Coleoptera: Scarabaeoidea). In: Nardi G, Whitmore D, Bardiani M, Birtele D, Mason F, Spada L, Cerretti P (Eds) Biodiversity of Marganai and Montimannu (Sardinia). Research in the framework of the ICP Forest network. Conservazione Habitat Invertebrati 5: 353–387.

Chiari S, Zauli A, Audisio P, Campanaro A, Donzelli PF, Romiti F, Svensson GP, Tini M, Carpaneto GM (2014) Monitoring presence, abundance and survival probability of the stag beetle, *Lucanus cervus*, using visual and odour-based capture methods: implication for conservation. Journal of Insect Conservation 18: 99–109. https://doi.org/10.1007/s10841-014-9618-8

Corezzola S, Bardiani M, Hardersen S, Spada L, Toni I (2012) Progetto di attività di monitoraggio della fauna invertebrata in attuazione del piano di gestione del Sito ITB20B0011 Bosco Fontana e definizione di protocolli di monitoraggio. Relazione finale, Mantova, 1–84.

Cortellessa S, Cianferoni F, Bartolozzi L (2014) Nuovi dati sulla distribuzione dei Lucanidi in Toscana (Coleoptera, Lucanidae). Onychium 10(2013): 68–77.

Cox K, Thomaes A, Antonini G, Zilioli M, De Gelas K, Harvey D, Solano E, Audisio P, McKeown N, Shaw P, Minetti R, Bartolozzi L, Mergeay J (2013) Testing the performance of a fragment of the COI gene to identify western Palaearctic stag beetle species (Coleoptera, Lucanidae). In: Nagy ZT, Backeljau T, De Meyer M, Jordaens K (Eds). DNA barcoding: a practical tool for fundamental and applied biodiversity research. Zookeys 365: 105–126.

Fabbri R (2010) Segnalazioni faunistiche n. 99–102. Quaderno di Studi e Notizie di Storia Naturale della Romagna, 29 (2009): 235–236.

Fernández de Gamboa R, Garzón A (2009) Primer registro de *Lucanus cervus* (Linné, 1758) f. *pontbrianti* (Mulsant, 1839) (Coleoptera: Lucanidae) para la Península Ibérica. Boletín Sociedad Entomológica Aragonesa 44: 533–534.

Franciscolo ME (1997) Fauna d'Italia. Vol. XXXV. Coleoptera Lucanidae. Calderini Edizioni, Bologna, I-XI+1–228.

Fremlin M, Hendriks P (2011) Sugaring for stag beetles – different feeding strategies of *Lucanus cervus* and *Dorcus parallelipipedus*. Bulletin of the Amateur Entomologist's Society 70: 57–67.

Fremlin M, Hendriks P (2014) Number of instars of *Lucanus cervus* (Coleoptera: Lucanidae) larvae. Entomologische Berichten 74(3): 115–120.

Fremlin M, Tanahashi M (2015) Sexually-dimorphic post-eclosion behaviour in the European stag beetle *Lucanus cervus* (L.) (Coleoptera: Lucanidae). Bulletin de la Societe Entomologique Suisse 88: 29–38.

Fujita H (2010) The Lucanid Beetles of the World. Iconographic Series of Insects 6, Mushi-Sha, Tokyo, 1–472.

Fusu L, Stan M, Dascălu MM (2015) Coleoptera. In: Iorgu IS (Ed.) Ghid Sintetic pentru Monitorizarea Speciilor de Nevertebrate de Interes Comunitar din România, București, 44–61.

GTLI (2005) 4.4 Abundance of *Lucanus cervus*. Available on: http://entomologia.rediris.es/gtli/engl/four/d/abunengl.htm

Harvey DJ, Gange AC (2006) Size variation and mating success in the stag beetle, *Lucanus cervus*. Physiological Entomology 31: 218–226. https://doi.org/10.1111/j.1365-3032.2006.00509.x

Harvey DJ, Gange AC, Hawes CJ, Rink M, Abdehalden M, Al-Fulaij N, Asp T, Ballerio A, Bartolozzi L, Brustel H, Cammaerts R, Carpaneto GM, Cederberg B, Chobot K, Cianferoni F, Drumont A, Ellwanger G, Ferreira S, Grosso-Silva J, Gueorguiev B, Harvey W, Hendriks P, Istrate P, Jansson N, Jelaska L, Jendek E, Jovic M, Kervyn T, Krenn H, Kretschmer K, Legakis A, Lelo S, Moretti M, Merkl O, Mader D, Palma R, Neculiseanu Z, Rabitsch W, Rodriguez S, Smit J, Smith M, Sprecher-Uebersax E, Telnov D, Thomaes A, Thomsen P, Tykarski P, Vrezec A, Werner S, Zach P (2011a) Bionomics and distribution of the stag beetle, *Lucanus cervus* (L) across Europe. Insect Conservation and Diversity 4: 23–38. https://doi.org/10.1111/j.1752-4598.2010.00107.x

Harvey DJ, Hawes CJ, Gange AC, Finch P, Chesmore D, Farr I (2011b) Development of non-invasive monitoring methods for larvae and adults of the stag beetle, *Lucanus cervus*. Insect Conservation and Diversity 4: 4–14. https://doi.org/10.1111/j.1752-4598.2009.00072.x

Hawes CJ (2005) The Stag Beetle *Lucanus cervus* (L.) (Coleoptera: Lucanidae) in the County of Suffolk (England): Distribution and Monitoring. Proceedings of the 3rd Symposium and Workshop on the Conservation of Saproxylic Beetles, Riga/Latvia, 7th-11th July 2004: 51–67.

Hawes CJ (2008) The stag beetle *Lucanus cervus* (Linnaeus, 1758) (Coleoptera: Lucanidae): a mark-release-recapture study undertaken in one United Kingdom residential garden. Revue d'Ecologie, Terre vie, 63: 131–138.

Hendriks P, Fremlin M (2012) How stag beetles pupate. http://maria.fremlin.de/stagbeetles/pupation/pupation_captivity.html.

Hines JE, MacKenzie DI (2004) PRESENCE, version 5.3 http://www.mbr-pwrc.usgs.gov/software.html

Jansson N (2011) Attraction of stag beetles with artificial sap in Sweden. Bulletin of the Amateur Entomologist's Society 70: 51–56.

Kervyn T (2006). Cerf-volant, lucane. In: Goffart P (Ed.) Cahiers "Natura 2000": Especes de l'Annexe II de la Directive Habitat presentes en Wallonie, CRNFB, Gembloux, 42–44.

Lapiana F, Sparacio I (2006) I Coleotteri Lamellicorni delle Madonie (Sicilia) (Insecta Coleoptera Lucanoidea et Scarabaeoidea). Naturalista siciliano (S. IV) 30(2): 227–292.

Malchau W (2006) Kriterien zur Bewertung des Erhaltungszustandes der Populationen des Hirskäfers *Lucanus cervus* (Linnaeus, 1778). In: Schnitter P, Eichen C, Ellenwanger G, Neukirchen M, Schröder E (Eds) Empfehlungen für die Erfassung und Bewertung von Arten als Basis für das Monitoring nach Artikel 11 und 17 der FFH-Richtlinie in Deutschland. Berichte des Landesamtes für Umweltschutz Sachsen-Anhalt (Halle), Sonderheft 2: 153–154.

Mason F, Cerretti P, Tagliapietra A, Speight MCD, Zapparoli M (2002) Invertebrati di una foresta della Pianura Padana, Bosco della Fontana, Primo contributo. Conservazione Habitat Invertebrati 1. Gianluigi Arcari Editore, Mantova, 1–176.

Mason F, Roversi PF, Audisio P, Bologna MA, Carpaneto GM, Antonini G, Mancini E, Sabbatini Peverieri G, Mosconi F, Solano E, Maurizi E, Maura M, Chiari S, Sabatelli S, Bardiani M, Toni I, Redolfi De Zan L, Rossi de Gasperis S, Tini M, Cini A, Zauli A, Nigro G, Bottacci A, Hardersen S, Campanaro A (2015) Monitoring of insects with public participation (MIPP; EU LIFE project 11 NAT/IT/000252): overview on a citizen science initiative and a monitoring programme (Insecta: Coleoptera; Lepidoptera; Orthoptera). Fragmenta entomologica 47(1): 51–52. https://doi.org/10.4081/fe.2015.134

Miksic R (1959a) Beitrag zur Kenntnis der Lamellicornia–fauna der Apenninen. II. Campanische Apenninen. Memorie del Museo Civico di Storia Naturale di Verona 7: 287–295.

Miksic R (1959b) Beitrag zur Kenntnis der Lamellicornen-Fauna der Apenninen. Ricerche zoologiche sul massiccio del Pollino, 29, Coleoptera 15. Annali dell'Istituto e Museo di Zoologia dell'Università di Napoli 11: 1–24.

Miksic R (1961) Beitrag zur Kenntnis der Lamellicornia–fauna der Apenninen. IV. Aspromonte. Memorie del Museo Civico di Storia Naturale di Verona 9: 5–25.

Moretti M, Sprecher-Uebersax E (2004) Űber das Vorkommen des Hirschkäfers *Lucanus cervus* (Coleoptera, Lucanidae) im Tessin: Eine Umfrageim Sommer 2003. Mitteilungen der Entomologischen Gesellschaft Basel 54: 75–82.

Nichols JD, Bailey LL, O'Connell AF Jr., Talancy NW, Campbell Grant EH, Gilbert AT, Annand EM, Husband TP, Hines JE (2008) Multi-scale occupancy estimation and modelling using multiple detection methods. Journal of Applied Ecology 45: 1321–1329. https://doi.org/10.1111/j.1365-2664.2008.01509.x

Nieto A, Alexander KNA (2010) European Red List of Saproxylic Beetles. Publications Office of the European Union, Luxemburg, 1–56.

Percy C, Bassford G, Keeble V (2000). Findings of the 1998 National Stag Beetle Survey. London, People's Trust for Endangered Species, London, 1–46.

Proyecto Ciervo Volante (1995) Proyecto ciervo volante. Boletín de la Sociedad Entomológica Aragonesa 11: 41–44.

Redolfi De Zan L, Bardiani M, Antonini G, Campanaro A, Chiari S, Mancini E, Maura M, Sabatelli S, Solano E, Zauli A, Sabbatini Peverieri G, Roversi PF (2017) Guidelines for the monitoring of *Cerambyx cerdo*. In: Carpaneto GM, Audisio P, Bologna MA, Roversi PF, Mason F (Eds) Guidelines for the Monitoring of the Saproxylic Beetles protected in Europe. Nature Conservation 20: 129–164. https://doi.org/10.3897/natureconservation.20.12703

Rink M, Sinsch U (2007) Radio-telemetric monitoring of dispersing stag beetles: implications for conservation. Journal of Zoology 272: 235–243. https://doi.org/10.1111/j.1469-7998.2006.00282.x

Rink M, Sinsch U (2011) Warm summers negatively affect duration of activity period and condition of adult stag beetles (*Lucanus cervus*). Insect Conservation and Diversity 4: 15–22. https://doi.org/10.1111/j.1752-4598.2009.00073.x

Romiti F, Redolfi De Zan L, Piras P, Carpaneto GM (2016) Shape variation of mandible and head in *Lucanus cervus* (Coleoptera: Lucanidae): a comparison of morphometric approaches. Biological Journal of the Linnean Society. https://doi.org/10.1093/biolinnean/blw001

Romiti F, Tini M, Redolfi De Zan L, Chiari S, Zauli A, Carpaneto GM (2015) Exaggerated allometric structures in relation to demographic and ecological parameters in *Lucanus cervus* (Coleoptera: Lucanidae). Journal of Morphology 276(10): 1193–1204. https://doi.org/10.1002/jmor.20411

Royle JA (2004) N-mixture models for estimating population size from spatially replicated counts. Biometrics 60: 108–115. https://doi.org/10.1111/j.0006-341X.2004.00142.x

Royle JA, Nichols JD (2003) Estimating abundance from repeated presence-absence data or point counts. Ecology 84: 777–790. https://doi.org/10.1890/0012-9658(2003)084[0777:EAFRPA]2.0.CO;2

Santoro F, Pivotti I, D'Allestro V, Fabrizi A, Di Veroli A, Di Giovanni MV, Corallini C, Mandrioli M, Goretti E (2009) *Lucanus cervus* e *Lucanus tetraodon* (Coleoptera – Lucanidae) in Umbria. Bollettino del Museo e Istituto di Biologia dell'Università di Genova 71: 202.

Smit JT, Krekels RFM (2006) Vliegend hert in Limburg Actieplan 2006–2010. EIS-Nederland en Bureau Natuurbalans-Limes duvergens; Leiden-Nijmegen/Nederland.

Smith MN (2003) National stag beetle survey 2002. People's Trust for Endangered Species, London, 1–14.

Smith MN (2011) Great Stag Hunt III: National stag beetle survey 2006 – 2007. People's Trust for Endangered Species, London, 1–23.

Solano E, Thomaes A, Cox K, Carpaneto GM, Cortellessa S, Baviera C, Bartolozzi L, Zilioli M, Casiraghi M, Audisio P, Antonini G (2016) When morphological identification meets genetic data: the case of *Lucanus cervus* and *L. tetraodon* (Coleoptera, Lucanidae). Journal of Zoological Systematics and Evolutionary Research 54: 197–205. https://doi.org/10.1111/jzs.12124

Sprecher-Uebersax E, Durrer H (1998) Über das Vorkommen des Hirschkäfers (*Lucanus cervus* L.) in der Region Basel. Mitteilungen der Entomologischen Gesellschaft Basel 48(4): 142–166.

Tanashi M, Kubota K, Matsushita N, Togashi K (2010) Discovery of mycangia and associated xylose-fermenting yeasts in stag beetles (Coleoptera: Lucanidae). Naturwissenschaften 97: 311–317. https://doi.org/10.1007/s00114-009-0643-5

Thomaes A (2008) Onderzoek en monitoring van het Vliegend hert [Study and Monitoring of the Stag Beetle]. Instituut Natuur-en Bosonderzoek, INBO.R.2008.2, Brussel, Belgium.

Tini M, Bardiani M, Campanaro A, Mason F, Audisio PA, Carpaneto GM (2017a) Detection of stag beetle oviposition sites by combining telemetry and emergence traps. In: Campanaro A, Hardersen S, Sabbatini Peverieri G, Carpaneto GM (Eds) Monitoring of saproxylic beetles and other insects protected in the European Union. Nature Conservation 19: 81–96. https://doi.org/10.3897/natureconservation.19.12678

Tini M, Bardiani M, Chiari S, Campanaro A, Maurizi E, Toni I, Mason F, Audisio PA, Carpaneto GM (2017b) Use of space and dispersal ability of a flagship saproxylic insect: a telemetric study of the stag beetle (*Lucanus cervus*) in a relict lowland forest. Insect Conservation and Diversity. https://doi.org/10.1111/icad.12260

Tini M, Bardiani M, Campanaro A, Mason F, Audisio PA, Carpaneto GM (in press) A stag beetle's life: sex-related differences in daily activity and behaviour of *Lucanus cervus* (Coleoptera: Lucanidae). Journal of Insect Conservation.

Trizzino M, Audisio P, Bisi F, Bottacci A, Campanaro A, Carpaneto GM, Chiari S, Hardersen S, Mason F, Nardi G, Preatoni DG, Vigna Taglianti A, Zauli A, Zilli A, Cerretti P (2013) Gli artropodi italiani in Direttiva Habitat: biologia, ecologia, riconoscimento e monitoraggio. Quaderni Conservazione Habitat 7. Cierre Grafica, Sommacampagna, Verona, 1–256.

Tronquet M (2014) Catalogue des Coléoptères de France. Revue de l'Association Roussillonaise d'Entomologie 23(Suppl.): 1–1052.

Vrezec A (2008) Phenological estimation of imagos occurrence in four saproxylic beetle species of conservation importance in Slovenia: *Lucanus cervus, Cerambyx cerdo, Rosalia alpina, Morinus funereus* (Coleoptera: Lucanidae, Cerambycidae). Acta Entomologica Slovenica 16: 117–126.

Vrezec A, Ambrožiĉ Š, Kapla A (2012) Supplementary studies of Natura 2000 species and monitoring of selected beetle species in year 2012: *Carabus variolosus*, *Lucanus cervus*, *Rosalia alpina*, *Rosalia alpina*, *Morimus funereus*, *Graphoderus bilineatus*. Final Report – Nationalni inštitut za biologijo, Ljubljana.

Vrezec A, Kapla A (2007) Quantitative beetle (Coleoptera) sampling in Slovenia: a reference study. Acta entomologica Slovenica 15: 131–160.

Worton BJ (1989) Kernel methods for estimating the utilization distribution in home-range studies. Ecology 70: 164–168. https://doi.org/10.2307/1938423

Zapponi L, Cini A, Bardiani M, Hardersen S, Maura M, Maurizi E, Redolfi De Zan L, Audisio P, Bologna MA, Carpaneto GM, Roversi PF, Sabbatini Peverieri G, Mason F, Campanaro A (2017) Citizen science data as an efficient tool for mapping protected saproxylic beetles. Biological Conservation 208: 139–145. https://doi.org/10.1016/j.biocon.2016.04.035

Zilioli M, Pittino R (2004) Un reperto eccezionale: *Lucanus tetraodon* Thunberg in Lombardia (Coleoptera, Lucanidae). Atti della Società Italiana di Scienze Naturali e del Museo Civico di Storia Naturale in Milano 145(2): 301–306.

Supplementary material 1

Field sheet

Authors: Marco Bardiani, Stefano Chiari, Emanuela Maurizi, Massimiliano Tini, Ilaria Toni, Agnese Zauli, Alessandro Campanaro, Giuseppe Maria Carpaneto, Paolo Audisio

Data type: field sheet

Explanation note: Field sheet to be compiled during each survey, for each transect. Each sighting is registered with a code, in the exact point of detection along the transect (path). Codes are reported on the right column of the field-sheet. Examples of codes: male in flight over 2 m – M2; female walk on the ground – F0; *L. cervus* with uncertain sex-determination, in flight below 2 m – U1.

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Guidelines for the monitoring of *Osmoderma eremita* and closely related species

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Abstract

Osmoderma eremita (Scopoli, 1763) is a saproxylic scarab beetle protected by the Habitats Directive in the European Union. The present paper is part of a special issue on monitoring of saproxylic beetles protected in Europe and starts with a revision of the current knowledge on systematics, ecology, ethology and conservation of *O. eremita* and its allied species, followed by experimental tests of different methods for monitoring its populations. Two methods were compared in several localities of central Italy: (1) the widely used pitfall traps into tree cavities and (2) black cross windows traps baited with a specific pheromone produced by male beetles. The first method, often used in northern and central Europe, did not give acceptable results in Italy probably because of the scarcity of veteran trees with large hollows. It could only be used successfully in areas where: 1) tree hollows were abundant, large enough and with sufficient amounts of wood mould for planting pitfall traps and 2) the team is composed of several operators in order to ensure the checking of at least 150 traps every two days during the whole period of mating activities (15 July–25 August). The second method, consisting of hanging 30 black cross window traps during the mating period and checking them every two days, turned out to be better for capturing a significant number of individuals but cannot be used every year because of the possible disturbance on mating activities of the species.

Keywords
Saproxylic beetles, old-growth forests, hollow trees, dead wood, trap sampling, Habitats Directive

Introduction

The hermit beetle, *Osmoderma eremita* (Scopoli, 1763), is a large saproxylic chafer (Coleoptera: Scarabaeidae: Cetoniinae) associated with hollow veteran trees of the European broadleaf woodlands. It is included in Annexes II and IV of the Habitats Directive (Council Directive 92/43/EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora). The Habitats Directive requires that Member States prepare, every six years, a report on the conservation status of the species listed in the Annexes. In order to facilitate the answer to this obligation, the Life Project "Monitoring of insects with public participation" (LIFE11 NAT/IT/000252: hereafter, MIPP) conducted experimental fieldwork to develop a standardised method for monitoring the saproxylic beetle species for the project: *Osmoderma eremita* (hermit beetle, Scarabaeidae), *Lucanus cervus* (European stag beetle, Lucanidae), *Cerambyx cerdo* (great capricorn beetle, Cerambycidae), *Rosalia alpina* (rosalia longicorn, Cerambycidae) and *Morimus asper/funereus* (morimus longicorn, Cerambycidae) (Mason et al. 2015).

The present paper is part of a special issue on the results for monitoring saproxylic beetles which are protected in Europe and it is dedicated to *Osmoderma eremita*. The paper starts with a significant revision of the current knowledge on systematics, distribution, ecology, ethology and conservation of *Osmoderma eremita* and allied species, mostly derived from a subsequent split of the species and hence worthy of the same protection level. Such a review is followed by the experimental test of methods for monitoring its populations.

Systematics and distribution

The genus *Osmoderma*, established in 1828 by Le Peletier de Saint-Fargeau and Audinet-Serville, is currently assigned to the superfamily Scarabaeoidea, family Scarabaeidae (scarab beetles) and subfamily Cetoniinae (fruit or flower chafers). Nevertheless, in many European scientific papers and databases produced in the second half of the last century, the subfamily was raised to family rank and *Osmoderma* was reported as a genus of the family Cetoniidae. This change in systematic arrangement caused some confusion in the documents of the Habitats Directive and is explained by the fact that some specialists, (i.e. authors of the most important works used for identification of the European scarab beetles (Balthasar 1963, Baraud 1977, 1992, Paulian and Baraud 1982, Martin-Piera and López-Colón 2000, etc.) revised the systematic subdivision, introduced by Westwood (1839), where these animals were divided into numerous families. Such extreme splitting classification was overcome by modern integrated studies of morphological and molecular analysis where the family Scarabaeidae was reconsidered in a wider sense (e.g. Lawrence and Newton 1995, Browne and Scholtz 1995, 1998, 1999, Scholtz and Grebennikov 2005, Smith et al. 2006, Ahrens 2006, Bouchard et al. 2011, Ahrens et al. 2014, McKenna et al. 2015).

Within the subfamily Cetoniinae, traditional taxonomy assigned the genus *Osmoderma* to the subtribe Osmodermina (two genera) of the tribe Trichiini, a large clade of Cetoniinae that includes the widespread genera *Trichius*, *Gnorimus* and others (Howden 1964, Krikken 1978, Smetana 2006, Schoolmeesters 2016). However, a recent molecular study (Šípek et al. 2016a, 2016b) suggested closer affinities between *Osmoderma* and other fruit chafers, more than with the above-mentioned genera and thus concluding that it should be assigned to the tribe Cetoniini. Such great instability at higher levels of systematic classification is also reflected at both genus and species level, due to an extensive debate on nomenclature and taxonomy which has been on-going for many years (cf. Tauzin 1994a, 1994b, 1996, 2000, 2002, 2006, Sparacio 1994, 2000, Massa 1995, Gusakov 2002, Audisio et al. 2007, 2009, Zauli et al. 2016a), with implications also concerning the conservation priority of *Osmoderma* populations. Although the validity of the genus name became controversial when some authors (Ádam 1994, Gusakov 2002) asserted that priority was due to a formerly used epithet, *Gymnodus* Kirby 1827, the International Commission on Zoological Nomenclature decided to adopt the name *Osmoderma* by a special resolution based on the principle of prevailing usage and the need for stability (Smith 2004, Krell et al. 2006, Audisio et al. 2007, Barclay 2007, ICZN 2007). The debate at species level involved both taxonomy and nomenclature of the eastern populations, since Motschulsky (1860) put in doubt the terra typica of *Osmoderma barnabita*, a species he had formerly described in 1845. Thus, from southern European Russia, the terra typica became eastern Siberia, setting off a series of subsequent taxonomic interpretations. Therefore, the name assigned to these populations changed over time according to different authors: *O. lassallei septentrionale* Tauzin, 1994, *O. coriarius* (De Geer, 1774) and then again *O. barnabita* (cf. Tauzin 1991, 1994a, 1994b, Gusakov 2002, Audisio et al. 2007, 2009).

At global level, the genus includes at least 12 species throughout the Holarctic Region (Howden 1964, Gusakov 2002, Smetana 2006, Audisio et al. 2007, Bezborodov 2016, Schoolmeesters 2016). The geographic range of the genus *Osmoderma*, such as the range of many deciduous forest-dwelling animals of the northern hemisphere, is subdivided into three disjunct sectors: northeastern and central United States of America (3 species according to Howden 1968), Europe with its Near East countries (4 in Europe, 1 in Anatolia, 1 in Georgia) and Far East Asia (3 species in NE China, S Siberia, Korea and Japan).

The North American sector includes mainly the potential range of the US eastern broadleaf deciduous forests and extends southwards up to the 35th parallel, where two species occur sympatrically: *O. eremicola* (Knoch, 1801) and *O. scabrum* (Palisot de Beauvois, 1805). A third species, *O. subplanatum* Casey, 1915, unexpectedly lives in the Great Plains, probably restricted to fragmented woodlands along lake shores and the river basins of the Mississipi and Missouri. All three of them overrun the southern border of Canada to a limited extent.

The three species living in the Asian Far East have recently been revised by Bezborodov (2016): *O. davidis* Fairmaire 1887, from Transbaikalia and NE China to Primorskii Territory and N Korea; *O. caeleste* (Gusakov 2002) from NE China, Primorskii Territory and N Korea; *O. opicum* Lewis 1887 from Primorskii Territory, N Korea and Japan. The different ranges of the three species overlap in the temperate broadleaf and mixed forests of the southern Primorskii Territory, near Vladivostok, where they live together and where they were also observed in the same tree (Bezborodov 2016). The range of *O. davidis* is wider than the other two species and reaches westwards to the forest-steppe ecotones on the border between Transbaikalia and Mongolia.

In order to address the great confusion in taxonomy and nomenclature of the European hermit beetles, a DNA-approach of species delimitation was attempted by Audisio et al. (2007, 2009) and resulted in the consideration of the occurrence of at least four species in Europe:

Osmoderma eremita (Scopoli, 1763) in most of western Europe;
Osmoderma barnabita Motschulsky, 1845 (sensu Audisio et al. 2007) in most of eastern Europe;
Osmoderma lassallei Baraud and Tauzin, 1991, endemic to northern Greece and European Turkey;
Osmoderma cristinae Sparacio, 1994, endemic to Sicily.

Genetic distance and parsimony analysis well supported the delimitation of two clusters, each formed by two species: the first cluster is restricted to western Europe (*O. eremita* and *O. cristinae*) and the second one to eastern Europe (*O. barnabita* and *O. lassallei*) (Figure 1). The northern trait of the boundary between the two clusters divides Sweden (*O. eremita*) and Finland (*O. barnabita*), while the southern trait, still poorly delimited, crosses Germany, Austria and Slovenia (where both *O. eremita* and *O. barnabita* seem to occur) (Audisio et al. 2007, 2009, Landvik et al. 2013) (Figure 1). Within *O. eremita*, preliminary morphological analyses delimited a putative Italian subspecies, *O. eremita italicum* Sparacio, 2000 whose presence was, until the present time, verified for only a few localities in three regions of southern Italy (Campania, Basilicata and Calabria).

The morphological traits used for delimiting the above mentioned species and subspecies are poorly marked and subject to a significant variation. They mainly concern the reliefs on head and pronotum, as well as the shape of male genitalia (Figures 2, 3). The lack of well-differentiated outlines can generate a high degree of uncertainty when the populations living in the areas of contact between the ranges of the various taxa are studied, e.g. in central Europe and in the Balkans, due to intermediate phenotypes which make difficulties in the assignment of individuals to two neighbouring taxa.

An integrated approach to the Italian populations, based on morphological and molecular analyses (Zauli et al. 2016a), suggested that both the genetic distance (COI and AFLP markers) and morphological divergence (particularly the shape of male genitalia) support the validation of species rank for *O. cristinae*, despite the close relationship between this Sicilian endemic taxon and the widespread *O. eremita*.

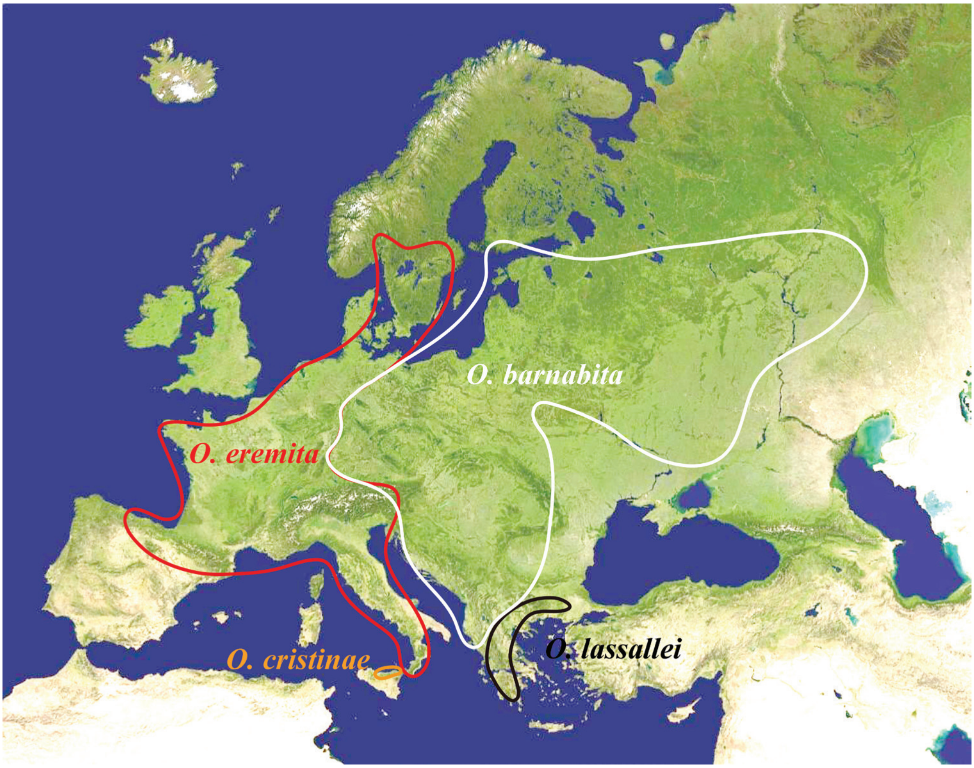

Figure 1. Distribution map of the genus *Osmoderma* in Europe (from Audisio et al. 2009, modified) (prepared by M. Liu).

Additional poorly investigated taxa occur from the Balkan peninsula to the Caucasus mountains (apart from *O. lassallei* and *O. barnabita*): at least one undescribed species or semispecies, related to *O. lassallei*, in southern Greece (P. Audisio, P. Petrakis and G.M. Carpaneto, unpublished data); the rare *O. brevipenne* Pic, 1904, described from the Taurus Mountains, southern Turkey; *O. richteri* Medvedev, 1953, from Georgia, Caucasus (only two females captured until now).

Morphology and comparison with similar taxa

The genus *Osmoderma* includes the largest fruit chafers (Cetoniinae) of the European fauna (body length up to 36mm, head included; greatest width of elytra up to 19mm). The head is small in comparison with the body, with a clypeus broader than long. Antenna is 10-segmented with a small terminal club formed by three short lamellae. The body is wide, suboval and more or less flattened dorsally. The prothorax is significantly narrower than the bases of the elytra, more or less convex, with lateral sides rounded or sharp-cornered just before the middle. The legs are strong, with moderately long tibiae

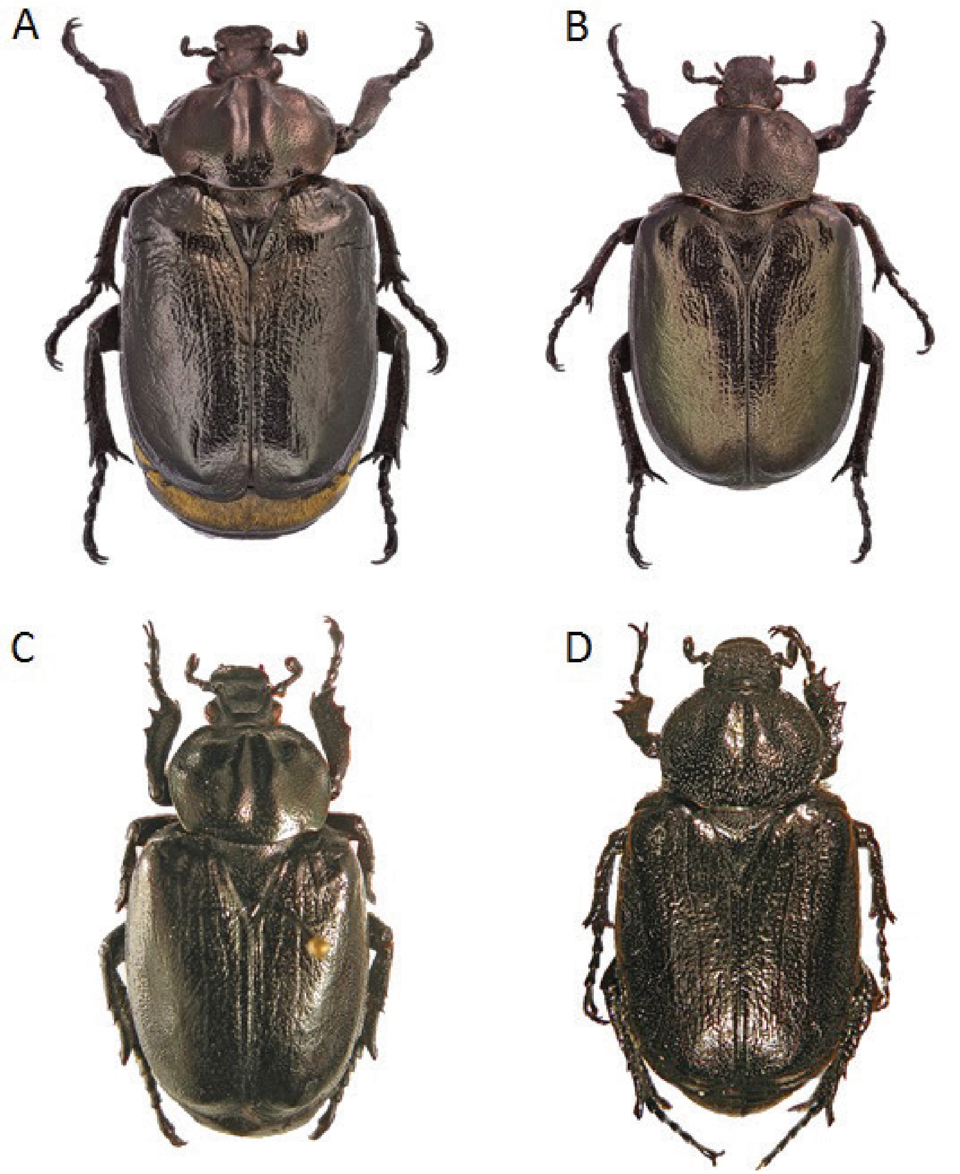

Figure 2. Habitus of *Osmoderma* in dorsal view. **A** *O. eremita* male **B** *O. eremita* female **C** *O. cristinae* male **D** *O. cristinae* female (photographs by A. Ballerio and M. Uliana, from Ballerio et al. 2010).

and short tarsi. Fore tibiae have three distinct teeth on their external side. The wings are well developed in both sexes. Colour is black to piceus or chestnut, often shiny with cupreous or green metallic lustre.

In the European species, males have a clypeus concave, more or less reflexed at apex, a slightly convex clypeo-frontal transition area and two lateral elevations above

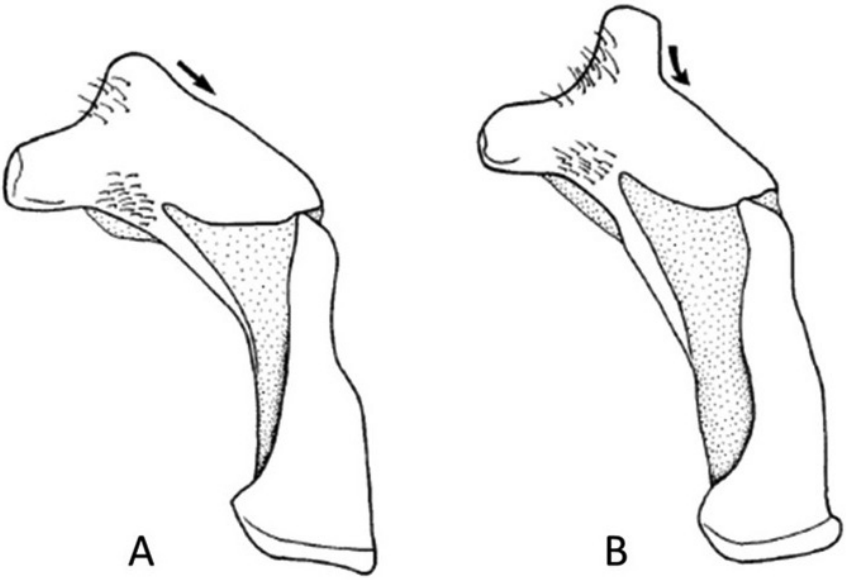

Figure 3. Lateral side of parameres, in *O. eremita* (**A**) and *O. barnabita* (**B**). The arrows indicate the lack or the presence of concavity under the protuberance (From Lüchte and Klausnitzer 1998, modified).

the antennal insertion that look like two horns. Females have a clypeus flat and not reflexed at apex, a tumid clypeo-frontal transition area and lack elevations at sides. Head surface is punctured in males and rugose in females. The pronotum of males has distinct basal angles, with lateral sides obtuse-cornered just before the middle; it is about one-third wider than long, strongly convex, with two distinct longitudinal carinae, parallel and separated by a more or less deep groove. The pronotum of females has basal angles rounded and an overall rounded outline, it is less convex than males and shows only vestigial ridges, almost vanished or reduced to two tubercles. Pygidium is large and strongly convex in males, smaller and slightly convex in female.

As already mentioned in the previous paragraph, the morphological traits used for delimiting the European species and subspecies are poorly marked and subject to significant variations. The morphological identification of *Osmoderma* is a task for specialists who have examined many specimens from different locations (in museums or other collections) and progressively acquired a deep knowledge of the intraspecific variation within single populations and throughout the geographic range of the species. Another problem is that the specimens occurring in many collections and museums cannot be used as a tool for making comparisons as they have often been improperly identified, owing to the extensive taxonomic confusion in the past decades. Luckily, the European species have different ranges and, in most cases, the populations can be simply assigned to a species by the locality. The problem arises when a locality is situated in the overlapping area between the ranges of two parapatric species, as in the case of *O. eremita* and *O. barnabita* in central Europe and the Balkans, where the possibility cannot be ruled out that they mated with each other. The less variable trait for separation of these two species is perhaps the shape of the parameres of the male genital organ (Krell 1997, Lüchte and Klausnitzer 1998, Sparacio 2000, Zauli et al. 2016a) according to the following key (see also Figure 3):

1. Parameres, on lateral side, show a strong upward protuberance like a triangular bump rounded at apex, followed by an almost straight or very slightly convex slope continuing up to their base. .... *O. eremita*
2. Parameres, on lateral side, show a very strong upward protuberance like a square bump rectangular at apex, followed by a strong concavity and then a straight slope continuing up to their base. .... *O. barnabita*

The male genitalia also allow the separation of *O. eremita* and *O. barnabita* from *O. cristinae*, as the latter does not show a real upward extension on parameres but only a moderate swelling followed by a straight or very slightly convex slope continuing up to the base (Figure 4). However, genitalia shape cannot be used for monitoring populations because it requires dead specimens. In these cases, identification must rely on molecular analyses (e.g. mtDNA cytochrome C oxidase I, extracted from the femoral or tarsus muscle from a living individual) (Audisio et al. 2009).

All morphological characters used to separate the European species of *Osmoderma* are quantitative and change between them without a sharp difference; furthermore, they are subjected to significant variation and cannot give certainty in identification. The sexual differences in the *O. barnabita/lassallei*-group are less prominent than in the *O. eremita/cristinae*-group.

Non-expert operators engaged in searching the remains of *Osmoderma* in wood mould inside tree cavities, may find it hard to distinguish them from those of *Gnorimus variabilis* (Linnaeus, 1758). However, the remains of the latter are clearly smaller and slender, with proportionately longer and fine tarsi and middle tibiae are strongly recurved in males. Moreover, the outer side of the fore tibiae have two well developed teeth (instead of three teeth in *Osmoderma*), while the scutellum is shorter and half-circle shaped (instead of the almost isosceles triangle-shaped scutellum of *Osmoderma*). Elytra of *G. variabilis* can have whitish spots on the dorsal surface.

The eggs of *Osmoderma* are globe-like and white, with a diameter of 4-5mm. Larvae are classified in the scarabaeiform type and usually named 'white grubs' or 'curl grubs' by applied entomologists. They are characterised by a stout and downward projecting (hypognathous) head and a large fat, white or blueish, C-shaped body (Hayes 1929, Boving and Craighead 1931, van Emden 1941, Ritcher 1966). In particular, the shape of hermit beetle larvae is very similar to that of other Cetoniinae, with whom they share the following characters: head small; labrum symmetrical; mandible with a ventral, oval, stridulatory area consisting of transverse ridges; maxilla with lacinia and galea fused and stridulatory teeth anteriorly directed; ninth and tenth abdominal segment fused together (Hayes 1929, Ritcher 1966, Shabalin and Bezborodov 2009). The

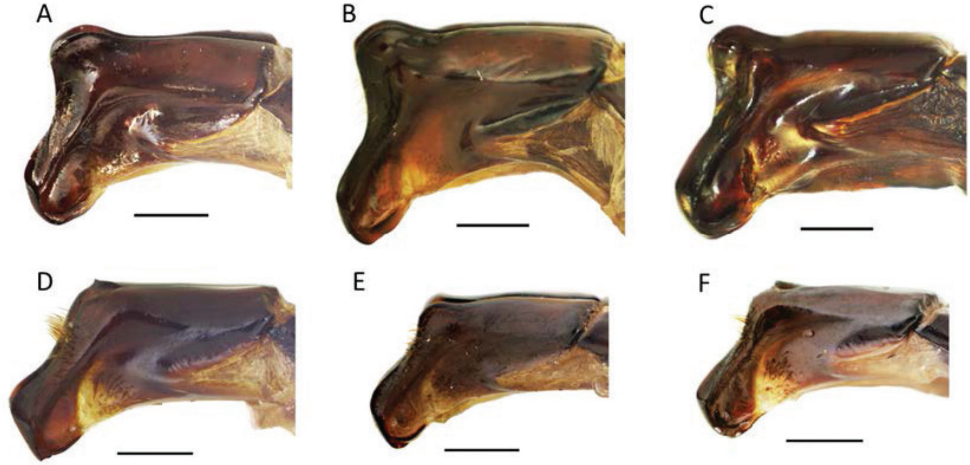

Figure 4. Lateral side of parameres, in *O. eremita* (**A–C**) and *O. cristinae* (**D–F**), showing intraspecific variation and difference on the parameres between the two species (photo by Federico Romiti).

head of the hermit beetle larvae is a red-orange capsule, with rugose punctures in the frons, a trilobed labrum and a pair of black and strong asymmetric mandibles. Legs are yellow-brownish and relatively short, each one ending with a wide-based, shortly subconical and blunted claw. The bristled ventral side (hereafter: 'raster', according to Boving and Craighead 1931) of the last abdominal segment lacks the longitudinal double row of spinules (hereafter: 'palidia', according to Ritcher 1966), which usually occur in Cetoniinae larvae (Korschefsky 1940) (Figure 5).

Larval characters are only used for the identification of wholly developed larvae (mainly belonging to the third stage, i.e. the last phase of their life cycle). A good combination of characters for separating hermit beetle larvae from those of all other European Cetoniinae is the following: 1) the absence of palidia across the raster, 2) the short, blunted and subconical terminal claw of tarsus and 3) the body size between 65 and 75mm, considering that the large larvae of *Protaetia speciosissima* are never longer than 65mm (Korschefsky 1940). In fact, almost all European saproxylophagous fruit chafers which live in dead wood (i.e. *Cetonia*, *Protaetia* and *Gnorimus*) have distinct palidia and legs which end without a claw or with a normally elongate and slender claw (never thick, subconical and blunted) (Figure 6). Amongst the fruit chafer larvae occurring in the same habitat, only those of the genus *Trichius* lack palidia but are easily recognisable from *Osmoderma* because of their small body size (up to 40mm long when mature), the normally elongate claw, the labrum rounded (instead of trilobed) and the last two abdominal segments well separated (instead of fused) (Shabalin and Bezborodov 2009).

The following key, mainly valid for third instar larvae was constructed by one of us (G. Carpaneto) and was based on information drawn from several authors (Hayes 1929, Böving and Craighead 1931, Korschefsky1940, Ritcher 1966, Klausnitzer and Krell 1997, Mico and Galante 2003a, 2003b, and Shabalin and Bezborodov 2009) as well as unpublished data. It may be useful for recognising larvae of hermit beetles from all other European Cetoniinae, with the aid of a pocket hand-held magnifier:

1. Tarsi ending with a claw (long or short, sharpened or blunted) **2**
– Tarsi rounded apically, without claws **4**
2. Tarsal claws slender, more or less sharpened and recurvate. Ninth and tenth abdominal terga separated in two single dorsal units. Raster with or without palidia. Anterior margin of labrum not distinctly trilobed, almost straight or slightly emarginate in the middle. Larvae in dead wood, up to 60mm **3**
– Tarsal claws neither sharpened nor recurvate, but like a black, short and thick conus with a large base and a blunted apex. Ninth and tenth abdominal terga fused into a single dorsal unit. Raster without palidia. Anterior margin of labrum distinctly trilobed. Larvae in dead wood, up to 75mm long............. .... ***Osmoderma***
3. Raster with two distinct palidia forming an oval Figure. Larvae in dead wood, up to 50 or 60mm long, according to the species .... ***Gnorimus***
_ Raster without palidia. Last abdominal segment normally shaped. Larvae in dead wood, up to 40mm long... ***Trichius***
_ Raster without palidia. Last abdominal segment ends with a pair of rounded areas delimited by a slight wrinkle. Larvae in dead wood, up to 25mm......... .... ***Valgus***
4. Larvae small (up to 25mm), amongst the roots of herbaceous plants............ .... ***Oxythyrea* and *Tropinota***
– Larvae large (25-65mm), amongst plant roots, humus and dead wood, according to species.. **5**
5. Raster with two well distinct palidia, each formed by18-28 spinules (mostly 22-24). Larvae up to 45mm long.. ***Cetonia***
– Raster with two well distinct palidia, usually formed by less than 22 spinules (usually 14-20, exceptionally up to 26 in the rare *P. fieberi*). Larvae, up to 65mm long.. ***Protaetia* and *Aethiessa***

Non-expert operators may have difficulty in recognising the larvae of hermit beetles from those of the other two large and widespread scarab beetles occurring in deadwood i.e.*Oryctes* (Scarabaeidae Dynastinae) and *Lucanus* (Lucanidae Lucaninae). Apart from microscopy characters involving antennae and mouth parts, often very hard to detect during the fieldwork, the larva of *Oryctes* is easily distinguished because its last abdominal segment is divided into two halves by a transverse narrowing, so that, in dorsal view, the abdomen seems to be formed by ten clearly observed segments. On the other hand, the larva of *Lucanus* is immediately recognisable by the abdominal terga not plicate, the longitudinal anal slit (instead of the transverse anal slit of *Osmoderma* and other Cetoniinae), the large but much narrower and elongate abdominal spiracles (see Bardiani et al. 2017) and the stridulating organs consisting of a series of teeth on

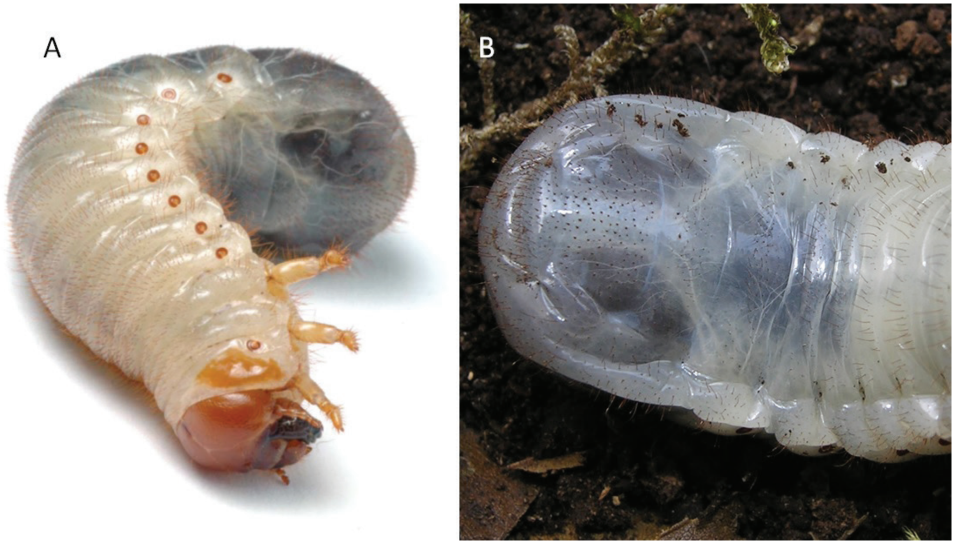

Figure 5. Larva of *Osmoderma eremita*. **A** Habitus and **B** Raster (ventral spiny area of the last abdominal segment). Note the lack of the palidia (the two longitudinal series of spinules occurring in the raster of most fruit chafers) and the end of the legs provided with a short and stout claw (see text for detailed explanation). (**A** photo by Sonia Dourlot, from Dubois 2009 **B** photo by Benjamin Calmont).

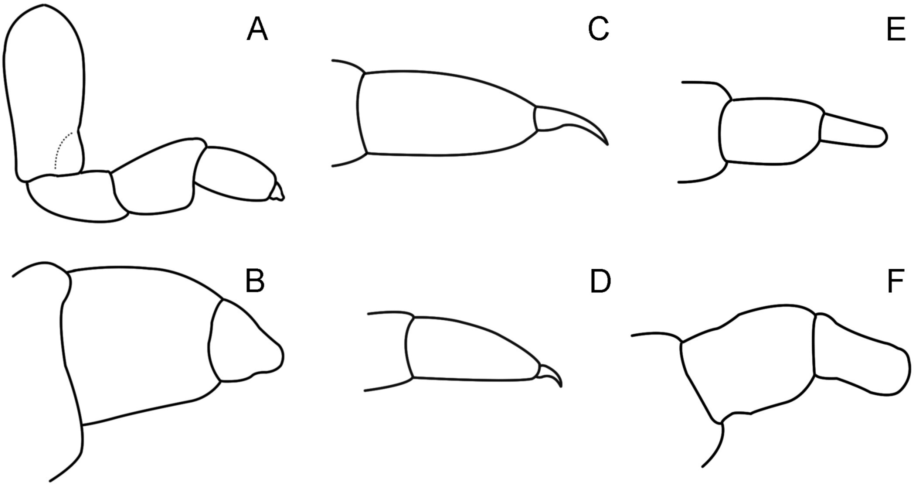

Figure 6. Shape of the terminal claw in European fruits chafer larvae. **A** leg of *Osmoderma eremita* **B** last two segments and claw of *O. eremita* **C** idem of *Gnorimus variabilis* **D** idem in *Trichius fasciatus* **E** last three segments in *Cetonia aurata* **F** last three segments in *Protaetia cuprea* (outlined by Federico Romiti).

second and third pairs of legs (instead of being present on the ventral side of mandibles and dorsal side of maxillae as in Cetoniinae).

Life cycle, food and chemical ecology

The freshly laid egg of *Osmoderma* is globe-like and opaque white but, after 2-3 weeks, it becomes yellowish and doubles its size, reaching a diameter of 4-5mm. Each female lays 20-80 eggs deeply in a tree cavity, between the inner wall and the wood mould, protected by a flexible coating of wood pulp and a variable number of larvae (from 12 to 18) hatch after 14-20 days. The newly hatched larvae measure just 6mm but they grow quickly, feeding on decaying wood and its mixture of rot-causing fungi. Larvae go through three different instars (L1, L2 and L3) across a developing time of 2-4 years and overwinter at their first or second instar, depending on the oviposition date and local microclimatic conditions (Tauzin 1994, Luce 1996, Schaffrath 2003a, Ranius et al. 2005). Laboratory and field studies conducted in Poland by Pawłowski (1961) showed that feeding activity of the larvae took place when the average daily temperature exceeded 13°C and they developed after three or four years. On the whole, the larvae were active only 30 weeks per year. Therefore, hibernation and developing time are directly affected by the temperature of the microhabitat in either tree hollows or vivarium. Similar field and laboratory observations over 3-4 years of development were made in other countries, such as Russia, Germany and Latvia (Nikitsky et al. 1996, Schaffrath 2003a, D. Telnov, in Ranius et al. 2005), while a shorter life cycle was reported for specimens kept indoors under favourable conditions without spending periods of feeding inactivity (M. Uliana, pers. comm. 2016). According to Dubois (2009), intra-clutch variation occurs in the development time of *O. eremita*: larvae hatched from the clutch of one female were bred in the same conditions and finished their development after two years (two males) and three years (10 males and one female) respectively. Such variation could be genetically based and may have a role in minimising inbreeding by avoiding mating between siblings.

At the end of the second or third summer, usually in September, when the third instar larva has reached its full development (75mm long), it builds an oval-shaped cocoon by clumping fine wood debris with its salivary and faecal secretions. Within this protecting case, the larva spends its last winter and then transforms into a pupa in the spring (normally in May) and into an adult (June) (Tauzin 1994). The emergence of the adults occurs in July and in early August, when many individuals show a shiny integument suggesting they had just emerged from the cocoon.

Tauzin (1994b, 2005), who reared *O. eremita* in the laboratory, observed that, in the case of mating, the lifespan of males was very short (10–20 days), while females lived for more than 90 days. Nevertheless, some males fed in captivity reached lifetimes of 90 days (Schaffrath 2003a) and a field study conducted on a Swedish population suggested males and females have the same life–time, with a maximum of about one month (Ranius 2001). Although hermit beetles seem unable to survive in natural habitats after the arrival of autumn, a hibernating adult female has once been found in January in the forest of Fontainebleau, France (Tauzin 1994b). The last observation seems to suggest that, under favourable conditions, females may have a longer lifespan and take advantage of the opportunity for increasing their breeding success, as evidenced by field research on other beetles, e.g. the longhorn beetle *Morimus asper* (Rossi de Gasperis et al. 2016).

The seasonal activity of the adults is correlated with climatic factors, such as altitude, latitude and habitat type. In Sweden, the adults are active from July to September, while in central and southern Europe, there have been several observations in May-June and even a few at the end of April (Stegner 2002, Schaffrath 2003a, Ranius et al. 2005, Vignon 2014). In France, the adults are active from the end of June to the end of August, with an emergence peak between 10 and 15 July (Vignon 2014). In Italy, the activity period is mostly from early June to mid-August with peaks in July (Carpaneto et al. 2010, Chiari et al. 2012, 2013a, 2013b, Giangregorio et al. 2015). In Valtellina (Lombardia, N Italy, Sondrio province), an emergence peak and an activity period, almost identical to those above reported for France (Vignon 2014), were observed during several years, with peaks of activity in the second half of July (P. Audisio, unpublished data). It may be concluded that, on the whole range of the species, phenology is from the end of April to September, but local variations occur due to different regimes of temperature and rainfall. However, most records were usually made from early July to early August.

One or two days after their emergence, the males of the genus *Osmoderma* start to emit a strong odour which can be perceived by humans at a distance of several metres, reported by French entomologists of the 19th century as "cuir de Russie", namely Russian leather (Latreille et al. 1825). This odour is a male-produced sexual pheromone which attracts females and induces them to gather into the tree hollow occupied by a male. Chemical analysis identified this compound as (*R*)-(+)-γ-decalactone (Larsson et al. 2003, Svensson et al. 2003, 2004, Zauli et al. 2014). Ecological investigations showed that this chemical is also an attractant for females of the rusty red click beetle, *Elater ferrugineus* Linnaeus, 1758, a large click-beetle whose larvae live in hollow trees and feed on the immature stages of other saproxylic insects, mostly those of fruit chafers, with a special preference for hermit beetles. Therefore, click-beetle females use the sexual pheromone of the hermit beetle males as a kairomone in order to lay eggs in suitable tree cavities, already inhabited by the favourite prey for their offspring (Svensson et al. 2004, Larsson and Svensson 2009, 2011, Zauli et al. 2014, 2016c). Field trials, scent collection and biochemical analysis on *O. barnabita* and *O. cristinae*, showed that these species use exactly the same sex pheromone compound as *O. eremita*, demonstrating a strong conservation of this sexual trait within the genus throughout Europe (Landvik et al. 2015, Zauli et al. 2016a). Besides, as suggested by Svensson et al. (2009), the male-produced pheromone in *Osmoderma* beetles may function as a territorial signal instead of a classical sex pheromone used for species discrimination and the selection pressure to change the signal may be weak or absent. From a conservation perspective, the use of (R)-(+)-γ-decalactone by allied species facilitates large-scale monitoring of the hermit beetles in Europe since it can be performed with the same target compound.

Many authors have indicated this scent to be similar to fermented fruit (peaches, plums or apricots) on which the adults were reported to feed. The French vernacular name "pique-prune" (= who picks the plum) (Vignon 2006) and the German one

"Aprikosenkäfer" (Ranius et al. 2005) are surely related to this odour and/or to the popular knowledge that this species likes to feed on plums, apricots and other fruit of the rose family. However, the feeding activity of adult hermit beetles in nature has been rarely observed by entomologists. Ranius et al. (2005) revised the scarce information occurring in literature and gathered some unpublished data on this subject. Literature data reported some findings of the species on sap flows of broadleaf trees in Germany, Poland, Estonia and Russia, as well as a few observations in the laboratory where some specimens were fed with apples and bananas (Schaffrath 2003b). Unpublished data gathered by Ranius et al. (2005) included field observations on flowers of *Leucanthemum* sp. and *Viburnum* sp.in Croatia, on flowers of *Sambucus nigra* in Spain, on unidentified umbelliferous plants in Germany and on ripe yellow plums (*Prunus* sp.) in Poland. Another field observation was reported for central Italy by Chiari et al. (2013b) and concerned a radio-telemetered female that flew out of a cork oak forest and covered a distance of almost 1500m before landing in an olive grove and staying at least one hour from 1300h to 1400h, to eat an overripe apricot (*Prunus armeniaca*) which had fallen on the main tree fork. After all, the popular French name "pique-prune" and the similarity in the odour of plums and pheromones cannot be entirely due to chance: the link between this species and *Prunus* fruits should be thoroughly investigated, even to evaluate the needs of the species for its survival in protected areas.

Habitat requirements and use of space

The primary habitat of hermit beetles consisted of broadleaf old-growth forests with an abundance of hollow trees and dead wood. In particular, the species seems to prefer areas where the canopy is not dense because of the age of the plants and the occurrence of open space left by the fall of veteran trees. In fact, according to many literature data and the authors' long personal experience, hermit beetles are saproxylophagous species whose larvae meet their habitat and food requirements in tree cavities rich in wood mould. Nevertheless, as it is more than a strictly forest dwelling species, the hermit beetle seems to be an ecotonal species of the forest edge and clearings which can be found in any context where there are old hollow trees, not only in mature forests but also in the agricultural and urban landscapes. In contrast to logs and snags, live hollow trees provide long-lasting resources (Ranius and Nilsson 1997) and a specific abiotic environment characterised by stable temperature, suitable moisture conditions and increased pH values (Müller et al. 2014). Owing to the alteration of natural forests by commercial management and the consequent rarity of hollow trees, the hermit beetles disappeared in many apparently suitable broadleaf forests but survived in artificial habitats, such urban parks, wooded grasslands, old orchards and tree rows along channels or country roads, even if embedded in unsuitable cultivated environments (Vignon et al. 2004, Baratelli 2004, Dutto 2005, Ranius et al. 2005, Oleksa 2007, Carpaneto et al. 2010, Giangregorio et al. 2015). Many populations of hermit beetles in France live in chestnut orchards and in the hedgerow network, where the number of old trees is higher than in forests due to local management practices (Vignon and Orabi 2003, Vignon et al. 2004, Vignon 2006, 2008, 2015). These artificial environments represent 'habitats of substitution' for *O. eremita* in France and are considered very important for its survival in areas where old-growth forests are extremely reduced or absent.

The altitudinal range of the hermit beetle varies from sea level (evergreen forest dominated by holm oak, *Quercus ilex* or cork oak, *Q. suber*) up to almost 1400m (mountain forests of beech tree, *Fagus sylvatica*), through all intermediate altitudinal belts (covered by mixed deciduous forests and woodlands of *Quercus* spp., *Acer* spp., *Fraxinus* spp., *Tilia* spp., *Ulmus* spp., *Carpinus* spp., *Salix* spp., *Populus* spp., *Betula* spp., *Alnus* spp., *Castanea sativa* etc.) (Ranius et al. 2005). However, an adult male of *Osmoderma eremita* ssp. *italicum* was recently collected in southern Italy (Mount Pollino Massif, Calabria) at 2000m in a hollow beech (A. Mazzei, pers. comm. 2016). Larvae can live in dead wood of both deciduous trees (oak, beech, maple, ash, lime, elm, hornbeam, willow, poplar, birch, alder, chestnut, apple, pear, plum, mulberry) and evergreen trees (holm and cork oak) and no clear preference was demonstrated for each of these species according to most observers (Schaffrath 2003a, 2003b, Tauzin 2005, Ranius et al. 2005, Chiari et al. 2014). Some scattered notes exist on the local association of hermit beetles with coniferous trees (*Taxus*, *Pinus* and *Abies*) but such information needs to be verified.

Hermit beetle larvae usually occur in hollow but still living, standing trees but they have also been found in dead standing trees as long as the dead wood is able to retain a suitable degree of moisture (Rainus et al. 2005). More rarely, the species has been observed in felled tree trunks or in stubs but it mainly occurs after a recent cut. The age of habitat trees suitable for hermit beetles depends on the features of wood. Fast-growing species such as poplars (*Populus* spp.) and willows (*Salix* spp.) may harbour hermit beetles when they are only a few decades old (Schaffrath 2003a), while fruit trees are usually 80–100 years old (Stegner 2002). According to Ranius et al. (2005), slow-growing trees like oaks are 150-400 years old in Sweden, while the pollarded trees of the same species in France are only 70-120 years (Vignon and Orabi 2003). Usually, if suitable hollow trees are abundant and rich in dead wood, the local populations of hermit beetles may consist of a high number of individuals, even where the forest surface is reduced.

Despite widespread opinion supporting the extreme polyphagy of hermit beetles, detailed studies by larval sampling in wood mould showed that these insects have preferred tree species at the local level. The northern-most populations of *Osmoderma* (*O. eremita* in Sweden and *O. barnabita* in Finland) exploit mainly the huge trees of pedunculated oaks (*Quercus robur*) which form small stands in the agricultural landscape (Ranius et al. 2005, Landvik et al. 2015). Across a total of 192 hollow trees examined by Landvik et al. (2015) in Finland, they found signs of *O. barnabita* in a total of 62 trees (i.e. in 32% of the trees examined): 67% of *Q. robur*, 21% of *Tilia* spp. and 6% of *Alnus glutinosa*. In addition to these core tree species, *Acer platanoides, Sorbus aucuparia* and *Populus nigra* also figured in the list of host plants with only one record. In Poland, the distribution of species (probably *O. barnabita*) was not random with respect to tree species (Oleksa 2007). Contrary to common belief, the hermit beetles of Poland did not prefer oaks, avoided *Acer platanoides* and *Carpinus betulus* and preferred *Tilia cordata* and *Alnus glutinosa*. Furthermore, they showed a significant predilection for trees of about 450cm in circumference at 1.3m height and did not show any dependence on the hollow entrance area and exposition (Oleksa 2007). A habitat study in central Italy (Chiari et al. 2012), conducted by wood mould sampling in 151 hollow trees belonging to eight broadleaf species, revealed that *O. eremita* was present in 22% of the trees, all belonging to four species, *Ostrya carpinifolia* (60%), *Quercus suber* (25%), *Acer obtusatum* (6.6%) and *Quercus pubescens* (1%). The number of larvae and remains was higher in cavities with more than 4 litres of wood debris, where the wood mould was relatively dry and had low soil contamination. Successful colonisation and breeding of the hermit beetle was obtained in an artificial habitat i.e. wooden boxes filled with oak leaves and sawdust, placed in natural conditions in Sweden and Poland (Jansson et al. 2009, Hilszczański et al. 2014, Carlsson et al. 2016).

Despite being able to fly, hermit beetles show a low dispersion distance. In Sweden, *O. eremita* showed a limited dispersal rate and mean dispersal distances of less than 200m, with only 15% of the adults leaving their natal hollow tree (Ranius and Hedin 2001, Hedin and Ranius 2002, Ranius 2006, Hedin et al. 2008, Svensson et al. 2011). In France, Dubois and Vignon (2008) reported for one individual of *O. eremita* a total dispersal of 700m. The dispersal patterns of *O. eremita* were studied in a cork oak (*Quercus suber*) woodland in central Italy, the southernmost part of its distribution range which was characterised by a hot and dry Mediterranean climate (Chiari et al. 2013b). The dispersal activity of 39 individuals (16 males, 23 females) was followed by a 2-year radio-telemetric approach and the results showed that hermit beetles spent their lifetime not only inside hollow trees but also in other habitats, such as under the ground surface. The beetles were more active at the beginning of the season but 81% of males and 69% of females continued to disperse during their lifetime. The dispersal distance over their lifetime did not differ between sexes and the longest dispersal was performed by a female that travelled 1,504m, while 39% per cent of dispersing individuals reached distances further than 250m. Both the dispersal rate and range are higher than that which has been found in northern and central Europe. The population size per tree is low, most of the adults leaving their natal tree and a high predation rate (at least 28%) was observed (Chiari et al. 2013b).

Tethered flight experiments were conducted in the laboratory by Dubois et al. (2010) where a flight mill was used to measure the flying activity for each individual. These experiments revealed a maximum single flight distance of 1,454m and a maximum total flight distance of 2,361m, also demonstrating a higher flight capacity in females than males. Investigations on the factors that may affect flying activity, e.g. temperature, humidity or feeding on fresh fruit as sources of energy such as plums (which are rare or absent in forest ecosystems), should be carried out for assessing the potential flight capacity of the species.

As the daily activity of hermit beetles varies according to light, temperature and humidity, they have been recorded as active both day and night by different studies. In

Sweden, during a long term research project, the adults were only found active at day-time, with flying individuals mainly seen in the early afternoon on warm, sunny days (Ranius 2005); on the contrary, in central-southern European countries (Germany and France), they have been observed to be active at dawn (Stegner 2002, Schaffrath 2003a). In central Italy, a telemetric study showed that hermit beetles are active both day and night, over a span of 24 hours (Chiari et al. 2013b). In Valtellina (north Italy, ca 500m asl) the patrolling activity of males outside the hollows was mostly observed from 1400h to 1900h and flying specimens (both males and females) were also observed in the early afternoon of July and August in warm, sunny days, as in Sweden (P. Audisio, unpublished data). All these phenological data seem to suggest that the daily activity can shift towards the hours when the beetles find the most favourable climatic conditions, e.g. preferring the day-time in cool and humid areas but night-time in warm and dry ones.

Threats and conservation measures

Hermit beetles represent one of the most important "flagship" species and "umbrella" species for the protection of the local saproxylic communities (Ranius 2002a, 2002b, 2002c, Audisio et al. 2003, Carpaneto et al. 2015). In 1992, *O. eremita* was listed as a priority species in Annex II and IV of the EU's Habitats Directive (Luce 1996, 2001, Galante and Verdú 2000, Audisio et al. 2003, Ranius et al. 2005), when the systematic knowledge of the group was still very poor. Until the present time, the only *Osmoderma* species formally protected by the Habitats Directive is *O. eremita*, even though the need for conservation of all related taxa derived from its splitting was expressed by several authors and is provided by the Directive itself (Audisio et al. 2008, 2009, 2014, Carpaneto et al. 2015).

The conservation status and threats of the European hermit beetle species have been assessed for the IUCN Red List: *O. eremita* (Nieto et al. 2010), *O. cristinae* (Nardi and Micó 2010), *O. barnabita* (Alexander et al. 2010). An overview of the opinions, expressed by several authors, has led to the recognition of the following threats, listed in order of importance:

1. Decreasing number of hollow veteran trees (habitat trees) in forest ecosystems due to commercial management which was not suitable for the conservation of biological diversity;
2. Fragmentation of old-growth forests and lack of connection amongst the habitat trees;
3. Felling of chestnut orchards or their transformation to coppices;
4. Transformation or felling of ancient orchards with old fruit trees of the rose family (especially *Prunus*);
5. Abandonment of pollarding practices on willows, poplars, mulberry trees and other species across the rural landscape;

6. Removal of dead wood and old trees from forests or hedgerows for civic use such as domestic fuel;
7. Increasing use of old trees and dead wood in general as biomass for industrial fuel production;
8. The lack of entomologists involved in risk management assessment of dangerous old trees in urban parks and country roads for ensuring both human safety and biodiversity conservation;
9. Risk of pheromone use by insect dealers for international trade of rare species.

The long-term research conducted in Sweden, where hermit beetles live aggregated into large hollow oaks within small tree stands, concluded that there is a considerable extinction risk for many populations as they mainly rely on only one or a few trees with large amounts of wood mould and 10% of the hollow trees harbour two thirds of the individuals (Ranius 2007, Ranius et al. 2009). According to Vignon (2015), many chestnut groves in France have not been renewed since the late 19th century and the habitat transformation from chestnut orchards to coppices represents the following serious threats for hermit beetles: (1) loss of a suitable habitat, (2) barriers for dispersal because the species is not able to fly in dense coppices and (3) colder climatic conditions for a species whose development is favoured by the sun heating the trees. According to Dubois and Vignon (2015), hermit beetles are damaged by dense woodlands, mainly coppices, as they are unable to avoid twigs and fall to the ground thereby risking reproductive failure. Indeed, remarkable aggregations of hermit beetles have been observed in French orchards, especially on apple trees and in open parts of the agricultural landscape where they preferred pollarded oaks of hedgerows (Dubois et al. 2009). According to Oleksa et al. (2007), hermit beetles do not require old-growth forests to survive whereas, on the contrary, they seem to avoid forests with a dense canopy. Open woodlands or densely wooded pastures with old hollow trees, seem to be the optimum habitat type for these beetles, provided that renewal of trees is ensured over the years to balance veteran tree death. As a matter of fact, hermit beetles occupy anthropogenic habitats whenever suitable trees are present. These facts and other findings suggest that hollow veteran trees in ecotones as forest edge, along country roads or channels might play a crucial role in the preservation of this species, while changes in traditional structures of agricultural landscapes (e.g. the cutting down of old hedgerow trees) could result in a rapid extinction of the species at the local level. Further studies are needed to investigate the occurrence of hermit beetles in different types of habitats to judge how far the species is connected with anthropogenic habitats or whether such environments play only a secondary role. The protection of hermit beetles requires the preservation of sites with a network of suitable trees in space and time where natural forests and rural habitats occur together and any preservation strategy must plan the balancing of long-term processes for cavity creation and disappearance in order that suitable cavities are always present. Tree management for saproxylic conservation should bear in mind that the creation of a new cavity can take several tens to hundreds of years in trees with harder wood, such as oak or beech, but this process is very much faster in softer wood, such as that of limes, alders, poplars or willows. Only a few oaks and beeches, younger than 150 years, develop large hollows suitable for hermit beetles (Nilsson et al. 2001) while limes of only 70 years have been observed to possess cavities occupied by these beetles (Oleksa et al. 2007). The low dispersal ability of hermit beetles and their preference for open woodlands seem to be the major causes for the sensitivity of these animals to tree management and vulnerability to habitat alteration (Ranius and Hedin 2001, Dubois 2009, Dubois et al. 2009). Artificial cavities, i.e. boxes full of wood mould, can be a useful strategy for filling gaps in time and space and making a fragmented landscape more suitable for *O. eremita* (Jansson et al. 2009).

Review of monitoring experience in European countries

Until the present time, several methods have been used to study and monitor the *O. eremita* populations, mainly based on: (1) searching or capturing the adults by either pitfall and pheromone traps or visual survey and (2) wood mould sampling, searching for the remains of adults and frass or larvae in hollow trees. These methods are however often used in combination to achieve a greater sampling efficacy. In the following paragraphs, an overview is presented of the monitoring methods used for the different European countries on the genus *Osmoderma*.

Sweden

In this country, the standard method for the monitoring of *O. eremita* populations was developed by using traditional methods, like pitfall trapping and/or wood mould sampling (Ranius and Nilsson 1997, Ranius 2000, 2001, Ranius and Jansson 2002), as well as more innovative pheromone-based approaches (Larsson et al. 2003, Svensson et al. 2003, Larsson and Svensson 2009).

Ranius and Nilsson (1997) tested wood mould sampling on 135 trees for three years. Every year, the sampling was undertaken in September. From each hollow, tree samples of wood mould between 2-8 litres were extracted and carefully examined for larvae, fragments and frass. In order to obtain information on population size, Ranius (2000) suggested the use of the pitfall traps inside hollow trees, with the entrance hole wide enough, < 5m from the ground and the wood mould surface not too far from the entrance hole. The traps were empty jars with a top diameter of 7cm, positioned with the openings at a level with the wood mould surface. The traps needed to be checked every day or every two days and hermit beetles searched for in the hollows and on trunks in order to increase the probability of detecting the species (Ranius 2000, 2001). Each beetle was marked on the elytra with an insect needle in a drill. The optimum monitoring period was from the 2nd or 3rd week of July until the end of August (Ranius 2001). Furthermore, Ranius (2000) and Ranius and Jansson (2002) suggested carrying out the wood mould sampling before the survey with pitfall traps in order to set the traps in tree hollows where fragments have been found. The occurrence of fragments is an accurate indicator of the presence of the species, since there is a strong correlation between occurrence of fragments and of living adults and frass from larvae in hollow oaks (Ranius and Nilsson 1997). However, the above- mentioned methods were considered labour intensive and time consuming (Svensson et al. 2012) and can be applied only for trees with certain characteristics.

The identification of the peach-like, fruity odour of the hermit beetles as (*R*)-(+)-γ-decalactone and the identification of its function as a sex pheromone released by males to attract females, allowed the development of different methods useful for monitoring the species. Svensson et al. (2003) used the air sampling method. The air was sampled from the cavity by using Micro Diaphram Gas Sampling Pumps connected to a charcoal filter and extracts analysed using gas chromatography and mass spectrometry. However, this method is limited in that females cannot be detected and the mainly problematic aspect is the need for costly equipment (Svensson et al. 2003). The air sampling method can however be a useful complement to other methods when trying to detect as many trees housing this beetle as possible.

Svensson and Larsson (2008) for the first time tested the "custom built trap", also called the pheromone trap, baited with the pheromone of the species. They positioned 12-20 replicates of traps for three years. The traps were suspended from tree branches at 2-4m height and at least 10m apart. The traps would be checked every second day. They suggested undertaking the monitoring from the end of June until the end of August (Svensson and Larsson 2008). Pheromone traps outside trees and pitfall traps inside tree hollows, facilitate the mark-recapture of beetles (Larsson and Svensson 2009, Svensson et al. 2011) and the estimation of both population size and dispersal rate of the species (Ranius 2001, Ranius and Hedin 2001, Hedin et al. 2008, Svensson et al. 2011).

Furthermore, in 2016, a national monitoring programme was carried out in 73 sites, from 19 July until 1 August using 198 pheromone traps and 114 pitfall traps. The traps were checked every three days. During the sampling, 56 hermit beetles from 43 pheromone traps and 29 beetles from 12 pitfall traps were detected respectively (N. Janssons, pers. com.).

Finland

In 2012, in order to achieve a comprehensive assessment of the national distribution of *O. barnabita*, a pheromone-based plan was designed and implemented by volunteers in several urban parks and in all major oak woodlands in Finland (Landvik et al. 2015). The volunteers were amateur and professional entomologists, contacted by advertisement; the protocol and the sampling set (trap, bait etc.) were supplied by the project. The sampling was carried out from 2012 until 2014 in 52 sites, during the flight activity of the species from mid July to mid August, using 2-4 traps per site. Despite the massive sampling effort of more than 3,500 trap-days, *O. barnabita* was only detected in the Ruissalo Island (SW Finland). The presence of *O. barnabita* at the level of host trees was evaluated by a standardised method: the site was divided into squares of 250 x 250m and, in each square, the hollow trees were detected and analysed by sieving the wood mould from any remains of adults, frass and larvae of the species (Landvik et al. 2015). Signs of hermit beetles were detected in 62 trees out of 192 total examined (32%) and the most preferred host was pedunculate oak (*Quercus robur*), with additional occurrences in lime (*Tilia*) and alder (*Alnus*). Large trees were more frequently occupied than smaller ones, with an incidence of 90% on the largest oaks.

Norway

During the last 100 years, live specimens of the *O. eremita* had not been found in Norway and the species was presumed regionally extinct (category "Extinct?") (Ranius et al. 2005 and reference therein, Kalas et al. 2006). Surprisingly, during the summer 2008, the species was detected in the cemetery of Tonsberg, in the county of Vestfonl (Flaten and Fjellberg 2008). The monitoring project of hermit populations was carried out using different methods. In July 2008, four adults were detected by using visual encounter surveys while 14 larvae of third stage and frass were found inside the hollow trees by wood mould sampling. During August 2009, "sweep cameras", set inside hollow trees, were added to other methods (Sverdrup-Thygeson et al. 2010). While in 2010, the hermit beetles were monitored by using the sweep camera and pheromone traps and marked with labels. Sverdrup-Thygeson et al. (2010) recommended the development of a strategy to care for and conserve the veteran trees where the beetle population currently lived. Efforts are also necessary to establish new host trees for future generations by using artificial hollow trees. Hermit populations should be monitored annually to follow population trends, using both a visual search for adults in hot summer evenings during their mating season and a special "sweep camera" to see inside tree cavities (Sverdrup-Thygeson et al. 2010). Furthermore, the action plan provides some activities in order to familiarise the citizens with the species.

Poland

Despite the large number of occurence sites of hermit beetles in Poland (see Oleksa 2009) and several studies carried out (Oleksa and Gawronski 2006, Oleksa et al. 2006, 2007, 2012, Byk et al. 2013, Hilszczanski et al. 2014), a national monitoring protocol on hermit beetle populations has still not been proposed. Oleska (2009) suggested measures for conservation focused not only on veteran trees in open areas, along avenues or channels and in old-growth forests, but also on the entire landscape inhabited by populations of hermit beetles in Poland. Therefore, an action plan for the species (locally represented only by *O. barnabita*) should include three steps: (1) localisation of core areas using both target species and structure-based indicators, such as the presence of old trees; (2) strict protection of corridors in fragmented landscapes (trees in roadsides and canal banks), including management to keep some areas open, e.g. by grazing or cutting and (3) restoration of stepping stones between core areas, planting trees in places where the continuity of habitat has been disturbed, as well as other measures enhancing the hollow continuity in space and time e.g. promoting hollow creation by pollarding trees or fungal inoculation.

Germany

In southern Germany, where both *O. eremita* and *O. barnabita* are known to occur, the monitoring method proposed is the wood mould sampling by using the "vacuum cleaner method", used for the first time by Bußler and Müller (2008). A suction tube of a vacuum cleaner (Nilfisk Backuum battery) was introduced into the cavity, extracting each time 3.5 litres of wood mould. The sample was checked for frass, pellets, adults, larvae and remains of the hermit beetles. Sampling was undertaken on 127 tree cavities during 2006 and 2007, in eight Nature 2000 areas in southern and northern Bavaria. Hermit beetles were recorded in 39 tree cavities. In particular, larvae were recorded in 11 trees and remains in 30 samples. This method allows good results to be obtained but has a significant impact on the microhabitat structure and negatively affects the saproxylic communities.

France

In France, several monitoring programmes for hermit beetle populations were undertaken by using different methods separately and in combination: wood mould sampling (Vignon and Orabi 2003, Vignon et al. 2004, Dubois et al. 2009); pitfall traps and telemetry (Dubois and Vignon 2008); wood mould sampling, pitfall traps (Vignon 2008) and telemetry (Dubois and Vignon 2008, Vignon 2015, Le Gouar et al. 2015). In all studies, the authors suggested the creation of an inventory of suitable hollow trees by mapping them by GPS and recording the features of the trees (see Ranius 2001). The rate of occupancy of the species was detected by using the wood mould sampling method, searching for the larval faecal pellets, larval stages, cocoons and adult body parts inside the cavities. This method was mainly applied during the winter (Dubois and Vignon 2008). Furthermore, the authors suggested the capture of adults using pitfall traps set in the cavities, one or two for each tree, checked at least every two days and most frequently once a day. In Dubois and Vignon (2008) and Le Gouar (2015), the traps were visited once per day, five days per week from the beginning of July until the end of August, when the daily total number of captures fell below one capture per day (Le Gouar et al. 2015). In order to estimate the size of populations, the capture-recapture method was applied by marking the individuals with micro-perforations made in elytra with a thin drill, with a combination of six perforations. Furthermore, adults were also searched for on wood-mould surfaces or at the entry to the cavity and on trunks during the routine time of visits to the traps. In the recent work published by Vignon (2015), the results are reported of the long term monitoring studies conducted for 16 years (1997–2013) in the Sarthe department in chestnut (*Castanea sativa*) orchards and for 7 years (2001–2008) in the Orne department in hedgerow networks. The surveys were carried out using pitfall traps and radio telemetry for 950 days with 14 persons capturing 612 individuals. The most relevant result was that the adults emerged from the end of June until the end of August with an emergence peak between 10 and 15 July; a second emergence peak was observed between 25 and 29 July.

Romania

In Romania, the standard method for the monitoring of *Osmoderma* (locally represented only by *O. barnabita* sensu Audisio et al. 2007, 2008) is based on a combination of methods and spread temporally throughout the seasons in order to monitor the species in the best manner (Iorgu et al. 2015). The occurrence of the species was established by wood mould sampling and the materials in the hollow trees were sieved out on to a white sheet of 1m^2, to search for frass, adults, remnants and larvae. Iorgu et al. (2015) established the screening of the hollow trees on a standard area of 1ha (e.g. a transect 500m long and 20m wide). Suitable trees were geo-referenced by using a GPS. The time spent for searching in the standard area was almost 2 hours with two operators. The populations of hermit beetle can be monitored by using the pheromone traps with γ-decalactone. The monitoring should be carried out to find larvae throughout the seasons and to find the adults between July and August (Iorgu et al. 2015).

Austria

In Austria, the monitoring protocol on hermit beetle populations has still not been proposed, although *O. eremita* was considered a highly endangered species from past knowledge and the species was thought to have become extinct due to habitat loss (Ranius et al. 2005). In the northeastern part of Lower Austria, from 2008 to 2011, the occurrence of *O. eremita* (s.l.) was studied by using wood mould sampling, searching for frass, adults, remnants and larvae in the cavities (Straka 2009, 2011). This method was applied without destroying the microhabitat of the species. Fifteen new occurrence sites were found, mainly in relicts of the historical cultural landscape, such as castle parks, avenues with pollarded trees and only one population was found in natural oak wood. The species was recorded in 49 old trees (sometimes > 200 years old), frequently in lime (*Tilia cordata*) and willows (*Salix alba*, *Salix fragilis*, *S. x rubens*) and rarely in black popular (*Populus nigra*), pedunculate oak (*Quercus robur*) and appletree (*Malus domestica*) with diameters ranging from 45 to 260cm. The surveys were carried out during the whole year.

Switzerland

In 2004, a management plan for old trees was developed to protect saproxylic beetles in urban and periurban areas (Juillerat and Vögeli 2004). The main aim of the plan was to maintain and to improve the sites for an extended period in order to protect the species, evaluating suitable measures of forest management. The action plan suggested combined monitoring for each tree of relict and urban forests, for all the saproxylic beetles. The plan recommended citizen participation to improve the knowledge on the distribution of the species. The monitoring of *O. eremita* was carried out by searching for presence signs within the cavities of the wood mould. The samples of mould were sieved to isolate frass produced by larvae (>7mm), larval stages and remnants of adults; the dark brown droppings and many large fragments (>10mm) attested to the recent presence of the species. The plan suggested the best time to undertake this research was from July to October (Juillerat and Vögeli 2004).

Slovenia

In this country, the monitoring method for *O. eremita* (s.l.) populations was published for the first time by Vrezec et al. (2014). The surveys were carried out by using baited traps with a racemic mixture of pheromone. Each selected site was monitored using different numbers of traps, between 1 and 199, for about two weeks depending on the workers involved for each area and the characteristics of the study site. However, Vrezec et al. (2014) suggested to use 20 traps for each site and to check the traps once a week. The monitoring should be carried out during July which is considered the activity period of the species in Slovenia (Vrezec 2008).

Italy

In Italy, Campanaro et al. (2011) proposed the first method for the monitoring of *O. eremita* based on the capture-mark-recapture procedure. The authors suggested capturing individuals mainly by using the pitfall traps (PT: i.e. empty jars placed inside the cavity of hollow trees) as it resulted in efficient capture of adults and also because it was the cheapest method and traps could more easily be constructed; moreover, they suggested the use of black cross window traps (BCWT: i.e. two black plastic sheets attached to a plastic funnel leading down to a plastic bottle, baited with a racemic mixture of γ-decalactone, placed on the trunk and suspended from tree branches) mainly in woods without hollow trees and during preliminary investigations; finally, they suggested the avoidance of interception air traps (IAT: i.e. a transparent plastic shield with a plastic dust-funnel leading down to a plastic bottle baited with a racemic mixture of γ-decalactone, placed on the trunk and suspended from tree branches) as it was less efficient in capturing adults of *O. eremita.* Chiari et al. (2013a) found a similar result by comparing four independent capture methods for the monitoring of *O. eremita* in central Italy: BCWT, PT, IAT and VES, visual encounter surveys (i.e. searching adults by visual inspection of hollows and trunks). Indeed, the PT appeared the best method in terms of high detection probability, low cost and least time for construction, low sensitivity to damage and less time for checking and restarting in the field (Chiari et al. 2013a). Regarding the BCWT, Zauli et al. (2014), by using these traps as a capture method for monitoring adults of *O. eremita* in Central Italy, could draw attention to two populations of this species reduced to a minimum number of individuals and exposed to local extinction. As well as for the other European species belonging to the genus *Osmoderma*, the BCWT baited with a racemic mixture of γ-decalactone attracted (also for *O. cristinae)* an endemic species of Sicily, in south Italy, suggesting that these types of traps can also be used for the monitoring of this species (Zauli et al. 2016a). A further method, the wood mould sampling, performed by Chiari et al. (2012) in central Italy, was used to detect presence/absence of *O. eremita* (larvae, frass and remains of dead individuals) by examining wood mould samples taken from the tree cavities.

Regarding the setting of the traps in sampling areas, Campanaro et al. (2011) proposed the arrangement of the PT and BCWT along linear transects, at the nodes of a grid (in both cases at a distance between 30 and 50m) or alternatively, in a random manner. Chiari et al. (2013a) used the random manner, setting the traps on 116 hollow trees randomly selected. Regarding timing of sampling, both groups of researchers indicated that the monitoring of *O. eremita* should be undertaken during the peak of its activity, i.e. from the middle of June until the middle of August, respectively, at sea level in northern Italy (Campanaro et al. 2011) or at a hilly area in Central Italy (Chiari et al. 2013a). Campanaro et al. (2011) also suggested undertaking a minimum of six monitoring sessions. Trizzino et al. (2013) and Bologna et al. (2016) shared the suggestion for the monitoring method of *O. eremita* proposed by Campanaro et al. (2011) and Chiari et al. (2013a).

Methods

Three methods were selected at the beginning of the project (for details on the project, see Mason et al. 2015 and Carpaneto et al. 2017), for testing over a period of three years (from 2014 to 2016) in two macroareas: the Foreste Casentinesi (hereafter: FC) and the Parco Nazionale d'Abruzzo Lazio e Molise (hereafter: PA). Two methods, pheromone and pitfall traps which were tested during the project, are described below while the detailed description of the third method, the use of a trained dog, is reported in this issue with Mosconi et al. (2017).

A preparatory phase was conducted to select the sites and the period for monitoring of hermit beetle populations. Firstly, an analysis of previous data from literature and the study of collections of museums and private owners were carried out for an overview of the presence of the species and its phenology. Moreover, preparatory surveys were made during autumn-winter 2013-2014 in order to select the best suited sites in the two macroareas (FC and PA) for setting traps in the coming years (considering accessibility, presence of the species, forest structure etc.). During these surveys all potential suitable trees were mapped by GPS and the main features were recorded to create an inventory.

Pheromone Traps

Black cross window traps (hereafter: BCWT) were used, functioning as interception air traps baited with the pheromone produced by males of *O. eremita* (Svensson and Larsson 2008, Chiari et al. 2013a). They consisted of two black plastic sheets (25cm height x 30cm width, 3mm thickness), arranged in a cross and attached perpendicularly to a white plastic funnel (upper diameter 30cm, neck diameter 4cm) leading down to a 0.5 litres white plastic bottle (Figure 7). Traps were set on tree branches at a height of 2-4m (Figure 7B).

Figure 7. BCWT for capturing *O. eremita*. **A** Outline of a trap with an eppendorf vial containing the pheromone **B** positioning on tree branch **C** insertion of the funnel into the bottle, with details of hermit beetle captured by the trap (note the four small holes in the base to avoid water filling) **D** *O. eremita* with double marking procedure on elytra: dimples made by a small drill and a numbered sticker (photographs by E. Capogna: **A–B**, F. Bernardini: **C,** S. Chiari: **D**).

Dispensers for pheromone were 1.5ml plastic eppendorf vials loaded with 1200µl of the racemic mixture of γ-decalactone (catalogue no. W236004, Sigma-Aldrich, USA). Cut strings of cotton dental rolls (Celluron, Paul Hartmann, S.A., France) were inserted as wicks into the dispenser. The dispensers were attached to the traps with a metal hook (Figure 7A). Traps were checked every second day to avoid the death of beetles inside the bottle. Moreover, the plastic bottles were equipped with several small holes at the base to avoid water filling by rainfall and possible death of the beetles (Figure 7C). The traps were mapped by GPS (Garmin 60Csx) and then marked by a label with information on the Project and the code of the trap.

Pitfall Traps

Pitfall traps (hereafter: PT) were used to catch beetles walking on the wood mould surface inside the cavities. PT consisted of empty jars, with an opening diameter of around 7cm, placed inside the wood mould with the opening at the surface of the mould (for details see Campanaro et al. 2014, Chiari et al. 2013b). PT, mapped by using GPS and marked by a label with the code of the trap, were checked every second day.

Capture release method

At the first capture, the sex of each hermit beetle was determined by examining head and pronotum morphology. Afterwards, each beetle was marked on the elytra by a numeric code following a double procedure (Figure 7D): 1) by a small drill (Dremel Lithium Cordless 8000JE) and 2) applying a numbered sticker by a very small drop of cyanoacrylate glue (®Loctite, Super Attack Power Flex Gel; Henkel, Dusseldorf, Germany). No beetle was damaged by the marking procedure.

Sampling plan

Within the macroarea FC, trap sampling was undertaken in two sites: Foresta della Lama (FL) and Camaldoli (CA) which were dominated by beech and chestnut respectively. Within the macroarea PA, traps were set in three study sites: Difesa di Pescasseroli (DP), Val Fondillo (VF) and the Riserva Naturale Feudo Intramonti e Colle di Licco (FI). The first two areas were dominated by beech forests and the third area was occupied by oak forests (for other information on the study areas, see Carpaneto et al. 2017).

In 2014, trapping was only undertaken in the macroarea PA (in two sites, DP and VF), during July (Table 1) by using 15 replicates of three BCWT. Each replicate was set at least 100m from each other and contained: a single trap baited with two eppendorf vials with 600μl of (R)-enantiomer; a single trap baited with one eppendorf vial with 1200μl of racemic mixture and one empty trap (without pheromone) as control. Inside each replicate, traps were hung on tree branches at 2-4m height, at least 10m apart and they were checked for the presence of beetles every second day, for 5 surveys in total. The relative position of traps within each replicate was changed every two days.

These field experiments addressed the following questions: (i) are the sites suitable for the species? (ii) what is the most efficient marking technique for hermit beetle populations? (iii) does the use of 1200μl in one or two dispensers affect the efficacy of the method? (iv) is the method suitable for detecting the species and estimating relative abundance?

In 2015, trapping was conducted in both macroareas: PA (in three sites DP, VF and FI) and FC (in two sites, FL and CA). For each site, 10 BCWT and 10 PT were

Table 1. Sampling design used during the MIPP project. Summary of the sampling design followed during the study of *O. eremita* in three years. (PA= Parco Nazionale d'Abruzzo Lazio e Molise; FC= Foreste Casentinesi; VF = Val Fondillo; DP = Difesa di Pescasseroli; FI= Feudo Intramonti e Colle di Licco; CA= Camaldoli; FL = Foresta della Lama).

Macroarea	Site	Method		Study period	# Survey
PA	VF	45 BCWT	–	21/07-31/07/2014	5
	DP	45 BCWT	–	21/07-31/07/2014	5
PA	VF	10 BCWT	10 PT	15/07-28/08/2015	21
	DP	10 BCWT	10 PT	15/07-28/08/2015	21
	FI	10 BCWT	10 PT	15/07-28/08/2015	21
FC	CA	10 BCWT	10 PT	15/07-18/08/2015	14
	FL	10 BCWT	10 PT	15/07-18/08/2015	14
PA	DP	10 BCWT	10 PT	18/07-12/08/2016	12
FC	FL	10 BCWT	10 PT	18/07-12/08/2016	12

used. BCWT were positioned on tree branches at 2-4m height and baited with one eppendorf vial containing 1200µl of racemic mixture. PT were positioned inside the tree hollows amongst suitable trees which had previously been investigated in 2014. The distance between BCWT and PT was at least 100m. Traps were checked for the presence of beetles every second day, three times a week (Table 1). Four site covariates were recorded for each site in 10 plots of 24m radius (Area= 1808m^2) (Redolfi De Zan et al. 2014 a,b) around the tree where the BCWT were set: (1) trees number (TN); (2) mean circumference (MC); (3) holes number (HN) and (4) microhabitat amount (MA). Starting from the habitat suitable for *O. eremita*, the number of trees (old trees with hollows) with a circumference greater than or equal to 157cm at breast height (DBH) was recorded in the area. For each tree, the number of cavities and the number of "microhabitat type" identified were also recorded. Following Winter and Möller (2008), "microhabitat type" was used as a method with which it is possible to obtain information on the structure of the forest investigated and also to correlate the presence of microhabitat data with the presence of saproxylic species. Amongst the 20 microhabitat types identified, according to the scheme proposed by Winter and Möller (2008), only those most suitable for the presence of *O. eremita* were taken into account from MA04 to MA10 (see Winter and Möller 2008).

These field experiments addressed the following questions: (i) between BCWT and PT, what is the better method to detect hermit beetles? (ii) is the colour numbered sticker a reliable method to mark hermit beetles? (iii) what is the lower limit of population abundance for using the capture-release method? (iv) what is the minimum sampling effort (in terms of trap and survey number) for detecting the species? (v) do environmental variables of the plot have a relationship with the species occurrence?

In 2016, trapping was again conducted in both macroareas: PA (in two sites DP and VF) and in FC (in one site FL). For each site, 10 BCWT and 10 PT were used.

The BCWT were positioned on tree branches at 2-4m height and baited with one eppendorf vial containing 1200µl of racemic mixture. PT were positioned inside the tree hollows, previously selected amongst suitable trees investigated in 2014. The distance between BCWT and PT was at least 100m. Traps were checked for the presence of beetles every second day, for three times a week for a total of 12 surveys (Table1). These field experiments addressed the following questions: (i) what is the best method to detect hermit beetles? (ii) is the colour numbered sticker a reliable method to mark hermit beetles? (iii) what is the population abundance by using capture-release method?

Statistical analysis

For the dataset of 2014, the Yates corrected χ^2 test was applied to investigate differences between male and female captures. Analysis was performed with the programme R version 3.2.5 (R Core Team, 2016), using a significance level of 0.05 to reject the null hypothesis.

The large-scale occupancy probability (ψ) was firstly explored by condensing detections from each capture method and fitting single species, single season and multimethod models. Then, the detection probability estimate ($\hat{p}$) was calculated applying the single species, single season and multi-method models separately to each capture method (Nichols et al. 2008). Detection probability was modelled as constant over time and detection methods (p) as time-independent but different amongst methods (p^s), as time-dependent but constant amongst methods (p_t), or with the method as an additive effect with time (p_{s+t}). Small-scale occupancy was modelled as either time-independent (θ^t) or time-dependent (θ^t).

For the dataset of 2015, the Yates corrected χ^2 test was used to investigate differences between sampling methods and sex. Analysis was performed as for the 2014 dataset.

Occupancy models for single species, single season were used to calculate site occupancy (ψ), i.e. an estimate of the probability that a randomly selected site of the study area is occupied and the detection probability (p), i.e. the probability of detecting the species by the applied monitoring method (MacKenzie et al. 2006). These parameters were modelled to be constant (.) or to vary with time ($_t$) during the first and the last survey for each study area. The recommended survey effort for monitoring schemes was calculated by applying the general advice reported by MacKenzie and Royle (2005). In particular, the simplifying assumption was applied that selected values of ψ and p are constant across both space and time for a standard design where all sites (s) are surveyed an equal number of times (K) with all surveys having the same cost. Finally, a general situation was assumed where the study is designed with a precision objective (standard error of the estimated level of occupancy) of 0.10 to determine what values of s and K are the most efficient to achieve the study's objective, given the assumed values of ψ and p. The assumed values of ψ and p were the means obtained from the four study sites where the species turned out to be more common (DP, VF, FI, FL) and the other two sites where the species turned out to be less common, both from the Lazio region: Monte Soratte (F. Bernardini, unpublished master's thesis) and Forcella Buana (Chiari et al. 2013a).

Craig's model for closed populations (Craig 1953) was used to estimate the population size for each study area. The closed population model was used due to the low number of recaptures. The Coefficient Variation (CV) was calculated as the standard error divided by the number of individuals estimated and indicates the precision of the population size estimate. Population size estimates for each study area were generated with male and female data pooled together.

The species habitat relationship was analysed pooling data from all the study areas. A set of *a priori* models were defined with the covariates varying in a way that could explain patterns of trap occupancy. Models with single, additive and multiplicative effects of site covariates (HN, MC, TN, MA) were hypothesised.

Occupancy models, for both 2014 and 2015 data analysis, were fitted and maximum-likelihood estimates were obtained using the programme PRESENCE (Hines and MacKenzie 2004). The Akaike Information Criterion (AIC) model selection procedure was used to rank the models and model averaging AIC weights (w) of candidate models ($\Delta AIC < 2$) was used to obtain a weighted average of the individual parameter estimates (Burnham and Anderson 2002).

Results

In 2014, a total of 17 captures of 15 individuals and 8 captures of 8 individuals of *O. eremita* were made in VF and DP respectively (Table 2). The female captures outnumbered male captures ($\chi^2_1 = 6.76$, $P = 0.01$). Only one male and one female were recaptured, both in VF and only once.

In both sites, the estimated large-scale probability of *O. eremita* occupancy was high, but considerably uncertain (VF $\psi = 0.55$, SE = 0.18; DP $\psi = 0.80$, SE = 0.57). Model selection statistics provided strong evidence that occupancy probabilities, in both sites, were firstly influenced by the sampling method (s) (VF $w_s = 0.98$; DP $w_s = 0.96$) (Table 3). The BCWT baited with one dispenser containing 1200µl of γ-decalactone of neat racemic mixture, performed better than those baited with two dispensers of 600µl or controls in detecting the presence of *O. eremita* in both sites (Table 4).

In 2015, in total 116 capture events of 105 individuals were registered by BCWT (Table 5). No capture events were recorded by PT. Females were significantly more abundant than males (Yates corrected $\chi^2 = 63.43$, df = 1, $P < 0.01$). Individuals were collected in all the study areas in a time interval between 14 July and 28 August (Figure 8).

The mean value amongst macroareas for ψ was 0.98 (SE = 0.03) and for p it was 0.10 (SE = 0.04) (Table 6).

For selection of a standard error for the estimated level of occupancy of 0.10, the most efficient allocation of resources is to use 30 BCWT, each checked 23 times (690 total number of surveys).

Amongst the marked beetles, not one lost the colour-numbered sticker.

Table 2. Dataset of mark recapture during 2014. Summary of capture-mark-recapture data of *O. eremita* obtained during 2014 in PA (PA = Parco Nazionale d'Abruzzo Lazio e Molise; VF = Val Fondillo; DP = Difesa di Pescasseroli).

Site	Sex	Marked	Captures		
			control	one dispenser	two dispensers
VF	male	4	0	3	2
	female	11	0	11	1
DP	male	1	0	1	0
	female	7	0	4	3

Table 3. Occupancy probabilities model selection. Summary of the model selection statistics for the study of *O. eremita* during 2014 in PA (PA = Parco Nazionale d'Abruzzo Lazio e Molise; VF = Val Fondillo; DP = Difesa di Pescasseroli).

Site	Model	*K**	-2Log (*L*)	ΔAIC	*w*
VF	ψ, θ^t, p_{s+t}	9	61.54	0.00	0.55
	ψ, θ^t, p^s	9	62.05	0.51	0.43
DP	ψ, θ^t, p_{s+t}	9	42.99	0.00	0.52
	ψ, θ^t, p^s	9	43.48	0.49	0.41

**K* represents the number of parameters in the model and -2Log (*L*) is twice the negative log-likelihood value. Small sample Akaike Information Criteria (AIC) were calculated for each model. Relative AIC values (ΔAIC = the difference in AICc value relative to the top model, Burnham and Anderson 2002) and Akaike weight (*w*), are reported for each model. Detection probabilities may vary amongst method (s) or sampling occasion (t).

Table 4. Detection probabilities estimates by monitoring during 2014. *O. eremita* detection probability estimates ($\hat{p}$) and associated standard errors (in parenthesis) are given for the plausible candidate models (ΔAIC < 2) (w = Akaike's weight for each model), during 2014 in PA (PA = Parco Nazionale d'Abruzzo Lazio e Molise; VF = Val Fondillo; DP = Difesa di Pescasseroli).

Site	Model	w	$\hat{p}_{control}$	(SE)	$\hat{p}_{one\ disp.}$	(SE)	$\hat{p}_{two\ disp.}$	(SE)
VF	ψ, θ^t, p_{s+t}	0.55	0.00*	(0.00)	0.34*	(0.16)	0.10*	(0.08)
	ψ, θ^t, p^s	0.43	0.00	(0.00)	0.67	(0.27)	0.20	(0.13)
DP	ψ, θ^t, p_{s+t}	0.52	0.00*	(0.00)	0.21*	(0.14)	0.07*	(0.07)
	ψ, θ^t, p^s	0.41	0.00	(0.00)	0.50	(0.35)	0.17	(0.15)

* Parameter estimate reported is the mean value amongst the 5 detection probability estimates for each detection method (control = BCWT with no bait; 1 disp. = BCWT baited with one dispenser with 1200µl of γ-decalactone neat racemic mixture; 2 disp. = BCWT baited with two dispensers with 600µl of γ-decalactone neat racemic mixture in each one).

The most abundant population was the one for VF 304±104 (C.V. 0.34), the less abundant being that of FL 22±5 (C.V. 0.23) (Table 7).

Trap occupancy was favoured by the additive effect of the hole number (w_{HN} = 0.72) and microhabitat amount (w_{MA} = 0.38) (Table 8). Increasing the HN and MT increased the trap occupancy (Figure 9).

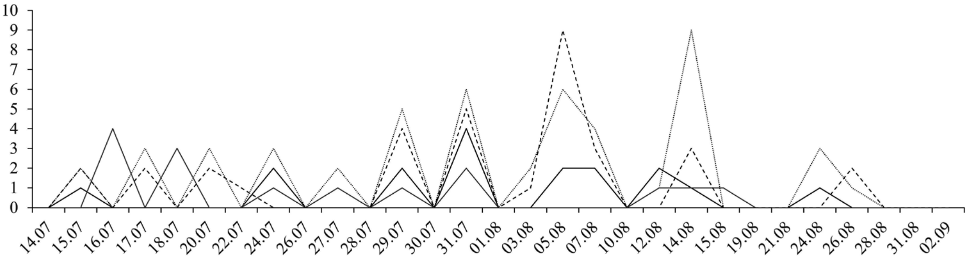

Figure 8. Time interval of capture events during 2015. Capture events of *O. eremita* during the MIPP project monitoring in 2015 in four Italian study sites (grey line = Foresta della Lama; black line = Feudo Intramonti e Colle di Licco; black dashed line = Val Fondillo; black dotted line = Difesa di Pescasseroli).

Table 5. Dataset of mark recapture during 2015. Summary of mark-recapture data obtained during the study of *O. eremita* in Italy with different capture methods during 2015 in the two macroareas PA and FC (BCWT = Black Cross Windows Traps; PT = Pitfall Traps; PA= Parco Nazionale d'Abruzzo Lazio e Molise; FC = Foreste Casentinesi; VF = Val Fondillo; DP = Difesa di Pescasseroli; FI = Feudo Intramonti e Colle di Licco; CA = Camaldoli; FL = Foresta della Lama).

Site	BCWT		PT	
	Marked beetles (♀/♂)	**Recapture events (♀/♂)**	**Marked beetles (♀/♂)**	**Recapture events (♀/♂)**
VF	31/3	2/0	0/0	0/0
DP	37/5	6/0	0/0	0/0
FI	12/1	4/0	0/0	0/0
CA	2/0	0/0	0/0	0/0
FL	8/2	3/0	0/0	0/0

Table 6. Occupancy and detection probability estimates by monitoring during 2015. *O.eremita* occupancy (ψ) and detection (*p*) probability estimates and associated standard errors (in parenthesis), are given for plausible candidate models (within 2 ΔAIC of the top model). The data were obtained by monitoring during 2015 in the two macroareas PA and FC (PA= Parco Nazionale d'Abruzzo Lazio e Molise; FC = Foreste Casentinesi; VF = Val Fondillo; DP = Difesa di Pescasseroli; FI = Feudo Intramonti e Colle di Licco; FL = Foresta della Lama).

Site	Model	*K**	-2Log (*L*)	ΔAIC	*w*	ψ	SE	*p*	SE
VF	ψ(.), *p*(.)	2	167.93	0.00	1.00	0.93	0.10	0.15	0.03
DP	ψ(.), *p*(.)	2	150.84	0.00	1.00	1.00	0.00	0.09	0.07
FI	ψ(.), *p*(.)	2	108.07	0.00	0.99	1.00	0.00	0.07	0.02
FL	ψ(.), *p*(.)	2	94.95	0.00	1.00	1.00	0.00	0.09	0.02

**K* represents the number of parameters in the model and –2Log (*L*) is twice the negative log-likelihood value. Relative Akaike Information Criteria (ΔAIC), i.e. the difference in AIC value relative to the top model modified for overdispersion (Burnham and Anderson 2002) and Akaike weight, *w*, are reported for each model. Both ψ and *p* were constant (.) within the time interval between the first and last survey of each study area.

In 2016, in total 32 captures of 31 individuals and 11 captures of 10 individuals of O. eremita were made, all by BCWT in PA and FC respectively (Table 9). No capture events were made by PT. Only two females were recaptured, one in DP and one in FL.

Table 7. Estimation of population size. *O. eremita* population size estimates obtained by monitoring during 2015 in the two macroareas PA and FC (PA= Parco Nazionale d'Abruzzo Lazio e Molise; FC = Foreste Casentinesi; VF = Val Fondillo; DP = Difesa di Pescasseroli; FI = Feudo Intramonti e Colle di Licco; FL = Foresta della Lama).

Site	Population size estimate	±95 % confidence interval	Coefficient of variation
VF	304	104	0.34
DP	173	45	0.26
FI	22	5	0.23
FL	19	2	0.10

Table 8. Dataset of environmental variables. Environmental characteristics (within 2 ΔAIC from the top model) affecting the occurrence of *O. eremita* in BCWT. The data were obtained during 2015 in the two macroareas PA and FC (PA= Parco Nazionale d'Abruzzo Lazio e Molise; FC = Parco Nazionale delle Foreste Casentinesi).

Model	*K*	-2Log (*L*)	ΔAIC	*w*
$\psi(_{\mathrm{HN}}), p(_t)$	18	492.66	0.00	0.50
$\psi(_{\mathrm{HN+MA}}), p(_t)$	19	492.29	1.63	0.22

*Occupancy (ψ) and detection (*p*) probabilities are considered. HN = holes number; MA = microhabitats amount. *K* represents the number of parameters in the model, *w* the Akiake weight and –2Log (*L*) is twice the negative log-likelihood value. Akaike Information Criteria (ΔAIC) were calculated for each model (Burnham and Anderson 2002).

Table 9. Dataset of mark-recapture during 2016. Summary of mark-recapture data obtained during the study of *O. eremita* in Italy with different capture methods during 2016 in the two macroareas, PA and FC (BCWT = Black Cross Windows Traps; PT = Pitfall Traps; PA= Parco Nazionale d'Abruzzo Lazio e Molise; FC = Parco Nazionale delle Foreste Casentinesi; DP = Difesa di Pescasseroli; FL = Foresta della Lama).

Site	BCWT		PT	
	Marked beetles (♀/♂)	Recapture events (♀/♂)	Marked beetles (♀/♂)	Recapture events (♀/♂)
DP	22/9	1/0	0/0	0/0
FL	5/5	1/0	0/0	0/0

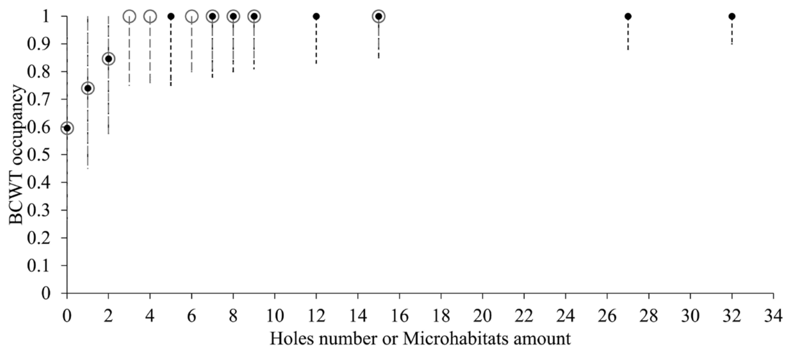

Figure 9. BCWT occupancy data. BCWT occupancy of *O. eremita* during 2015 in relation to the number of holes (grey empty circles) and microhabitat amount (black dots). Vertical bars indicate the standard error.

Discussion

The standard method for the monitoring of *O. eremita*

Comparison of the results obtained from all methods allowed the best standard method for the monitoring of *O. eremita* to be selected. The analysis of the total number of hermit beetles captured by the two trap types during the sampling of 2015 and 2016 in the two macroareas (FC and PA) showed that only BCWT captured the beetles. The use of PT provided zero captures in this research, notwithstanding this method was successfully used in different environmental contexts, such as in Sweden and France (Ranius 2000, 2001, 2007, Vignon 2015) until the identification of the sex pheromone of *O. eremita* and its now consolidated use as bait for BCWT (Larsson et al. 2003, Larsson and Svensson 2009, 2011). In the past years, many researchers chose to use a combination of BCWT and PT, as the first method is able to capture a higher number of individuals (with a high prevalence of females), while the second is able to capture a high number of males (Ranius 2001, Ranius and Hedin 2001, Svensson et al. 2011, Chiari et al. 2013a). Previous research in central Italy (Chiari et al. 2013a) reported that PT can be considered a good and cheap method, but it required much field effort and time in terms of setting and checking traps. In fact, PT was successful to a limited extent in Italy when a very high number of traps was used, as reported in Chiari et al. (2013a), 116 PT being used and checked every second day from the middle of June to the first week of August 2009, obtaining only 19 individuals. This low number of individuals, despite the high field effort and the 0 value obtained by the MIPP sampling (present paper) is probably due to the low density of Italian populations in the southern part of the hermit beetle geographic range and the reduced volume of tree hollows (associated with the young age of trees). In Sweden, where the habitat conditions are more favourable for the species (older trees and larger hollows with plenty of wood mould), the number of hermit beetles collected by PT is usually higher (Ranius et al. 2005).

These results suggested that, to carry out monitoring of a southern population of the hermit beetle (at least for the Apennine range), a number of 30 BCWT checked every second day for eight weeks (from early July to the end of August) is the most efficient method; traps can also be placed on trees without hollows due to the high attraction of the pheromone on the species. The activity period can vary according to altitude and latitude, other than seasonal weather conditions and then the sampling period for monitoring could be shorter where there is a greater knowledge of the life cycle of the species at local level (e.g. Figure 8). If no direct information is available on the activity period at local level, a preliminary study should be conducted in the previous year to obtain information. The BCWT showed differences in capture of the sexes, with a significant bias towards females (Tables 2, 5 and 9). Such strong attraction on females may produce a disturbing effect on breeding activity and behaviour, with the risk of reducing the birth rate of the local population. For this reason, precautionary measures should be taken even if the cause and effect relationships between disturbance by pheromone and population density of hermit beetles are not fully established scientifically. The authors' advice is to conduct BCWT monitoring every three years (one year of sampling separated by two years without sampling).

A high number of PT (at least 150) placed in the same number of tree cavities (preferably one trap per tree) may be used as an alternative method. In fact, this method is cheaper and less invasive (due to the breeding activities by pheromones not being disturbed), but may be used only in areas where: 1) tree hollows are abundant, large enough and with sufficient amounts of wood mould to set pitfall traps and 2) the team is composed of several people in order to ensure the checking of so many traps every two days.

According to N. Jansson (pers. com.), the bottom of the traps (both BCWT and PT) should be lined with wet moss, in order to reduce temperature, drought, stress and energy consumption of the beetles captured.

Protocol, materials and equipment

The first step in carrying out the monitoring plan is to choose a suitable area inhabited by *O. eremita*. Ideally, an area should be investigated in detail, in order to map the hollow tree by GPS, to take photographs and to record the characteristics of the trees (e.g. species, size, DBH, height, number of hollows etc.). This information, gathered in the field, will be useful for setting the traps. Afterwards, the BCWT would be built during the spring, before starting the monitoring. (For details and materials about the BCWT, see Methods and supplementary file 1.) The traps are set in the field at least a week before starting the sampling, without both pheromone and plastic collecting jars. The BCWT could be positioned randomly in a woodland or along a transect (e.g. along tree-lined streets, rows of pollarded trees bordering rivers and channels etc.) or in a grid (e.g. 5 rows with 6 traps in each one; the size of the grid is about 500m long and 600m wide), setting the traps at least 100m from each other. Every trap should be mapped by GPS and marked by a label with its number.

The monitoring can be carried out by two operators checking the traps every two days for 8 weeks, from July to September. The surveys can be conducted in two different ways:

Three times a week (Monday, Wednesday and Friday), the traps can be activated the day before starting the check (Sunday) and deactivated after the third weekly check, on Friday. The BCWT is activated when the caps of the plastic collection jar are removed and the eppendorf vial with pheromone is baited; in the opposite case, deactivation means that the collection jar is closed and the eppendorf vial has been removed. The activation and deactivation of the traps is crucial to avoid the death of individuals.

Every two days without any breaks, continuously; the traps can be activated the day before to start the check and never deactivated until the end of sampling.

The plastic eppendorf vials (capacity of 1.5ml) loaded with 1200μl of the racemic mixture of γ-decalactone can be prepared before starting the sampling and preserved in a freezer. It is suggested that the pheromone vials be changed once a week.

The protocol requires the presence of two operators who should fill the field sheet of each survey with the date, the weather conditions (rain and cloud) and the starting time (see supplementary file 2). After that, the operators check the traps and, once an adult is found, it is temporarily placed into the plastic jar with the cap to prevent escape. Once the check of BCWT has been completed, the number of individuals collected is counted, specifying the number of males and females for each trap in the field sheet (see supplementary file 2). Afterwards, the beetles were released in the same hollow tree from where they were captured. At the end of each survey, the operators recorded the weather conditions (rain and cloud) and the finishing time (see supplementary file 2).

The equipment required is: GPS, clipbord, field sheet, pencil, clock, plastic eppendorf vial with pheromone, cotton dental rolls, tweezers and camera.

Constraints, spatial validity and possible interferences

O. eremita is a species with a low dispersal rate. In fact, the majority of the individuals can move only a few hundred metres (from 100m to 250m), while some have a dispersal rate exceeding 2000m, as reported in several studies summarised by Chiari et al. 2013b. The dispersal rate seems to vary with latitude from southern to northern Europe, in relation to the mean temperatures and to the characteristics of the habitat (Larsson and Svensson 2009, 2011, Ranius et al. 2011, Chiari et al. 2013b). This means that the spatial validity of the method must be evaluated in relation to the local dispersal rate for different populations.

The major risk associated with the use of the BCWT is related to adverse weather conditions; in fact, in case of heavy rains, the plastic collection bottle, despite the presence of drainage holes, may be filled by water, resulting in an obvious danger to the specimens trapped inside. In case of heavy rainfall, it is recommended to increase the rate of checks, verifying daily if there are specimens inside the traps and emptying water from the collection chambers and from any material that can clog the holes (e.g. leaves).

Finally, *O. eremita* is a rare and elusive species, very sought after by collectors and the specimens trapped inside BCWT may represent easy prey for them. Thus, to avoid risks related to unauthorised collections, it is recommended to set the traps on higher branches with the help of an extension, so that the traps are not easily accessible.

Counting, quantification and data sharing

In order to identify the population trend for *O. eremita* at local level during the years, a reference value can be calculated as follows:

1. For each survey, *sum* the total number of individuals (males + females) captured by all 30 BCWT.
2. Calculate the *mean value* of the number of individuals from all surveys, excluding the survey with the lowest number. Removing the lowest number,

as proposed for other insect species (FLA 2014, Hardersen and Toni 2015), allows the elimination of eventual outlier values due to adverse climatic conditions (e.g. low temperature and/or rainfall) or other factors, that may affect the activity of the individuals and increase data heterogeneity.

Table 10 reports an example of calculation of the mean value obtained for identifying a population trend. This value allows the comparison of long-term data from the same location and to identify a population trend.

Table 10. Example of calculation of mean value of captured beetles. Subset of traps and surveys used for calculation of the reference value of the monitoring trend of *O. eremita* population in one site (BCWT: Black Cross Window Trap).

Survey	BCWT 1	BCWT 2	BCWT 3	BCWT 4	BCWT 5	BCWT 6	BCWT 7	Total
1	1	2	4	3	2	3	1	16
2	3	6	5	5	6	3	3	31
3	3	5	7	7	7	4	5	38
4	2	4	3	2	2	0	0	13
5	4	7	8	5	3	5	4	36
Average value for the four counts with the highest average total								**30.25**

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References

Ahrens D (2006) The phylogeny of Sericini and their position within the Scarabaeidae based on morphological characters (Coleoptera: Scarabaeidae). Systematic Entomology 31: 113–144. https://doi.org/10.1111/j.1365-3113.2005.00307.x

Ahrens D, Schwarzer J, Vogler AP (2014) The evolution of scarab beetles tracks the sequential rise of angiosperms and mammals. Proceedings of the Royal Society B 281: 1470. https://doi.org/10.1098/rspb.2014.1470

Alexander K, Buche B, Dodelin B, Schlaghamersky J (2010) *Osmoderma barnabita*. The IUCN Red List of Threatened Species 2010: e.T157901A5169119.

Audisio P, Ballerio A, Carpaneto GM, Antonini G, Mancini E, Coletti G, Piattella E, De Biase A (2003) *Osmoderma eremita* s.l. in Europa meridionale: stato delle conoscenze e problemi di conservazione (Coleoptera, Cetoniidae). In: Mason F, Nardi G, Tisato M (Eds) Atti del Simposio Internazionale Legno morto: una chiave per la biodiversità / Proceedings of the International Symposium Dead wood: a key to biodiversity (Mantova, 29–31 May 2003). Sherwood 95, 57–60.

Audisio P, Brustel H, Carpaneto GM, Coletti G, Mancini E, Piattella E, Trizzino M, Dutto M, Antonini G, De Biase A (2007) Updating the taxonomy and distribution of the European *Osmoderma*, and strategies for their conservation (Coleoptera, Scarabaeidae, Cetoniinae). Fragmenta Entomologica 39: 273–290. https://doi.org/10.4081/fe.2007.124

Audisio P, Brustel H, Carpaneto GM, Coletti G, Mancini E, Trizzino M, Antonini G, De Biase A (2009) Data on molecular taxonomy and genetic diversification of the European Hermit beetles, a species-complex of endangered insects (Coleoptera: Scarabaeidae, Cetoniinae, *Osmoderma*). Journal of Zoological Systematics and Evolutionary Research 47: 88–95. https://doi.org/10.1111/j.1439-0469.2008.00475.x

Audisio P, Baviera C, Carpaneto GM, Biscaccianti AB, Battistoni A, Teofili C, Rondinini C (2014) Lista Rossa IUCN dei Coleotteri saproxilici Italiani. Comitato Italiano IUCN e

Ministero dell'Ambiente e della Tutela del Territorio e del Mare, Roma, 132 pp. http://www.iucn.it/classe-insecta.php.

Ballerio A, Rey A, Uliana M, Rastelli M, Rastelli S, Romano M, Colacurcio L (2010) Coleotteri Scarabeoidei d'Italia. Marco Serra Tarantola Editore, Rodegno Saiano.

Balthasar V (1963) Monographie der Scarabaeidae und Aphodiidae der Paläarktischen und Orientalischen Region (Coleoptera: Lamellicornia). Band 1. Tschechoslowak Akademie der Wissenschaften, Praha, 287 pp.

Baratelli D (2004) Note sulla presenza di *Osmoderma eremita* Scopoli, 1763, in un biotopo umido prealpino, e interventi gestionali mirati alla conservazione della specie. Bollettino della Società Ticinese di Scienze Naturali 92: 83–90.

Baraud J (1977) Coléoptères Scarabaeoidea. Faune de l'Europe occidentale: Belgique, France, Grande-Bretagne, Italie, Péninsule ibérique. Supplément à la Nouvelle Revue d'Entomologie 7: 1–352.

Baraud J (1992) Coléoptères Scarabaeoidea d'Europe. Fédération française des Sociétés de Sciences naturelles, Paris, & Société linnéenne de Lyon, 873 pp.

Bărbuceanu D, Niculescu M, Boruz V, Niculescu L, Stoleriu C, Ursu A (2015) Protected saproxylic Coleoptera in "the Forests in the southern part of the Cândeşti Piedmont", a Romanian Natura 2000 Protected Area.

Barclay MVL (2007) Comment on the proposed conservation of the generic names *Gnorimus* Le Peletier de Saint-Fargeau & Serville, 1828 and *Osmoderma* Le Peletier de Saint-Fargeau & Serville, 1828 (Insecta, Coleoptera) (Case 3349; BZN 63: 177–183). Bulletin of Zoological Nomenclature 64: 122.

Bezborodov VG (2016) The Genus *Osmoderma* (Coleoptera, Scarabaeidae, Trichiinae) in Siberia and the Russian Far East. Entomological Review 95: 1088–1098. https://doi.org/10.1134/S0013873815080151

Bologna MA, Rovelli V, Zapparoli M (2016) *Osmoderma eremita* (Scopoli, 1763) (Scarabeo eremita), *O. italicum* Sparacio, 2000, *O. cristinae* Sparacio, 1994. In: Stoch F, Genovesi P (Ed.) Manuali per il monitoraggio di specie e habitat di interesse comunitario (Direttiva 92/43/CEE) in Italia: specie animali. ISPRA, Serie Manuali e linee guida, 141/2016, 64–65.

Bouchard P, Bousquet Y, Davies AE, Alonso-Zarazaga MA, Lawrence JF, Lyal CHC, Newton AF, Reid CAM, Schmitt M, Slipinski SA, Smith ABT (2011) Family-group names in Coleoptera (Insecta). ZooKeys 88: 1–972. https://doi.org/10.3897/zookeys.88.807

Boving AG, Craighead FC (1931) An illustrated synopsis of the principal larval forms of the Order Coleoptera. Brooklyn Entomological Society, New York, 351 pp.

Brandmayr P, Casale A, Puzzo F, Scalercio S (2005) Analisi cronogeonemica: alcuni esempi con riferimento alle specie della fauna italiana incluse nella Direttiva Habitat. In: Ruffo S, Stoch F (Eds) Checklist e distribuzione della fauna italiana. Memorie del Museo Civico di Storia Naturale di Verona (2 serie), Sezione Scienza della Vita 16: 41–45.

Britton EB (1956) Coleoptera Scarabaeoidea. Handbooks for the Identification of British Insects 5: 1–29.

Browne DJ, Scholtz CH (1995) Phylogeny of the families of Scarabaeoidea (Coleoptera) based on characters of the hindwing articulation, hindwing base and wing venation. Systematic Entomology 20: 145–173. https://doi.org/10.1111/j.1365-3113.1995.tb00089.x

Browne DJ, Scholtz CH (1998) Evolution of the scarab hindwing articulation and wing base: a contribution toward the phylogeny of the Scarabaeidae (Scarabaeoidea: Coleoptera). Systematic Entomology 23: 307–326. https://doi.org/10.1046/j.1365-3113.1998.00059.x

Browne DJ, Scholtz CH (1999) A phylogeny of the families of Scarabaeoidea (Coleoptera). Systematic Entomology 24: 51–84. https://doi.org/10.1046/j.1365-3113.1999.00067.x

Burnham KP, Anderson DR (2002) Model selection and multimodel inference: a practical information–theoretic approach. Springer, Berlin, 488 pp. https://doi.org/10.1007/b97636

Bußler H, Müller J (2009) Vacuum cleaning for conservationists: a new method for inventory of *Osmoderma eremita* (Scop., 1763)(Coleoptera: Scarabaeidae) and other inhabitants of hollow trees in Natura 2000 areas. Journal of Insect Conservation 13: 355–359. https://doi.org/10.1007/s10841-008-9171-4

Byk A, Borowski J, Mazur S, Mokrzycki T, Rutkiewicz A (2013) Waloryzacja lasów Leśnego Kompleksu Promocyjnego" Lasy Spalsko-Rogowskie" na podstawie struktury zgrupowań chrząszczy saproksylicznych. Studia i Materiały Centrum Edukacji Przyrodniczo-Leśnej 15: 35.

Campanaro A, Bardiani M, Spada L, Carnevali L, Montalto F, Antonini G, Mason F, Audisio P (2011) Linee guida per il monitoraggio e la conservazione dell'entomofauna saproxilica. Quaderni Conservazione Habitat 6, Cierre Grafica (Verona, 1–8. [CD-ROM]

Carlsson S, Bergman K-O, Jansson N, Ranius T, Milberg P (2016) Boxing for biodiversity: evaluation of an artificially created decaying wood habitat. Biodiversity and Conservation 25: 393–405. https://doi.org/10.1007/s10531-016-1057-2

Carpaneto GM, Piattella E, Valerio L (2005) Insecta Coleoptera Scarabaeoidea. In: Ruffo S, Stoch F (Eds) Checklist e distribuzione della fauna italiana. Memorie del Museo Civico di Storia Naturale di Verona (2 serie), Sezione Scienza della Vita 16, 193–197.

Carpaneto GM, Mazziotta A, Coletti G, Luiselli L, Audisio P (2010) Conflict between insect conservation and public safety: the case study of a saproxylic beetle (*Osmoderma eremita*) in urban parks. Journal of Insect Conservation 14: 555–565. https://doi.org/10.1007/s10841-010-9283-5

Carpaneto GM, Baviera C, Biscaccianti AB, Brandmayr P, Mazzei A, Mason F, Battistoni A, Teofili C, Rondinini C, Fattorini S, Audisio P (2015) A Red List of Italian Saproxylic Beetles: taxonomic overview, ecological features and conservation issues (Coleoptera). Fragmenta entomologica 47: 53–126.

Carpaneto GM, Campanaro A, Hardersen S, Audisio P, Bologna MA, Roversi PF, Sabbatini Peverieri G, Mason F (2017) The LIFE Project "Monitoring of insects with public participation" (MIPP): aims, methods and conclusions. In: Carpaneto GM, Audisio P, Bologna MA, Roversi PF, Mason F (Eds) Guidelines for the Monitoring of the Saproxylic Beetles protected in Europe. Nature Conservation 20: 1–35. https://doi.org/10.3897/natureconservation.35.12761

Cerretti P (2008) *Osmoderma eremita*. In: Manuale di indirizzo per la gestione delle aree tutelate del Friuli Venezia Giulia, Progetto "SARA Sistema Aree Regionali Ambientali - Costituzione del Sistema regionale delle aree naturali". Allegato B. http://www.regione.fvg.it/rafvg/cms/RAFVG/

Chiari S, Carpaneto GM, Zauli A, Marini L, Audisio P, Ranius T (2012) Habitat of an endangered saproxylic beetle, *Osmoderma eremita*, in Mediterranean woodlands. Ecoscience 19: 299–307. https://doi.org/10.2980/19-4-3505

Chiari S, Zauli A, Mazziotta A, Luiselli L, Audisio P, Carpaneto GM (2013a) Surveying an endangered saproxylic beetle, *Osmoderma eremita*, in Mediterranean woodlands: a comparison between different capture methods. Journal of Insect Conservation 17: 171–181. https://doi.org/10.1007/s10841-012-9495-y

Chiari S, Carpaneto GM, Zauli A, Zirpoli GM, Audisio P, Ranius T (2013b) Dispersal patterns of an endangered saproxylic beetle, *Osmoderma eremita*, in Mediterranean woodlands. Insect Conservation and Diversity 6: 309–318. https://doi.org/10.1111/j.1752-4598.2012.00215.x

Chiari S, Zauli A, Audisio PA, Carpaneto GM (2014) Interactions between larvae of the threatened saproxylic beetle *Osmoderma eremita* and other flower chafers in Mediterranean woodlands: Implications for conservation. Insect Conservation and Diversity 7(5): 462–469. https://doi.org/10.1111/icad.12069

Dubois G (2009) Écologie des coléoptères saproxyliques: Biologie des populations et conservation d'*Osmoderma eremita* (Coleoptera: Cetoniidae). Thèse PhD, Université de Rennes 1, École doctorale Vie-Agro-Santé, Paimpont, 262 pp.

Dubois G, Vignon V (2008) First results of radio tracking of *Osmoderma eremita* (Coleoptera: Cetoniidae) in French chestnut orchards. In: Vignon V, Asmodé J-F (Eds) Proceedings of the 4th Symposium and workshop on the conservation of Saproxylic Beetles (Vivoin, France, 27th–29th June, 2006). Revue d'Écologie (Terre et Vie) 63: 123–130.

Dubois GF, Vignon V, Delettre YR, Rantier Y, Vernon P, Burel F (2009) Factors affecting the occurrence of the endangered saproxylic beetle *Osmoderma eremita* (Scopoli, 1763) (Coleoptera: Cetoniidae) in an agricultural landscape. Landscape and urban Planning 91: 152–159. https://doi.org/10.1016/j.landurbplan.2008.12.009

Dubois G, Le Gouar P, Delettre Y, Brustel H, Vernon P (2010) Sex-biased and body condition dependent dispersal capacity in the endangered saproxylic beetle *Osmoderma eremita* (Coleoptera: Cetoniidae). Journal of Insect Conservation 14: 679–687. https://doi.org/10.1007/s10841-010-9296-0

Dutto M (2005) Osservazioni biologiche su *Osmoderma eremita* (Scopoli, 1753) ed altri Scarabaeoidea Pleurosticta in vecchi pioppi del Cuneese (Coleoptera Scarabaeoidea). Naturalista siciliano 29: 89–91.

Freude H, Harde KW, Lohse GA (1969) Kafer Mitteleuropas. Band 8. Teredilia (Lyctidae - Ptinidae), Heteromera (Oedemeridae – Boridae), Lamellicornia (Scarabaeidae, Lucanidae). Spektrum Akademischer Verlag, Berlin, 388 pp.

Giangregorio P, Audisio P, Carpaneto GM, Marcantonio G, Maurizi E, Mosconi F, Campanaro A (2015) Updated distribution of *Osmoderma eremita* in Abruzzo (Italy) and agro-pastoral practices affecting its conservation (Coleoptera: Scarabaeidae). Fragmenta Entomologica 47: 139–146. https://doi.org/10.4081/fe.2015.142

Gusakov AA (2002) Scarabaeid beetles of the subfamily Trichiinae (Coleoptera, Scarabaeidae) in the Fauna of Russia – Species of the genus *Gymnodus* Kirby, 1827. Eidos, Kaluga, 1–55. [In Russian]

Hedin J, Ranius T (2002) Using radio telemetry to study dispersal of the beetle *Osmoderma eremita*, an inhabitant of tree hollows. Computers and Electronics in Agriculture 35: 171–180. https://doi.org/10.1016/S0168-1699(02)00017-0

Hedin J, Ranius T, Nilsson SG, Smith HG (2008) Restricted dispersal in a flying beetle assessed by telemetry. Biodiversity and Conservation 17: 675–684. https://doi.org/10.1007/s10531-007-9299-7

Hilszczański J, Jaworski T, Plewa R, Jansson N(2014) Surrogate tree cavities: boxes with artificial substrate can serve as temporary habitat for Osmoderma barnabita (Motsch.) (Coleoptera, Cetoniinae). Journal of Insect Conservation 18: 855–861. https://doi.org/10.1007/s10841-014-9692-y

Hines JE, MacKenzie DI (2004) PRESENCE, vers. 4.0. http://www.mbrpwrc.usgs.gov/software.html

Howden HF (1968) A review of the Trichiinae of North and Central America (Coleoptera: Scarabaeidae). Memoirs of the Entomological Society of Canada 54: 1–77. https://doi.org/10.4039/entm10054fv

ICZN (2007) Opinion 2186 (Case 3349). *Gnorimus* Le Peletier de Saint-Fargeau & Serville, 1828, and *Osmoderma* Le Peletier de Saint-Fargeau & Serville, 1828 (Insecta, Coleoptera, Scarabaeidae): conserved. Bulletin of Zoological Nomenclature 64: 265–266.

Iorgu IS, Surugiu V, Gheoca V, Popa OP (2015) Ghid Sintetic pentru Monitorizarea Speciilor de Nevertebrate de Interes Comunitar din România. București, 159 pp.

Joy NH (1932) A practical Handbook of British Beetles. Witherby, London, 194 pp.

Jansson N, Ranius T, Larsson A, Milberg P (2009). Boxes mimicking tree hollows can help conservation of saproxylic beetles. Biodiversity and Conservation 18: 3891–3908. https://doi.org/10.1007/s10531-009-9687-2

Jönsson N, Méndez M, Ranius T (2004) Nutrient richness of wood mould in tree hollows with the Scarabaeid beetle *Osmoderma eremita*. Animal Biodiversity and Conservation 27: 79–82.

Juillerat L, Vögeli M (2004) Gestion des vieux arbres et maintien des Coléoptères saproxyliques en zone urbaine et periurbaine. Centre Suisse de Cartographie de la Faune, Neuchatel, 20 pp.

Jurc M, Ogris N, Pavlin R, Borkovic D (2008) Forest as a habitat of saproxylic beetles on Natura 2000 sites in Slovenia. Revue d'Écologie (Terre et Vie) 63: 53–66.

Kalas JA, Viken Å, Bakken T (2006) Norwegian Red List: 416. Norwegian Biodiversity Information Centre, Trondheim. [in Norwegian and English]

Klausnitzer BD, Krell FT (1997) Uberfamilie: Scarabaeoidea. In: Klausnitzer BD (Ed.) Die Larven der Kafer Mitteleuropas. Band 3, Polyphaga, Teil 2. Gustav Fischer Verlag, Jena, 11–89.

Kraus D, Bütler R, Krumm F, Lachat T, Larrieu L, Mergner U, Paillet Y, Rydkvist T, Schuck A, Winter S (2016) Catalogue of tree microhabitats – Reference field list. European Forest Institute, 16 pp.

Krell FT (1997) Zu Taxonomie, Chorologie und Eidonomie einiger westpalaearktischer Lamellicornia (Coleoptera). Entomologische Nachrichten und Berichte 40: 217–229.

Krell FT, Ballerio A, Smith ABT, Audisio P (2006) Case 3349. *Gnorimus* Le Peletier de Saint-Fargeau & Serville, 1828, and *Osmoderma* Le Peletier de Saint-Fargeau & Serville, 1828 (Insecta, Coleoptera): proposed conservation of the generic names. Bulletin of the Zoological Nomenclature 63(3): 177–183.

Krikken J (1984) A new key to the suprageneric taxa in the beetle family Cetonidae, with annotated lists of the known genera. Zoologische Verhandelingen 210: 1–75.

Landvik M, Wahlberg N, Roslin T (2013) The identity of the Finnish *Osmoderma* (Coleoptera: Scarabaeidae, Cetoniinae) population established by COI sequencing. Entomolia Fennica 24: 147–155.

Landvik M, Niemelä P, Roslin T (2016) Opportunistic habitat use by *Osmoderma barnabita* (Coleoptera: Scarabaeidae), a saproxylic beetle dependent on tree cavities. Insect Conservation and Diversity 9: 38–48. https://doi.org/10.1111/icad.12141

Larsson MC, Hedin J, Svensson GP, Tolasch T, Francke W (2003) Characteristic odor of *Osmoderma eremita* identified as a male-released pheromone. Journal of Chemical Ecology 29: 575–587. https://doi.org/10.1023/A:1022850704500

Larsson MC, Svensson GP (2009) Pheromone monitoring of rare and threatened insects: exploiting a pheromone-kairomone system to estimate prey and predator abundance. Conservation Biology 23:1516–1525. https://doi.org/10.1111/j.1523-1739.2009.01263.x

Larsson MC, Svensson GP (2011) Monitoring spatiotemporal variation in abundance and dispersal by a pheromone-kairomone system in the threatened saproxylic beetles *Osmoderma eremita* and *Elater ferrugineus*. Journal of Insect Conservation 15: 891–902. https://doi.org/10.1007/s10841-011-9388-5

Latreille PA, Lepeletier de Saint-Fargeau A, Guérin-Méneville FE (1825) Entomologie, ou Histoire Naturelle de Crustacées, des Arachnides et des Insectes (10th edn). Agasse, Paris, 833 pp.

Lawrence JF, Newton AF (1995) Families and subfamilies of Coleoptera (with selected genera, notes, references and data on family-group names). In: Pakaluk J, Slipínski SA (Eds), Biology, Phylogeny, and Classification of Coleoptera. Papers Celebrating the 80th Birthday of R. A. Crowson. Muzeum i Instytut Zoologii PAN, Warszawa, 779–1006.

Le Gouar PJ, Dubois GF, Vignon V, Brustel H, Vernon P (2015) Demographic parameters of sexes in an elusive insect: implications for monitoring methods. Population Ecology 57: 227–236. https://doi.org/10.1007/s10144-014-0453-2

Lepeletier de Saint-Fargeau A, Audinet-Serville JG (1828) Scarabé. In Latreille PA, Entomologie, ou Histoire naturelle des Crustacées, des Arachnides et des Insectes. Encyclopedie methodique 10, Paris, 346–382.

Luce J-M (1995) Écologie des cétoines (Insecta: Coleoptera) microcavernicoles de la forêt de Fontainbleau: niches écologiques, relations interspécifiques et conditions de conservation des populations. Thèse de doctorat en Ecologie générale. École doctorale Sciences de la nature et de l'Homme, Muséum National d'Histoire Naturelle, Paris, 1–166.

Luce J-M (1996) *Osmoderma eremita* (Scopoli, 1763). In: van Helsdingen PJ, Willemse L, Speight MCD (Eds) Background information on invertebrates of the Habitats Directive and the Bern Convention. Part I: Crustacea, Coleoptera and Lepidoptera. Council of Europe, Strasbourg, 64–69.

Luce J-M (2001) La Cétoine protégée *Osmoderma eremita* (Scopoli, 1763) peut elle être un outil de gestion de la biodiversité? In: Robert J-C, Guilbot R, Dommanget J-L, Maurin H (Eds) Inventaire et Cartographie des invertebrés comme contribution à la gestion des milieu naturels français. Actes du séminaire tenu à Besançon les 8, 9 et 10 Juillet 1999, Patrimoines naturels, Muséum National d'Histoire Naturelle, Besançon, 243–263.

Lüchte W, Klausnitzer B (1998) Die Käfer Mitteleuropas. 4 Supplementband. Goecke & Evers, Krefeld -Im Gustave Fischer Verlag, Jena, Stuttgart, Lübeck, Ulm, 398 pp.

MacKenzie DI, Royle JA (2005) Designing occupancy studies: general advice and allocating survey effort. Journal of Applied Ecology 42:1105–1114. https://doi.org/10.1111/j.1365-2664.2005.01098.x

MacKenzie DI, Nichols JD, Royle JA, Pollock KH, Bailey LL, Hines JE (2006) Occupancy Estimation and Modeling: Inferring Patterns and Dynamics of Species Occurrence. Academic Press, New York

Martin-Piera F, López-Colón JI (2000) Fauna Iberica, Vol. 14. Coleoptera Scarabaeoidea I. Museo Nacional de Ciencias Naturales, Consejo Superior de Investigaciones Científicas, Madrid.

Mason F, Roversi PF, Audisio P, Bologna MA, Carpaneto GM, Antonini G, Mancini E, Sabbatini Peverieri G, Mosconi F, Solano E, Maurizi E, Maura M, Chiari S, Sabatelli S, Bardiani M, Toni I, Redolfi De Zan L, Rossi de Gasperis S, Tini M, Cini A, Zauli A, Nigro G, Bottacci A, Hardersen S, Campanaro A (2015) Monitoring of insects with public participation (MIPP; EU LIFE project 11 NAT/IT/000252): overview on a citizen science initiative and a monitoring programme (Insecta: Coleoptera; Lepidoptera; Orthoptera). Fragmenta entomologica 47: 51–52. https://doi.org/10.4081/fe.2015.134

Massa B (1995) *Osmoderma heremitum meridionale* Tauzin 1994, sinonimo di *Osmoderma cristinae* Sparacio 1994. Naturalista siciliano 19: 307–308.

McKenna DD, Farrell BD, Caterino MS, Farnum CW, Hawks DC, Maddison DR, Seago AE, Short AEZ, Newton AF, Thayer MK (2015) Phylogeny and evolution of Staphyliniformia and Scarabaeiformia: forest litter as a stepping stone for diversification of nonphytophagous beetles. Systematic Entomology 40: 35–60. https://doi.org/10.1111/syen.12093

Medvedev SI (1953) A new species of *Osmoderma* Serville (Coleoptera, Scarabaeidae) from Georgia. Entomologicheskoe Obozrenie 32: 297–299.

Micó E, Galante E (2003a) Larval morphology and biology of four *Netocia* and *Potosia* species (Coleoptera: Scarabaeoidea: Cetoniidae: Cetoniinae). European Journal of Entomology 100: 131–142. https://doi.org/10.14411/eje.2003.023

Micó E, Galante E (2003b) Biology and new larval descriptions for three cetoniine beetles (Coleoptera: Scarabaeidae: Cetoniinae: Cetoniini: Cetoniina, Leucocelina). Annals of the Entomological Society of America 96(2): 95–106. https://doi.org/10.1603/0013-8746(2003)096[0095:BANLDF]2.0.CO;2

Mosconi F, Campanaro A, Carpaneto GM, Chiari S, Hardersen S, Mancini E, Maurizi E, Sabatelli S, Zauli A, Mason F, Audisio P (2017) Training of a dog for the monitoring of *Osmoderma eremita*. In: Carpaneto GM, Audisio P, Bologna MA, Roversi PF, Mason F (Eds) Guidelines for the Monitoring of the Saproxylic Beetles protected in Europe. Nature Conservation 20: 237–264. https://doi.org/10.3897/natureconservation.20.12688

Müller J, Jarzabek-Müller A, Bussler H, Gossner MM (2014) Hollow beech trees identified as keystone structures for saproxylic beetles by analyses of functional and phylogenetic diversity. Animal Conservation Animal Conservation 17: 154–162. https://doi.org/10.1111/acv.12075

Nardi G, Micó E (2010) *Osmoderma cristinae*. The IUCN Red List of Threatened Species 2010: e.T157875A5164314.

Nichols JD, Bailey LL, O'Connell AF Jr, Talancy NW, Grant EHC, Gilbert AT, Annand EM, Husband TP, Hines JE (2008) Multiscale occupancy estimation and modelling using multiple detection methods. Journal of Applied Ecology 45: 1321–1329. https://doi.org/10.1111/j.1365-2664.2008.01509.x

Nieto A, Alexander KNA (2010) European Red List of Saproxylic Beetles. International Union for Conservation of Nature and Publications Office of the European Union, Luxembourg, 1–46.

Nieto A, Mannerkoski I, Putchkov A, Tykarski P, Mason F, Dodelin B, Tezcan S (2010) *Osmoderma eremita*. The IUCN Red List of Threatened Species 2010: e.T15632A4926651. Downloaded on 07 November 2016.

Nüssler H (1986) Eine von *Osmoderma eremita* (Scop.) abweichende Weibchen-Form aus Südwestbulgarien (Insecta, Coleoptera, Scarabaeidae). Faunistiche Abhandlungen Staatlisches Museum für Tierkunde Dresden 13:113–117.

Oleksa A (2009) Conservation and ecology of the hermit beetle *Osmoderma eremita* s.l. in Poland. In: Buse J, Alexander KNA, Ranius T, Assmann T (Eds) Saproxylic Beetles - their role and diversity in European woodland and tree habitats. Proceedings of the 5th Symposium and Workshop on the Conservation of Saproxylic Beetles. Pensoft Series Faunistica 89, 177–188.

Oleksa A, Gawronski R (2006) Forest insects in an agricultural landscape - presence of old trees is more important than the existence of nearby forest. Ecological Questions 7: 29–36.

Oleksa A, Ulrich W, Gawronski R (2007) Host tree preference of hermit beetles in a network of rural avenues in Poland (*Osmoderma eremita* Scop., Coleoptera: Scarabaeidae). Polish Journal of Ecology 55: 315–323. https://doi.org/10.1007/s10841-012-9499-7

Oleksa A, Chybicki IJ, Gawroński R, Svensson GP, Burczyk J (2013) Isolation by distance in saproxylic beetles may increase with niche specialization. Journal of Insect Conservation 17: 219–233.

Paulian R, Baraud J (1982) Faune des Coléoptères de France, II. Lucanoidea et Scarabaeoidea. Éditions Lechevalier, Paris, 477 pp.

Parisi V, Busetto A (1992) Scarabaeidae, Lucanidae, Cerambycidae. In: Zara (Ed.) Revisione dei coleotteri presenti nella collezione "A. Leosini". Museo di Storia Naturale, Università di Parma, Parma, 70 pp.

Pic M (1904) Les "Osmoderma" paléarctiques. L'Échange, Revue Linnéenne 20 (239): 83–84.

Pignataro C, Vicidomini S (2007) *Osmoderma italicum* Sparacio (Coleoptera): ritrovato nei pressi del locus typicus dopo circa un secolo (Campania). Proceedings XXI Congresso Nazionale Italiano di Entomologia (Campobasso 11–16 Giugno 2007), Accademia Nazionale Italiana di Entomologia, 78.

Porta A (1932) Fauna Coleopterorum Italica. Vol. V, Rhynchophora-Lamellicornia. Stabilimento Tipografico Piacentino, Piacenza, 476 pp.

Prunier D (1999) Quelques observations sur la biologie d'*Osmoderma eremita* Scop. Le Coléopteriste 35: 23–24.

Ranius T (2000) Minimum viable metapopulation size of a beetle, *Osmoderma eremita*, living in tree hollows. Animal Conservation 3: 37–43. https://doi.org/10.1111/j.1469-1795.2000.tb00085.x

Ranius T (2001) Constancy and asynchrony of *Osmoderma eremita* populations in tree hollows. Oecologia 126: 208–215. https://doi.org/10.1007/s004420000515

Ranius T (2002a) *Osmoderma eremita* as an indicator of species richness of beetles in tree hollows. Biodiversity and Conservation 11: 931–941. https://doi.org/10.1023/A:1015364020043

Ranius T (2002b) Population ecology and conservation of beetles and pseudoscorpions living in hollow oaks in Sweden. Animal Biodiversity and Conservation 25: 53–68.

Ranius T (2002c) Influence of stand size and quality of tree hollows on saproxylic beetles in Sweden. Biological Conservation 103: 85–91. https://doi.org/10.1016/S0006-3207(01)00124-0

Ranius T (2006) Measuring the dispersal of saproxylic insects: a key characteristic for their conservation. Population Ecology 48:177–188. https://doi.org/10.1007/s10144-006-0262-3

Ranius T (2007) Extinction risks in metapopulations of a beetle inhabiting hollow trees predicted from time series. Ecography 30: 716–726. https://doi.org/10.1111/j.2007.0906-7590.05134.x

Ranius T, Hedin J (2001) The dispersal rate of a beetle, *Osmoderma eremita*, living in tree hollows. Oecologia 126: 363–370. https://doi.org/10.1007/s004420000529

Ranius T, Jansson N (2000) The influence of forest regrowth, original canopy cover and tree size on saproxylic beetles associated with old oaks. Biological Conservation 95: 85–94. https://doi.org/10.1016/S0006-3207(00)00007-0

Ranius T, Jansson N (2002) A comparison of three methods to survey saproxylic beetles in hollow oaks. Biodiversity and Conservation 11: 1759–1771.https://doi.org/10.1023/A:1020343030085

Ranius T, Nilsson SG (1997) Habitat of *Osmoderma eremita* Scop. (Coleoptera: Scarabaeidae), a beetle living in hollow trees. Journal of Insect Conservation 1: 193–204. https://doi.org/10.1023/A:1018416000766

Ranius T, Aguado LA, Antonsson K, Audisio P, Ballerio A, Carpaneto GM, Chobot K, Gjurašin B, Hanssen O, Huijbregts H, Lakatos F, Martin O, Neculiseanu Z, Nikitsky NB, Paill W, Pirnat A, Rizun V, Ruicănescu A, Stegner J, Süda I, Szwalko P, Tamutis V, Telnov D, Tsinkevich V, Versteirt V, Vignon V, Vögeli M, Zach P (2005) *Osmoderma eremita* (Scopoli, 1763) (Coleoptera: Scarabaeidae: Cetoniinae) in Europe: a Short Summary of a Pan-European Project. Proceedings of the 3th Symposium and Workshop on the Conservation of Saproxylic Beetles. Riga/Latvia, 07th–11th July, 2004: 79–82.

Ranius T, Aguado L A, Antonsson K, Audisio P, Ballerio A, Carpaneto GM, Chobot K, Gjurašin B, Hanssen O, Huijbregts H, Lakatos F, Martin O, Neculiseanu Z, Nikitsky NB, Paill W, Pirnat A, Rizun V, Ruicănescu A, Stegner J, Süda I, Szwalko P, Tamutis V, Telnov D, Tsinkevich V, Versteirt V, Vignon V, Vögeli M, Zach P (2005) *Osmoderma eremita* (Coleoptera: Scarabaeidae: Cetoniinae) in Europe. Animal Biodiversity and Conservation 28: 1–44.

Ranius T, Svensson GP, Berg N, Niklasson M, Larsson MC (2009) The successional change of hollow oaks affects their suitability for an inhabiting beetle, *Osmoderma eremita*. Annales Zoologici Fennici 46: 205–216. https://doi.org/10.5735/086.046.0305

R Core Team (2016). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/

Redolfi De Zan L, Battisti C, Carpaneto GM (2014a) Bird and beetle assemblages in relict beech forests of central Italy: a multi-taxa approach to assess the importance of dead wood in biodiversity conservation. Community Ecology 15: 235–245. https://doi.org/10.1556/ComEc.15.2014.2.12

Redolfi De Zan L, Bellotti F, D'Amato D, Carpaneto GM (2014b) Saproxylic beetles in three relict beech forests of central Italy: Analysis of environmental parameters and implications for forest management. Forest Ecology and Management 328: 229–244. https://doi.org/10.1016/j.foreco.2014.05.040

Ritcher PO (1966) White Grubs and their Allies. A study of North American Scarabaeoid Larvae. Oregon State University Press, Corvallis, 219 pp.

Robertson BA, Hutto RL (2006) A framework for understanding ecological traps and an evaluation of existing evidence. Ecology 87: 1075–1085. https://doi.org/10.1890/0012-9658(2006)87[1075:AFFUET]2.0.CO;2

Ruffo S, Stoch F (Eds) (2005) Checklist e distribuzione della fauna italiana. Memorie del Museo Civico di Storia Naturale di Verona, Sezione Scienze della Vita 16: 1–307.

Schaffrath U (2003a) Zu Lebensweise, Verbreitung und Gefährdung von *Osmoderma eremita* (Scopoli, 1763) (Coleoptera; Scarabaeoidea, Cetoniidae, Trichiinae), Teil 1. Philippia 10: 157–248.

Schaffrath U (2003b) Zu Lebensweise, Verbreitung und Gefährdung von *Osmoderma eremita* (Scopoli, 1763) (Coleoptera; Scarabaeoidea, Cetoniidae, Trichiinae), Teil 2. Philippia 10: 249–336.

Scholtz C, Grebennikov VV (2005) Scarabaeiformia (pp. 345–425). In: Beutel R, Leschen RAB, Handbook of Zoology, Volume 4: Arthropoda, Insecta; Volume 1: Coleoptera, Beetles: Morphology and systematics (Archostemata, Adephaga, Myxophaga, Polyphaga partim). Walter de Gruyter (Berlin): 1–567.

Schoolmeesters P (2016) Scarabs: World Scarabaeidae Database. In: Roskov Y, Abucay L, Orrell T, Nicolson D, Flann C, Bailly N, Kirk P, Bourgoin T, DeWalt RE, Decock W, De Wever A (Eds) Species 2000 & ITIS Catalogue of Life, 28th September 2016. Species 2000, Naturalis, Leiden. http://www.catalogueoflife.org/col [version Jul 2016]

Shabalin SA, Bezborodov VG (2009) Larvae of Scarab Beetles of the Subfamily Trichiinae (Coleoptera, Scarabaeidae) from the Russian Far East. Zoologicheskii Zhurnal 88: 1201–1206. https://doi.org/10.1134/s0013873809090139

Šípek P, Fabrizi S, Eberle J, Ahrens D (2016a) Why *Osmoderma* is not a member of Trichiini and what else can we learn from a molecular phylogeny of rose chafers (Coleoptera: Scarabaeidae: Cetoniinae). Forschungs Museum Koenig, Bonn, April 2016. https://doi.org/10.13140/RG.2.1.4124.7603.

Šípek P, Fabrizi S, Eberle J, Ahrens D (2016b) A molecular phylogeny of rose chafers (Coleoptera: Scarabaeidae: Cetoniinae) reveals a complex and concerted morphological evolution related to their flight mode. Molecular Phylogenetics and Evolution 101: 163–175. https://doi.org/10.1016/j.ympev.2016.05.012

Smetana A (2006) Cetoniinae Leach, 1815. In: Löble I, Smetana A (Eds) Catalogue of Palaearctic Coleoptera, Volume 3: Scarabaeoidea - Scirtoidea – Dascilloidea – Buprestoidea – Byrrhoidea. Apollo Books, Stenstrup, 283–313.

Smith ABT (2004) Clarification on the nomenclatural status of six genus-group names in the tribe Trichiini (Coleoptera: Scarabaeidae: Cetoniinae). The Coleopterists Bulletin 58(2): 285–291. https://doi.org/10.1649/704

Smith ABT, Hawks DC, Heraty JM (2006) An overview of the classification and evolution of the major scarab beetle clades (Coleoptera: Scarabaeoidea) based on preliminary molecular analyses. Coleopterists Society Monograph 5: 35–46. https://doi.org/10.1649/0010-065X(2006)60[35:AOOTCA]2.0.CO;2

Sparacio I (1994) *Osmoderma cristinae* n. sp. di Sicilia (Insecta Coleoptera: Cetoniidae). Naturalista siciliano 17: 305–310.

Sparacio I (2000) Osservazioni sulle *Osmoderma* Le Peletier et Audinet-Serville europee con descrizione di una nuova specie dell'Italia meridionale (Coleoptera Cetoniidae). Il Naturalista Siciliano 24 (3–4): 225–239.

Stegner J (2002) Der Eremit, *Osmoderma eremita* (Scopoli, 1763) (Col., Scarabaeidae), in Sachsen: Anforderungen an Schutzmaßnahmen für eine prioritäre Art der FFH–Richtlinie. Entomologische Nachrichten und Berichte 46: 213–238.

Straka U (2011). Untersuchungen zur Biologie des Juchtenkäfers (*Osmoderma eremita* Scopoli, 1763; Coleoptera) in Niederösterreich. Beiträge zur Entomofaunistik 12: 3–24.

Svensson GP, Larsson MC, Hedin J (2003) Air sampling of its pheromone to monitor the occurrence of *Osmoderma eremita*, a threatened beetle inhabiting hollow trees. Journal of Insect Conservation 7: 189–198. https://doi.org/10.1023/B:JICO.0000020896.71333.f6

Svensson GP, Larsson MC, Hedin J (2004) Attraction of the larval predator *Elater ferrugineus* to the sex pheromone of its prey, *Osmoderma eremita*, and its implication for conservation biology. Journal of Chemical Ecology 30: 353–363. https://doi.org/10.1023/B:JOEC.0000017982.51642.8c

Sverdrup-Thygeson A, Hanssen O, Ødegaard F (2010) Faglig grunnlag for handlingsplan for eremitt *Osmoderma eremita*. NINA Rapport 631: 1–44.

Tauzin P (1994a) Le genre *Osmoderma* Le Peletier et Audinet–Serville 1828 (Coleopt., Cetoniidae, Trichiinae, Osmodermatini). Systématique, biologie et distribution (Première partie). L'Entomologiste 50 (3): 195–214.

Tauzin P (1994b) Le genre *Osmoderma* Le Peletier et Audinet–Serville 1828 (Coleopt., Cetoniidae, Trichiinae, Osmodermatini). Systématique, biologie et distribution (Deuxième partie). L'Entomologiste 50: 217–242.

Tauzin P (1996) Complément à la note sur le genre *Osmoderma* (Coléoptères, Cetoniidae, Trichinae). L'Entomologiste 52: 105–106.

Tauzin P (2000) Localités connues de *Osmoderma eremitum* Scopoli en France. Le Coléoptériste 39: 133–136.

Tauzin P (2002) *Osmoderma eremitum*: complèments sur sa distribution en France et nouvelle information sur la taxonomie du genre (Coleoptera, Cetoniidae, Trichiinae, Osmodermatini). L'Entomologiste 58: 145–151.

Tauzin P (2005) Ethology and distribution of the "hermit beetle" in France. Cetoniimania 4: 131–153.

Tauzin P (2006) Nouveau statut pour la variété castaneum de *Osmoderma barnabita* Motschoulsky, 1845 (Coleoptera, Scarabaeoidea, Cetoniidae, Trichiinae, Osmodermatini). Cetoniimania 1–2, 39–49.

Trizzino M, Audisio P, Bisi F, Bottacci A, Campanaro A, Carpaneto GM, Chiari S, Hardersen S, Mason F, Nardi G, Preatoni DG, Vigna Taglianti A, Zauli A, Zilli A, Cerretti P (2013) Gli artropodi italiani in Direttiva Habitat: biologia, ecologia, riconoscimento e monitoraggio. Quaderni Conservazione Habitat, 7. CFS-CNBFVR, Centro Nazionale Biodiversità Forestale. Cierre Grafica, Sommacampagna, Verona, 256 pp.

Van Emden van FI (1941) Larvae of British Beetles II. A key to the British Lamellicornia larvae. Entomological Monthly Magazine 77: 117–127, 181–192.

Vernik M (2014) Collecting data of distribution for four Natura 2000 species of beetles (Coleoptera) in Slovenia (Poster), 4. Slovenski entomološki simpozij, 9. in 10. maja 2014, Fakulteti za naravoslovje in matematiko Univerze v Mariboru, Koroška. http://www.sporocivrsto.si

Vignon V (2006) Le pique-prune: histoire d'une sauvegard. Catiche Productions, Nohanent; Office de génie écologique, Saint-Maur_des_Fossés; Cofiroute, Sévres Cedex, France: 1–32.

Vignon V (2008) Comparing size of *Osmoderma* populations and habitat quality in different French localities: conservation perspectives. Revue d'Écologie (Terre et Vie) (supp. 10): 123–129.

Vignon V (2015) Parts of the life history of *Osmoderma eremita*'s metapopulations in two study areas in the west of France (Coleoptera, Cetoniidae). Bulletin de la Société Entomologique Suisse 88: 39–48.

Vignon V, Orabi P (2003). Exploring the hedgerows network in the west of France for the conservation of saproxylic beetles (*Osmoderma eremita, Gnorimus variabilis, Lucanus cervus, Cerambyx cerdo*) (pp. 36–38) In: Bowen CP (Ed) Proceedings of the second pan–European conference on saproxylic beetles, Royal Holloway, University of London, 25th–27th June 2002. People's Trust for Endangered Species (London).

Vignon V, Asmode J-F, Rapaport P (2004) Heterogeneity of the spatial distribution of *Osmoderma eremita* (Scopoli, 1763) populations in a hedgerow network (France, Normandy). Proceedings of the 3rd Symposium and Workshop on the Conservation of Saproxylic Beetles (Riga/Latvia, 07–11 July 2004): 109–115.

Vögeli M (2002) Der Eremit (*Osmoderma eremita*) in der Schweiz – eine naturschutzorientierte Analyse von Verbreitung und Habitat. Unveröffentlichte Diplomarbeit. Eidgenössische Technische Hochschule (Zürich).

Vrezec A, Pirnat A, Kapla A, Denac D (2008) Zasnova spremljanja stanja populacij izbranih ciljnih vrst hroščev vključno z dopolnitvijo predloga območij za vključitev v omrežje NATURA 2000. *Morimus funereus*, *Rosalia alpina*, *Cerambyx cerdo*, *Osmoderma eremita*, *Limoniscus violaceus*, *Graphoderus bilineatus* Nacionalni inštitut za biologijo (Ljubljana).

Vrezec A, Ambrožič Š, Kapla A (2012) An overview of sampling method tests for monitoring schemes of saproxylic beetles in the scope of Natura 2000 in Slovenia. In: Jurc M (Ed.) Saproxylic beetles in Europe: monitoring, biology and conservation. - Ljubljana: Slovenian Forestry Institute, Silva Slovenica, Studia Forestalia Slovenica 13: 73–90.

Vrezec A, Ambrožič Š, Kapla A, Bertoncelj I, Bordjan D (2014) Izvajanje spremljanja stanja populacij izbranih ciljnih vrst hroščev v letu 2013 in 2014. Končno poročilo. Nacionalni inštitut za biologijo (Ljubljana).

Westwood JO (1839) An Introduction to the modern classification of insects founded on the natural habits and corresponding organisation of the different families. Volume 1. Longman, Orme, Brown, Green, and Longmans (London): 1–462.

Winter S, Möller GC (2008) Microhabitats in lowland beech forests as monitoring tool for nature conservation. Forest Ecology and Management 255: 1251–1261. https://doi.org/10.1016/j.foreco.2007.10.029

Zauli A, Chiari S, Hedenstrom E, Svensson GP, Carpaneto GM (2014) Using odour traps for population monitoring and dispersal analysis of the threatened saproxylic beetles *Osmoderma eremita* and *Elater ferrugineus* in central Italy. Journal of Insect Conservation 18: 801–813. https://doi.org/10.1007/s10841-014-9687-8

Zauli A, Carpaneto GM, Chiari S, Mancini E, Nyabuga FN, Redolfi De Zan L, Romiti F, Sabbani S, Audisio P, Hedenström E, Bologna MA, Svensson GP (2016a) Assessing the taxonomic status of *Osmoderma cristinae* (Coleoptera: Scarabaeidae), endemic to Sicily, by genetic, morphological and pheromonal analyses. Journal of Zoological Systematics and Evolutionary Research 54: 206–214. https://doi.org/10.1111/jzs.12127

Zauli A, Maurizi E, Carpaneto GM, Chiari S, Svensson G, Di Giulio A (2016b) Antennal fine morphology of the threatened beetle *Osmoderma eremita* (Coleoptera: Scarabaeidae), revealed by scanning electron microscopy. Microscopy Research and Technique 79: 178–191. https://doi.org/10.1002/jemt.22618

Zauli A, Maurizi E, Carpaneto GM, Chiari S, Merivee E, Svensson GP, Di Giulio A (2016c) Scanning electron microscopy analysis of the antennal sensilla in the rare saproxylic beetle *Elater ferrugineus* (Coleoptera, Elateridae). Italian Journal of Zoology 83: 338–350. https://doi.org/10.1080/11250003.2016.1211766

Supplementary material 1

Scheme of protocol suggested by MIPP to monitoring the *O. eremita* population and list of materials to building the pheromone traps, BCWT

Authors: Emanuela Maurizi, Alessandro Campanaro, Stefano Chiari, Michela Maura, Fabio Mosconi, Simone Sabatelli, Agnese Zauli, Paolo Audisio, Giuseppe M. Carpaneto

Data type: protocol

Link: https://doi.org/10.3897/natureconservation.20.12685.suppl1

Supplementary material 2

Field sheet to fill during each survey and its legend

Authors: Emanuela Maurizi, Alessandro Campanaro, Stefano Chiari, Michela Maura, Fabio Mosconi, Simone Sabatelli, Agnese Zauli, Paolo Audisio, Giuseppe M. Carpaneto

Data type: field sheet

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Guidelines for the monitoring of *Cerambyx cerdo*

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Abstract

Cerambyx cerdo is a longhorn beetle widely distributed in southern and central Europe. This saproxylic beetle is generally associated with oak forests where there are mature or partially dead and sun-exposed trees. Its populations are currently threatened by forest practices such as the removal of partially dead trees and the decline in the number of old oak trees situated in open or semi-open landscapes. Thus, *C. cerdo* has been included in Annexes II and IV of the Habitats Directive. The present paper is part of a special issue on monitoring of saproxylic beetles which are protected in Europe, based on the research carried out during the LIFE-MIPP project, with a revision of the current knowledge on systematics, ecology and conservation of *C. cerdo*. The main aim of the present paper is to test different monitoring methods in order to develop a quick and reproducible protocol for the conservation of this species. The methods tested were: artificial sap attracting the adults, baited traps, VES (visual encounter survey) and collecting remains of predation along transects. Based on these results, a detailed monitoring method for *C. cerdo* using baited trap is proposed in this paper, together with a discussion on its constraints, spatial validity and possible interferences. In order to assess the conservation status of populations of *C. cerdo* in Europe and to compare populations over time, a method for the calculation of a reference value, based on the monitoring method, is provided.

Keywords

Habitats Directive, Saproxylic beetles, Coleoptera, Cerambycidae, Monitoring methods, Forest biodiversity, Baited trap

Introduction

The great capricorn beetle, *Cerambyx cerdo* Linnaeus, 1758, is a large longhorn beetle (Coleoptera: Cerambycidae), generally associated with oak forests where there are mature or partially dead and sun-exposed trees. It is listed in Annexes II and IV of the Habitats Directive (Council Directive 92/43/EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora). The Habitats Directive provides that Member States of Europe prepare, every six years, a report on the conservation status of the threatened species listed in the Annexes. In order to address this obligation, the Life Project "Monitoring of insects with public participation" (LIFE11 NAT/IT/000252) (hereafter, MIPP) conducted experimental fieldwork to develop standardised methods for monitoring of the saproxylic beetle species of the project: *Osmoderma eremita* (hermit beetle, Scarabaeidae), *Lucanus cervus* (European stag beetle, Lucanidae), *Rosalia alpina* (rosalia longicorn, Cerambycidae), *Morimus asper funereus* (morimus longicorn, Cerambycidae) and *Cerambyx cerdo* (great capricorn beetle, Cerambycidae).

The present paper is part of a special issue on monitoring the abundance of saproxylic beetles protected in Europe and is dedicated to *C. cerdo*. Therefore, it starts with an extensive revision of the current knowledge on systematics, distribution, ecology, ethology and conservation of this species. The review is followed by a detailed account of the fieldwork carried out during the MIPP project and concludes with a description of the proposed monitoring method.

Systematics and distribution

The genus *Cerambyx* includes 13 species in the Palaearctic region, at least 7 species of which occur in Europe (Müller 1950, Švácha and Danilevsky 1987, Bense 1995, Özdikmen and Turgut 2009).

Cerambyx cerdo (Linnaeus, 1758), *C. scopolii* Fuesslins, 1775, *C. miles* Bonelli, 1823 and *C. welensii* (Küster, 1846) are more or less widely distributed in Europe, with the last two taxa mainly restricted to the southernmost countries, whereas *C. nodulosus* Germar, 1817*, C. dux* (Faldermann, 1837) and *C. carinatus* Kuster, 1846 occur only in eastern European countries, especially in the Balkan-Mediterranean habitats (Bense 1995).

C. cerdo occurs in Europe, Caucasus and in the Middle East up to northern Iran. This species is widespread in most parts of Europe (northwards to southern Sweden and Great Britain eastward to Belorussia, Moldavia, Ukraine and Crimea) but is more common in the Mediterranean region (Sama 1988). In Italy, it is widely distributed, occurring also in the Tyrrhenian islands but being absent in the northwestern part (the Aosta Valley) (Sama 1988, 2002). *C. cerdo* is known to live sympatrically with *C. welensii* and to have an ecological niche similar to the latter, as in Southern Spain (Torres-Vila et al. 2012, 2013) and in the MIPP study area of Bosco della Fontana, in the Po valley. *C. welensii* (often still quoted under the synonym *C. velutinus* Alfieri, 1916) is widespread in southern Europe, reaching eastwards to Ukraine and Iran.

Different subspecies are described under the taxon *C. cerdo*: *C. cerdo pfisteri* (Stierl, 1864), *C. c. acuminatus* Motsch, 1852, *C. c. mirbecki* Lucas, 1849 and *C. c. iranicus* Heyrovský, 1951 (Müller 1950, Villiers 1978, Sama 1988, Özdikmen and Turgut 2009). However, the validity of most of these subspecies has been cast in doubt by Sama (2002).

Morphology

The adult specimens of *C. cerdo* are 17–56 mm long (excluding the antennae) and 8–14 mm wide, with a body overall blackish and elytra reddish-brown towards the distal portions. The head is provided with strong mandibles and is transversally rugose on the upper side (vertex). The antennae are long, as in most species of the Cerambycidae family: in females, the antennae are long like the main body length (last antennal segments reaching at least the distal part of the elytra), while in males, the antennae are much longer than the body (the last 3 or 4 segments of the antennae exceed the distal margin of the elytra). In males, the last segment of the antennae is much longer than the previous one, while in females, the last segment is as long as the previous one or shorter. The pronotum is heavy sculptured and shows a conspicuous thorn laterally on both sides. Elytra are rugose, densely punctate, with rugosity decreased in the distal part and are truncated at their apex (Rudnew 1936, Villiers 1978, Bense 1995, Harde 1996, Özdikmen and Turgut 2009).

The larvae of *C. cerdo* look like those of many other longhorn beetles, with generally creamy-white-yellowing body and reduced legs. The full grown larvae are up to 70–90 mm long, 18–20 mm broad; head white-yellow with widely pigmented and strongly sclerotised black-pitchy-brown mouth frame and black mandibles. Pronotum is provided with sclerotised shield; legs are very short but distinct (Rudnew 1936, Villiers 1978, Švácha and Danilevsky 1987).

Identification and comparison with similar taxa

Five species belonging to the genus *Cerambyx* often occur together in forest ecosystems of Italy and other south-central or western European countries: *C. cerdo*, *C. miles*, *C. scopolii* and *C. welensii* (Figure 1). *C. nodulosus* (an eastern species related to *C. miles*) occurs only in Friuli Venezia Giulia (Sama and Rapuzzi 2011).

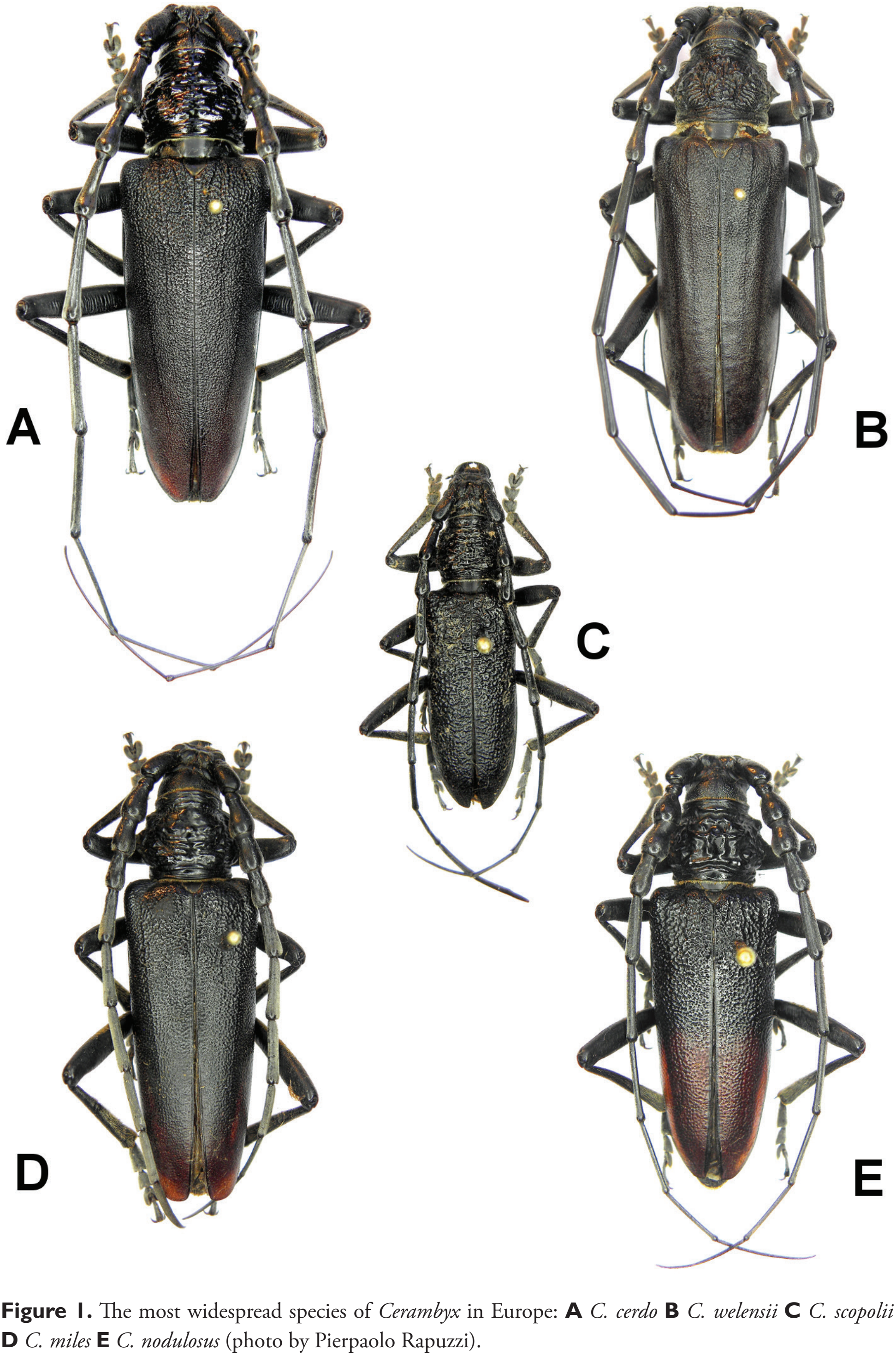

Figure 1. The most widespread species of *Cerambyx* in Europe: **A** *C. cerdo* **B** *C. welensii* **C** *C. scopolii* **D** *C. miles* **E** *C. nodulosus* (photo by Pierpaolo Rapuzzi).

C. welensii and *C. cerdo* can be distinguished from *C. miles* and *C. scopolii* by the shape of the inner elytral apex which bears a small acuminate tooth. Furthermore, *C. scopolii* is small (17–28 mm) and entirely black, often found on flowers of elders (*Sambucus*) and other shrubs. The elytra of *C. cerdo* have the anterior portion deeply sculptured, black, shiny, almost glabrous, tendentially restricted and subtruncated at apex; those of *C. welensii* are evenly brownish, weakly sculptured, covered with minute setae, sub-parallel and rounded at apex. *C. cerdo* and *C. miles* have the elytra black and shiny, deeply sculptured and with red apex; however, the latter does not have the terminal elytral tooth and shows the first four or five antennal segments short and thick. In males of *C. welensii*, antennae exceed the body length with the last three antennal segments (never with the last four as in some *C. cerdo* males). Females of these two species are distinguishable by the length of antennae, extended to the apical third of elytra in *C. cerdo* and only to the middle in *C. welensii* (Pesarini and Sabbadini 1994). In eastern European countries, the presence of additional species, whose identification is often hard and the taxonomic status questioned, makes the fieldwork more problematic for non-expert operators. The detailed and updated distribution of the species in Europe can be found in the TITAN Cerambycidae database (Tavakilian and Chevillotte 2017).

Ecology

C. cerdo is a polyphagus saproxylic species that usually lives in deadwood of standing living veteran oak trees (*Quercus* spp.) and other deciduous species such as *Castanea sativa*, *Juglans regia*, *Fraxinus* spp., *Salix* spp., *Ulmus* spp., *Fagus sylvatica*, *Platanus* spp., *Prunus* spp. (Müller 1950). *C. cerdo* generally occurs in semi-open wood stands which also match the habitat requirements of other saproxylic species like *Osmoderma eremita* Scopoli, 1763 (Coleoptera Scarabaeidae) and some other highly endangered beetles (Ranius and Nilsson 1997, Buse et al. 2007).

Habitats of *C. cerdo* are lowland and hilly forests comprising various species of *Quercus* (Sama 1988). Viable populations of *C. cerdo* can also be found in tree avenues or parks (Buse et al. 2007, Carpaneto et al. 2015). Colonised trees can be identified by visible holes made by the larvae on the trunk or thick branches (Müller 2001). These holes can persist over many years or even decades; typical signs of recent activity are wood meal and fresh holes with red-coloured interior sides (Buse et al. 2007). Studies on *C. cerdo* habitat preferences highlighted that the bark depth of the trees is one of the most significant predictors for species presence and increasing age and diameter of the oak trees improve the probability of occurrence. The vitality of the trunk, the insolation of the tree and the habitat openness appeared to be other important parameters for predicting the presence of this saproxylic beetle (Buse et al. 2007). Factors affecting the distribution of *C. cerdo* amongst individual trees have been studied by Albert et al. (2012). The authors demonstrated how the presence of exit holes in the trunks of large old oaks was positively associated with the diameter of the trunk and insolation of the tree, while the number of exit holes decreased with the height above the ground. When a tree is attacked by *C. cerdo*, the tree may survive over long periods, although its survival will be determined by the increasing amount of dead wood on it. Furthermore, the larvae create extensive galleries (Palm 1959), which may act as entrances and habitats for other species. Buse et al. (2008a, 2008b) compared the saproxylic assemblage of colonised and uncolonised trees in order to study the functional role of *C. cerdo* as an ecosystem 'engineer' by using the interceptor traps. Results suggested that *C. cerdo* can alter its own habitat to create favourable habitat conditions for entire assemblages of saproxylic insects.

Larval ecology

The larval development of *C. cerdo* mainly takes place in fresh wood of oaks (*Quercus* spp.) and lasts about 3-4 years, producing an irregular pattern of larval galleries (Pavlović et al. 2012). During the first year, the larvae feed under the bark layer, while in the second year, they move deeper into the wood (Neumann 1985, Bense 1995). Larval development was observed not only in tree trunks but also inside large branches (Campanaro et al. 2011a). Larvae, after completing their development, excavate a last tract of gallery directed toward the surface of the trunk and then back into the tree, where they are protected by any contact with the external environment; here, pupation occurs generally during May or June. The adult remains inside the pupation chamber or close to the exit hole until the following spring. During spring and summer, adults emerge from colonised trees by producing large and ellipsoidal exit holes into the bark (Horák et al. 2010). Exit holes are easily detectable but are not species-specific and can be confused with holes dug by other *Cerambyx* species. The wood is perforated by deep, broad and winding galleries and emergence holes are characterised by their large size and oval shape (length 3 cm, width 1.5 cm on average) (Luce 1997). In central Europe, the larvae usually develop in *Q. robur* or *Q. petraea*, but in southern countries, they have also been found on *Q. pubescens*, *Q. ilex*, *Q. suber* and *Castanea sativa* (Luce 1997). Although suitable trees have a sun-exposed trunk with at least 60 cm in diameter (Buse et al. 2007), in central Italy, smaller trees suitable for *C. cerdo* have been reported by Marianelli et al. (2011).

Adult ecology

The adults remain sheltered in their chambers during the winter (Horák et al. 2010) and emerge between May and August, depending on local climatic conditions relating to altitude and latitude. In central and southern France, where the species is more widespread, the adults are active for a longer period, from June to September (Bensetti and Gaudillat 2002). In Lower Saxony (Germany), the emergence of the adult great capricorn beetle generally occurs from the end of May until the beginning of August, although later observations have been reported throughout August and September (Buse et al. 2007). In south-western Hungary, the adults are active by mid-spring (early May), remaining active into mid-summer (Keszthelyi 2015). In the Iberian Peninsula (in the northern part of the province of Alicante), they are active during spring and summer, particularly from early May to late August. In some cases however, adult activity spans from February to June, due to higher average temperatures in south-eastern Spain (Peris-Felipo and Jiménez-Peydró 2011).

Old oaks are the preferred habitat of *C. cerdo*. In France, the beetle colonises oaks and chestnut trees below the altitude of 900 m a.s.l. (Horák et al. 2010). In Italy, the great capricorn develops mainly in various species of *Quercus* and has also been reported in other hardwood trees (Sama 1988). In Germany, the species developed exclusively in oak species: *Q. robur* is the preferred development tree, followed by *Q. petrea* (Neumann and Schmidt 2001) and *Q. rubra* (Nessing 1988). According to Neumann and Schmidt (2001), emergence holes belonging to *C. cerdo* have also been recorded on *Q. coccinea*, a non-native oak species introduced from North America in 1691. In Hungary, this species lives mainly in turkey oak woodlands on hill and lower mountain areas. Important populations occur in wood pastures and parklands where huge, old, solitary oaks occur. In Romania, it has been reported from all of oak species present in the country. In the European part of Turkey, larvae of *C. cerdo* developed in trees species belonging to the genus *Acer*, *Carpinus*, *Castanea*, *Cupressus*, *Fraxinus*, *Fagus*, *Platanus*, *Prunus*, *Salix*, *Quercus* and *Ulmus* (Horák et al. 2010). Adults of *C. cerdo* are mainly nocturnal and have a lifetime of a few days up to two months and they feed on mature fruit and oak sap (Weckwerth 1954, Döhring 1955, Neumann 1985).

The dispersal biology of the species is poorly known, the adults flying mainly after dusk when the temperature exceeds 18°C (Neumann 1985), but sometimes they have also been spotted in the afternoon (Müller 1950, Campanaro et al. 2011a).

In literature, no information has been found on predators of adults. It is however very likely that some mammal and bird species regularly prey on them.

Life cycle

Mating takes place during summer, when females lay their eggs in tree bark crevices or damaged parts of previously colonised oaks. Laboratory tests demonstrate that the maximum daily fecundity ranged widely, depending on the egg-laying day and female size from about 5 to 15–20 eggs/day, with some large females laying up to 30 eggs in a single day (Torres-Vila 2017). In the same study, Torres-Vila (2017) reports the measurements of the eggs of *C. cerdo*, (length × width: 3.7×1.9 mm^2) with mean dimensions slightly longer than those previously reported by other authors: 3.3×1.6 mm^2 (Marović 1973), 2.5–3×1 mm^2 (El Antry 1999) and 3.1×1.5 mm^2 (Vitali 2001). Larvae hatched from eggs have a more phloem-feeding habitus in the first year of life (they feed in the cortical part of the trunk) and then, in the following year, they start to dig tunnels that penetrate deeper into the xylem, altering sap flow and triggering leaf fall and tree decay (Neumann 1985, Bense 1995). According to Marović (1973), it is possible to rear *C. cerdo* larvae in the laboratory where the larval development can be approximately one third shorter than in the wild (Nenadović et al. 1999, Pavlović et al. 2012). The transformation from larva to adult takes place inside the pupal cell near to the surface of the trunk. The pupae are large and light coloured initially, gradually darkening until the appearance of the adult in the autumn, usually in October.

Threats and conservation

Over the last century, European populations of *C. cerdo* have suffered a dramatic decline in the number of populations and in population sizes in the whole of central Europe (Klausnitzer et al. 2003). This is mainly due to a decline in the number of old oak trees situated in open or semi-open landscapes, this representing the main threat for this species (Luce 1997, Dupont and Zagatti 2005, Buse et al. 2008a); in the Great Britain and Swedish mainland, the species is today considered extinct (Alexander 2002, Ehnström and Axelsson 2002). *C. cerdo* is classified as Vulnerable (VU), globally Vulnerable, according to the IUCN Red List of Threatened Species (World Conservation Monitoring Centre 1996). This species is listed in: (i) Appendix II of the Bern Convention as a strictly protected species, (ii) Annex II of the European Habitats Directive as a non-priority species and (iii) Annex IV of the European Habitats Directive.

Review of monitoring experience in European countries

Although *C. cerdo* is considered a threatened species in most parts of its range, a long-term monitoring programme has never been conducted. As reported by Campanaro et al. (2011a), several European countries highlighted the necessity to develop monitoring methods for this species in order to obtain information about its populations' consistency, demographic trends and habitat range, as required by the Habitats Directive (92/43/EEC). Actually, until now, no long-term monitoring projects have been carried out, except in Slovenia (Vrezec et al. 2007) and in Germany (Schnitter et al. 2006). On the other hand, some protocols for monitoring have been proposed and tested. These methods are briefly discussed below.

Capture-Mark-Recapture (CMR)

Campanaro et al. (2011a) proposed preliminary guidelines for the conservation and monitoring of *C. cerdo* and other saproxylic species in Italy by using the capture-recapture protocol. Briefly, adults were trapped with air traps baited with a mixture of vinegar (or wine or beer) and fruit (e.g. banana) during the flight period (May-August). The traps were positioned on trees where recent exit holes of beetles were found.

Beetles were marked with individual codes (colours or/and numbers) on the ventral side of the specimen (thoracic sternites or/and abdomen) using a permanent, non-toxic and odourless fine-tip marker. This method allows the estimation of the size of the population, but requires a significant sampling effort and a large number of person-hours for repeated sessions of capture and recapture. On the basis of the marked and recaptured individual ratio, it is possible to estimate the number of individuals in the population (Amstrup et al. 2005, Hill et al. 2005, Campanaro et al. 2011a, Trizzino et al. 2013). Capture-recapture estimates were also carried out on the closely related species *C. welensii* by López-Pantoja et al. (2008) and Torres-Vila et al. (2012, 2013) in Spain. The capture-recapture protocol using feeding traps (red wine, vinegar and sugar), integrated with nocturnal observation, was undertaken by Torres-Vila et al. (2012, 2013), while nocturnal observation was the method used by López-Pantoja et al. (2008).

Surveying the exit holes

In literature, there are essentially two kinds of contributions that focus on exit holes;

(i) the paper of Albert et al. (2012), who investigated the pattern of distribution of *C. cerdo* within the old open-grown oaks at two sites in Bohemia (Czech Republic). The colonised trees were identified by exit holes (Buse et al. 2007) for a total of 30 oaks with 4,279 holes. Each tree was climbed and the number of exit holes and environmental variables were recorded in relation to trunk sections (height 2 metres) and segments (orientation of the trunk). This study revealed that *C. cerdo* prefers trunks of large and old trees in open areas, especially in the sun-exposed lower parts of the trunks. "Searching the exit holes method" facilitates the detection of the presence of the species in an easy manner and it can be proposed as an effective method, although the sampling effort in climbing trees is significant.

(ii) the works of Buse et al. (2007, 2008a and 2008b). Buse et al. (2007) studied the distribution modelling pattern of the populations of *C. cerdo* in Germany in order to understand the species–habitat relationships and its environmental requirements. The presence data of the species were obtained by searching the exit holes on trees in two sites in Lower Saxony. They investigated more than 250 trees and identified that 67 of these had recently been colonised by *C. cerdo*. The results of this habitat suitability model showed that oak tree-level parameters, such as trunk insolation, presence of sap, bark depth and the distance from the next colonised tree, were able to predict the presence of *C. cerdo* much better than landscape-level predictors. It was concluded that, to facilitate the survival of the populations of *C. cerdo* and other similar saproxylic species, the management strategies should focus on semi-open woodstands (Ranius and Nilsson 1997, Buse et al. 2007).

In Germany, a standard monitoring approach based on the survey of the exit hole has been performed every five years since 2006. The field activity to estimate the population size is performed before the flight period of the adult, from September to April of the following year, by counting the number of exit hole on selected trees (e.g. n=10) per area. The number of selected trees depends on the number of colonised trees; in case the number of suitable trees is less than six, all trees should be considered (Schnitter et al. 2006).

Methods

The methods used during MIPP

During MIPP, several methods were tested for monitoring *C. cerdo*: (i) Artificial sap, (ii) Baited traps, (iii) Collecting remains of predation along transects and (iv) Visual Encounter Surveys (VES). These methods are discussed below:

Artificial sap

Manna is the sap extracted from the bark of several ash tree species (*Fraxinus*), particularly from *F. ornus* (manna ash). Many saproxylic beetles at the adult stage (including several longicorns), feed on mature fruit and on sap that flows out from the bark of trees (Neumann 1985). Observations showing that *C. cerdo* is attracted by oak sap on trees were reported by Weckwerth (1954) and Döhring (1955). Exploiting the characteristic smell of fermentation, as in baited traps, the method was aimed at mimicking the chemical component of oak sap and testing the attraction of the manna. Jansson (2011), for a similar study on *Lucanus cervus* (Linnaeus, 1758), used artificial sap prepared from pieces of oak bark and equipment for homemade wine. It was decided to use manna obtained from *Fraxinus* due to its commercial availability. As manna is generally sold in solid form as pieces of medium size, it was reduced to small pieces with a mixer and afterwards, water was added to obtain a final solution with a creamy consistency. The right consistency of the solution was obtained by mixing 150 g of manna reduced to small pieces with 60 cm^3 of water. This product was smeared with a brush on to the tree bark of selected trees to produce some 'feeding stations'. Each feeding station corresponded to a bark surface of 5 × 15 cm of smeared solution. The solution was added to bark in the afternoon and during the night between 21:30h and 22:30h, when the surveys were undertaken. Trees were selected for the presence of emergence holes of *Cerambyx* spp. (evidence of breeding) and for the absence of holes (as control) (Figure 2).

Baited traps

The baited traps used for the present research were the same as built by Campanaro et al. (2011a) and Bardiani et al. (2017a, 2017b) for monitoring *Cerambyx cerdo* and

Figure 2. Tree with manna solution smeared on the bark, used as feeding station for longhorn beetles (Photo by L. Redolfi De Zan).

Lucanus cervus respectively. They were obtained by modifying a previous aerial and baited trap model invented by Allemand and Aberlenc (1991) for trapping saproxylic beetles and chosen by several other authors for monitoring *C. cerdo* (Mason et al. 2002,

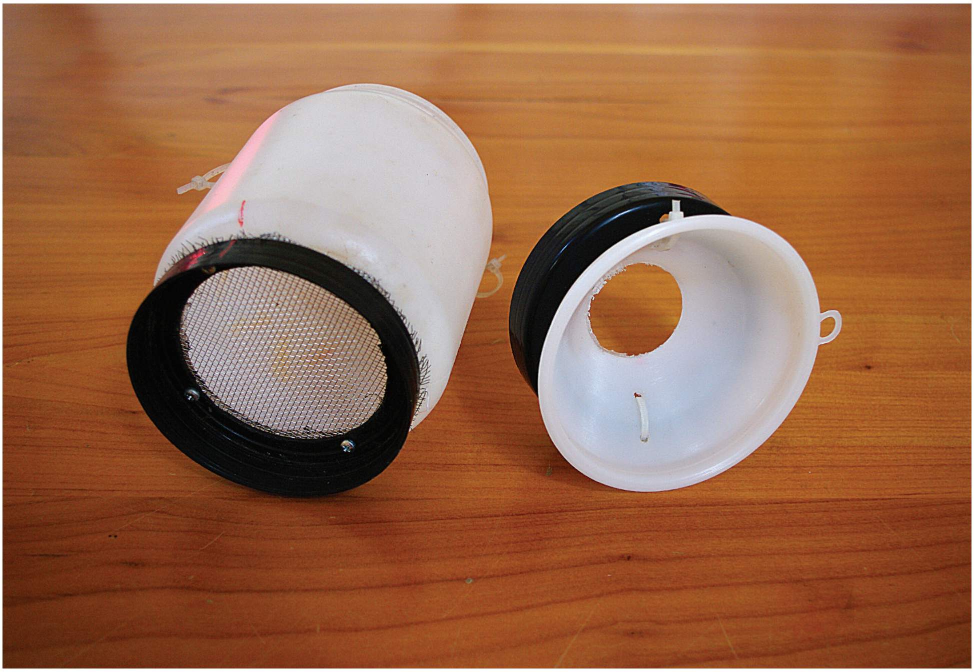

Figure 3. The upper jar of the baited trap modified with a wire insect net and a modified lid in which a plastic funnel has been inserted to collect insects falling into the trap (Photo by M. Bardiani).

Vrezec and Kapla 2007), *C. welensii* (Torres-Vila et al. 2012) and other beetles. The method was based on the attractive power of a bait (e.g. a food source that simulates a fermented sap, such as red wine + beer + sugar or squashed banana) poured into a plastic cylindrical container which was closed by a funnel (Allemand and Aberlenc 1991). Nevertheless, for the purpose of monitoring this protected species, these traps were modified (Campanaro et al. 2011a, Corezzola et al. 2012) to be less invasive and to prevent the death of the animal in the attractive liquid. Traps were all handcrafted and composed of two stacked HDPE plastic jars (1000 cm^3 each) fitted together one above the other, with a wire insect net (2 × 2 mm mesh) between them (in the upper bottle, the bottom was previously drilled with a hole of 76 mm diameter) (Figure 3). The lower jar contained the bait as a liquid mixture and the upper one represented the capture chamber. The wire net ensured that individuals survived in the trap by avoiding any contact with the liquid of the bait. The upper jar had the lid modified with a plastic funnel (diameter: 10 cm; with the stem cut out to create an opening of 4 cm diameter). Two mixtures were tested as baits: (i) red wine, beer and mashed banana ($R_wB_eB_a$) previously tested by several authors (Allemand and Aberlenc 1991, Campanaro et al. 2011a) and (ii) red wine, white wine and sugar (R_wW_wS) (suggested by P. Rapuzzi, a long horn beetles taxonomist). The mixtures were prepared a week before the setting of traps. To each litre of mixture made up by 50% of each of the two liquids

Figure 4. Baited traps set at two heights: **A** on a branch over 10 m high **B** on trunk, 1.5–2 m above understory level and **C** an example of capture of two individuals of *Cerambyx cerdo*. In picture **A** the ropes to lower and lift up the trap are visible, green and brownish respectively (Photo by M. Bardiani).

(red wine + beer or red wine + white wine), 220–330 g of banana or 220 g of sugar were added. The final volume of the bait per trap was about 500 cm^3. Traps were set at two heights: on the trunk at about 1.5–2 m high, to investigate the underground layer and on branches over 10 m high to investigate the lower canopy (the compact design and low weight of the traps allowed an easy lift up between the branches) (Figure 4). For canopy trap setting, a tree-climb slingshot (BigShot by Sherrill tree) was used for the launch of a rope, to which the trap was then tied. This rope allowed the trap to be lifted. Another rope was tied to the bottom of the trap to lower it. Trap checking was performed daily (to avoid any cases of death of collected individuals), in the morning (from 08:00h to 13:00h). Traps were activated on Monday, remained active for 4 days (i.e. surveys) and deactivated on Friday. No interception devices (e.g. panels in windows traps) were mounted on the traps, as the aim of this test was to evaluate only bait attraction efficacy and to reduce passive capture of both *C. cerdo* and other species.

Collecting remains of predation along transects

This method was based on the search and collection of remains of *C. cerdo*, in a similar manner to several monitoring and sampling studies on *L. cervus* (Campanaro et al. 2011b, Harvey et al. 2011, Bardiani et al. 2017a). The collection of remains was undertaken along transects of standard length (500 m long) and described in detail by Bardiani et al. (2017a). The collection of remains was carried out by one surveyor during daytime. All remains were collected and preserved dry in paper bags. The date, identification code of the bag, transect and sector of collection, number of specimens (counting is performed considering all the body parts reasonably belonging together as one specimen) and type of remains collected (e.g. elytra, pronotum, head, whole specimens) were recorded for each bag.

VES

This method was based on detecting active adults of *C. cerdo* on trunks (Figure 5). For this reason, a headlight was necessary (Tikka XP2, Petzl) or a torch (Led Lenser M7R) to light up trunks and lower parts of the canopy respectively. Binoculars were used to check higher parts of the tree. Sightings were divided according to the height of obser-

Figure 5. An individual of *Cerambyx cerdo* observed on tree selected for the VES (Visual Encounter Surveys) (Photo by S.G. Muñoz).

vation: up to 2 m and over 2 m. In case of co-occurrence of more *Cerambyx* species, individuals not identified were indicated as *Cerambyx* sp.

Sampling plan

The methods explained above, were tested in two study areas: Bosco della Fontana and Bosco della Mesola (Figures 6 and 7), both in the Po valley, during 2014, 2015 and 2016 (see Carpaneto et al. 2017 in this issue, for the description of the study areas).

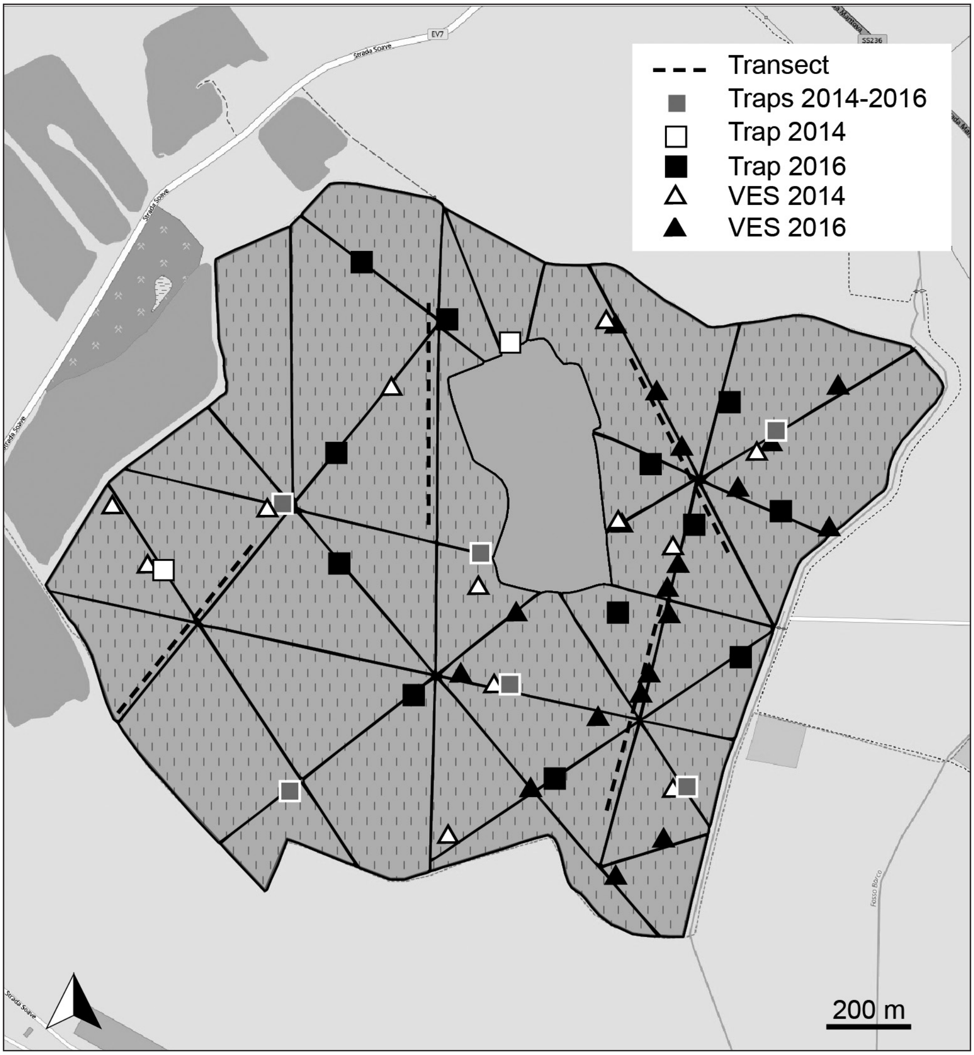

Figure 6. Map of the study area Bosco della Fontana.

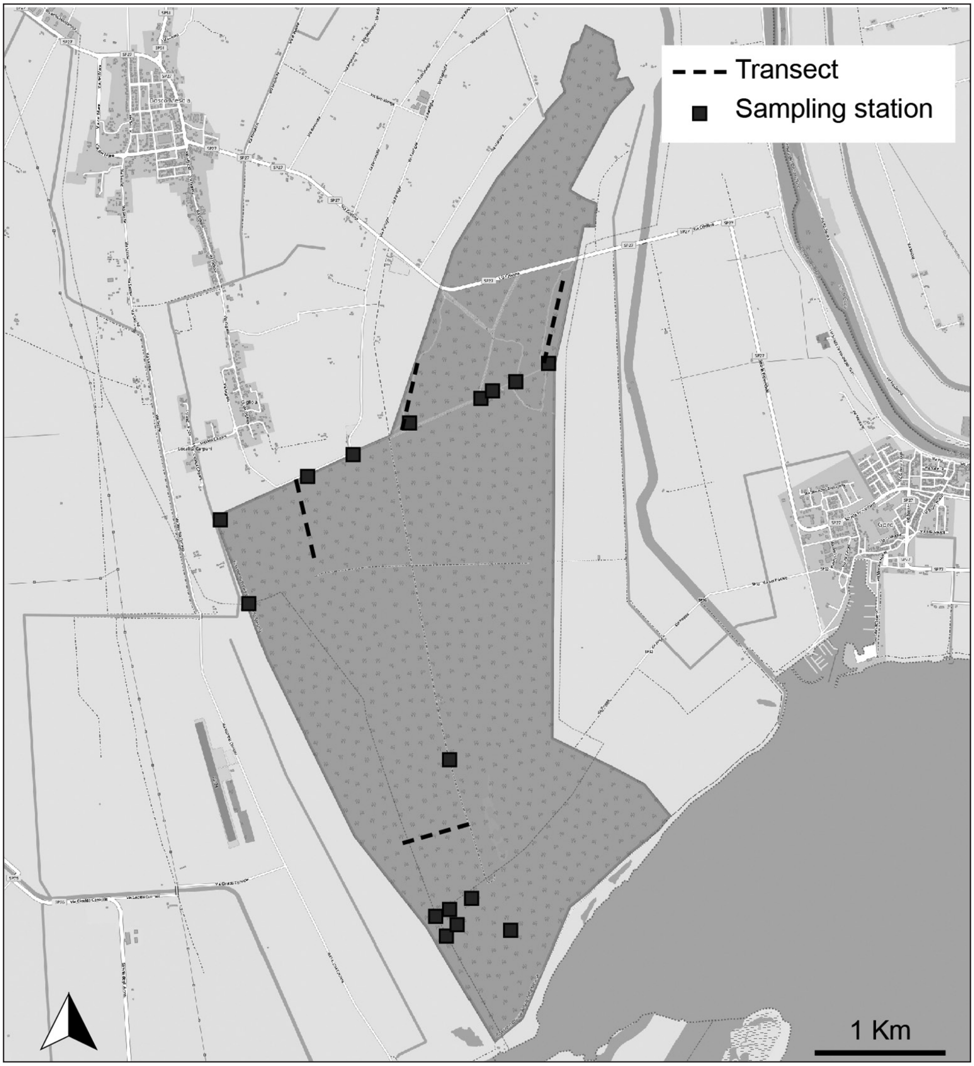

Figure 7. Map of the study area Bosco della Mesola.

The method "Artificial Sap" was tested only in 2015 at Bosco della Mesola, from 25 May to 17 July (Table1). Two kinds of feeding station were used: (i) on trunks of a potentially suitable tree for the presence of *C. cerdo* and (ii) on trunks of a tree without signs of *C. cerdo*. All trees selected were oaks (*Quercus ilex* and *Q. robur*) and suitability was established on the base of vitality and openness of the canopy, presence of exit holes, presence of sap and bark status (Buse et al. 2007). The feeding stations were distributed in 16 replicates: each replicate had both types of feeding stations (overall 32 oaks were selected). Each replicate was checked once a week (eight replicates on Tuesday and eight on Thursday) from 21:30h to 22:30h. In the afternoon (about 18:00h-19:00h), the manna of each feeding station was moistened by spraying water on it.

Table 1. Sampling plan at Bosco della Fontana (BF) and Bosco della Mesola (BM) during the three years of monitoring. N = number of transects or traps; S = number of surveys; * indicates the number of surveys for each transect.

Site	Method	2014			2015			2016		
		N	S	Dates	N	S	Dates	N	S	Dates
BF	Baited traps	48	32	27.5–18.7	-	-	-	54	24	31.5–8.7
	Remains	-	-	-	4	10*	20.5–22.7	4	7*	1.6–14.7
	VES	16	7	3.6–16.7	-	-	-	20	6	31.5–7.7
BM	Artificial sap	-	-	-	32	8	25.5–17.7	-	-	-
	Baited traps	-	-	-	32	32	25.5–17.7	-	-	-
	Remains	-	-	-	4	8*	25.5–17.7	-	-	-
	VES	-	-	-	16	8	25.5–17.7	-	-	-

The method "Baited traps" was tested in different numbers and in different years in the two study areas (Tables 1 and 2). During 2014, at Bosco della Fontana, the degree of attraction of the baits and height positions of the traps were tested. Overall 48 traps were set, arranged in eight replicates. Each replicate consisted of six traps, set in pairs on three oaks (*Q. cerris* and/or *Q. robur*) with a diameter at breast height (DBH) greater than 50 cm measured at 130 cm from the ground: one trap was set at canopy level, between 10 m and 20 m high and another at ground level on the lower part of the trunk (1.5–2 m height). For each replicate, two baits were tested: the $R_wB_eB_a$ in the first pair of traps and the R_wW_wS in the second pair (the traps of the third pair were left empty and used as control). Traps were checked for eight weeks from 27 May to 18 July (Table 1). At the beginning of each week, the pairs of traps were moved between the trees selected to change position within the replicate. In 2015, traps were set only at Bosco della Mesola: the overall number of traps was 32, on 16 trees (*Q. ilex* and/or *Q. robur*), with the same trap-setting for each tree selected. Traps were checked for eight weeks, from 25 May to 17 July (Table 1). The R_wW_wS was the only bait used (Table 2). For each trap, at the beginning of the 5th week, the bait was replaced by a fresh one. In 2016, traps were tested at Bosco della Fontana. Overall, 54 traps were set over 10 m high (no traps were set at ground level) and R_wW_wS was the only bait tested. Each trap was set on its own tree (*Q. cerris* and/or *Q. robur*, with a DBH of at least 50 cm). Traps were arranged in 18 trap replicates (3 traps per replicate) and, for each one, bait was tested at 3 different ageing stages (one per trap): (i) the mixture was never changed during the study season (A_{NC}), (ii) the mixture was replaced every three weeks (B_{3Ws}) and (iii) the mixture was replaced every two weeks (C_{2Ws}) (Table 2). Traps were checked for 6 weeks to test bait ageing. Testing for two extra weeks was undertaken after the 6th week, to investigate the last part of the season. Overall 32 surveys were undertaken from 31 May to 22 July. During the season, the position of the baits within the replicates never changed.

The method "Collecting of remains of predation along transect" was undertaken in 2015 in both study areas, whereas only at Bosco della Fontana in 2016. For each study area, four transects were selected. For both years, at Bosco della Fontana, the

Table 2. Overview of the trap sample and number of traps set in the two study areas, Bosco della Fontana (BF) and Bosco della Mesola (BM), during the three years of monitoring. Baits: $R_wB_eB_a$ (Red Wine, Beer, Banana); R_wW_wS (Red Wine, White Wine, Sugar); Control (empty traps used as control). Height at which traps were set: High (above 10 m), Low (1.5–2.0 m). Variation in ageing of the mixture: Never (mixture never changed during the season); 3 weeks and 4 weeks (mixture changed once during the season: after three or four weeks); 2 weeks (mixture changed twice during the season: every 2 weeks).

Bait	Height	Ageing	BF		BM
			2014	**2016**	**2015**
$R_wB_eB_a$	High	Never	8	-	-
	Low	Never	8	-	-
R_wW_wS	High	Never	8	18	-
		4 weeks	-	-	16
		3 weeks	-	18	-
		2 weeks	-	18	-
	Low	Never	8	-	-
		4 weeks	-	-	16
		3 weeks	-	-	-
		2 weeks	-	-	-
Control	High	-	8	-	-
	Low	-	8	-	-

transects were the same used by Bardiani et al. (2017a) for the monitoring of *L. cervus*. Each transect was monitored once a week and the survey undertaken in the same day.

The method “VES” was undertaken in all three years (Table1): 2014 and 2016 at Bosco della Fontana; 2015 at Bosco della Mesola. In the first year, 2014, 16 oaks (*Q. cerris* and/or *Q. robur*) were chosen: eight replicates close to the baited traps and eight in the rest of the Reserve. Following Buse et al. (2007), oaks were potentially suitable for colonisation by *C. cerdo*, selected on the basis of at least one of the following parameters: vitality and openness of the canopy, presence of exit holes, presence of sap, and bark status. For all trees, DBH was higher than 50 cm. Each tree was checked in four time-slots (09:00h–11:00h; 14:00h–16:00h; 19:00h–21:00h; 23:00h–01:00h), once a week (usually carried out in two slots on Tuesday and two slots on Thursday) from 3 June to 16 July (Table 1). Sixteen oaks (*Q. ilex* and/or *Q. robur*) were chosen in 2015 at Bosco della Mesola, following the same parameters for 2014 (with the exception of the value of tree diameters, as in very few trees, it was higher than 50 cm). Each tree was checked from 25 May to 17 July (Table 1), once a week (eight trees on Tuesday and the other eight on Thursday) but only in one single time-slot (21:30h–22:30h). Finally, in 2016, 20 oaks were selected following the same parameters of 2014. Each tree was checked in one single time-slot (21:30h–22:30h), once a week (10 trees on Tuesday and 10 on Thursday) from 31 May to 7 July (Table 1).

For all methods, suitability parameters of each tree were recorded and reported on field sheets (See Suppl. material 1: Field sheet 1).

Data analysis

Occupancy models were applied on captures data obtained at Bosco della Fontana only using the method “Baited Traps”; the methods “VES” and “Collecting of remains of predation along transect” did not provide sufficient data to permit statistical analysis. The Chi-Square test was used to investigate differences between the number of females and males captured, this analysis being undertaken using STATISTICA 7.0 (StatSoft Inc.), with a significance level of 0.05 to reject the null hypothesis. At Bosco della Mesola, the sampling activities carried out did not result in the detection of any individual of *C. cerdo*.

Closed vs open occupancy models

Single species and single season for closed and open occupancy models (MacKenzie et al. 2006, Kendall et al. 2013) were applied to test whether the population was open or closed. The following models were used: capture probability constant ($\hat{p}$, i.e. detection probability constant between surveys), full time (p_t, i.e. detection probability changes between surveys) or constrained time dependence (p_c, i.e. detection probability within the constrained time intervals is different from detection probabilities of time intervals before and after that constraint). Constant and full time dependence hypothesis refer to closed models. Instead, the constrained models take into account the entry probability (*e*, i.e. the emergence and/or colonisation of new individual) and the departure probability (*d*, i.e. the death and or emigration of individuals). For this analysis, the pooled data set, obtained from all traps during the entire study periods for both 2014 and 2016, was used.

Multi-method occupancy model

In 2014, the effects of different baits in relation to the height of the traps above ground were tested. In 2016, based on the results obtained in 2014, only traps with the best bait and at the best height were set; additionally, a different ageing of the bait was tested. Single species, single season and multi-method occupancy models (Nichols et al. 2008) were applied to estimate the detection probability ($\hat{p}$) for each sampling method ($_s$). Detection probability was modelled as constant over time and baits or ageing (p), as time-independent but different amongst baits or ageing (p_s), as time-dependent but constant amongst baits or ageing (p_t), or with baits or ageing as an additive effect with time-period (p_{s+t}). Small-scale occupancy, which corresponds to the presence of the species at the local sample station, was modelled as either time-independent (θ) or time-dependent (θ_t). For these models, the data set of bottle traps with different baits at different heights for 2014 and the data set of traps with different states of ageing for 2016 were used. Both data sets refer to the time interval between the first and the last capture of each study period. The data set for 2014 refers to the time interval between the first and the last capture of the study period whereas the data set for 2016 refers to the surveys from numbers 13 to 24. This period was selected to test the effect of the age of bait as, only from the 13th survey onwards, the baits A_{Nc} and B_{3Ws} had sufficiently aged to permit meaningful comparison.

Covariates effects

Single species, single season occupancy models (MacKenzie et al. 2006, Kendall et al. 2013) were applied to test the magnitude of the effect of site covariates. In 2014, two site covariates were registered during the study: Tree Diameter (TD) measured at 1.3 m height, and Tree Species (TS), *Q. cerris* or *Q. robur*. In 2016, only one site covariate was registered: Number of Suitable Neighbourhoods (NSN). The magnitude of the effect of each survey covariate was modelled as constant ($p_{\text{SURVEY COVARIATE}}$) or time dependent ($p_{t\text{SURVEY COVARIATE}}$). The relationship between *C. cerdo* occupancy (ψ) and survey covariates was tested for the data from 2014 on first and second order polynomial models (TD, TS; $TD+TD^2$, $TS+TS^2$), hypothesising single, additive (TD+TS; $TD+TD^2+TS+TS^2$) and multiplicative effects (TD*TS; $TD+TD^{2}*TS+TS^2$). For the data of 2016, only first order polynomial models were considered (NSN). For these models, reduced data sets obtained from the best bait at the best height for 2014 and from the best state of ageing for 2016 were used. Both data sets refer to the time interval between the first and the last capture of *C. cerdo* of the respective best method.

Models were ranked according to their values of AIC (Akaike Information Criterion), with models having low AIC value (i.e. more support) being ranked first (Burnham and Anderson 2002). Analyses were carried out using the programme PRESENCE (Hines and MacKenzie 2004).

To evaluate the survey effort necessary to achieve a standard error (SE) of 0.05 for the occupancy estimator $\hat{\psi}$ given the calculated ψ and p, the value of s (number of sites to investigate, i.e. in this case the number of traps) and K (number of surveys) were evaluated according to the equation of MacKenzie and Royle (2005):

$$var(\hat{\psi}) = \frac{\psi}{s}\left[(1-\psi) + \frac{(1-p^*)}{p^* - Kp(1-p^*)^{K-1}}\right]$$

where $p^* = 1 - (1 - p)^K$ is the probability of detecting the species at least once during K surveys of an occupied site.

The purpose of this analysis was to determine what values of s and K could be used to most efficiently achieve the desired level of precision for the value of occupancy ($\hat{\psi}$) using the different trap types. The values of $\hat{\psi}$ and p in the equation were the ones resulting from the best model previously selected.

Results and Discussion

Capture data

In 2014, VES did not provide any sightings whereas baited traps provided 29 captures of 28 individuals of *C. cerdo*, with no significant difference between female (16) and male (13) captures ($\chi^2_1 = 0.31$, $P > 0.05$); no beetle was found dead inside the traps. In 2015, the only method undertaken, Collecting of remains, provided three specimens. In 2016, VES provided the sightings of four individuals whereas Collecting of remains gave three specimens of *C. cerdo*. The baited traps provided 256 captures, with no significant difference between female (115) and male (141) captures ($\chi^2_1 = 1.32$, $P > 0.05$); five beetles were found dead inside the traps. Table 3 shows captures data performed by baited traps during the sampling season at Bosco della Fontana.

The phenology of the species for both years is shown in Figure 8. In 2014, the first capture was undertaken on 6 June (23rd week of the year), while the last capture was undertaken on 1 July (27th week of the year). In 2016, the first capture was undertaken on 3 June (22nd week of the year) while the last capture was undertaken on 22 July (29th week of the year).

Occupancy models

Closed vs open occupancy models

For both years, the hypothesis of constrained time dependence for capture probability was strongly supported (Table 4, *Closed vs open models*). In both years, the highest

Table 3. Summary of captures data for *Cerambyx cerdo* recorded in 2014 and 2016 at Bosco della Fontana, using baited traps with different baits ($R_wB_eB_a$ = red wine, beer, banana; R_wW_wS = red wine, white wine, sugar), different ageing (never = mixture never changed within comparison period; 2 weeks = mixture changed every 2 weeks, i.e. twice; 3 weeks = mixture changed every 3 weeks, i.e. once; over = mixture went over the comparison period), located at different heights (High = 10 m; Low = 1.5–2 m).

Year	Bait	Ageing	High	Low
2014	$R_wB_eB_a$	never	3	0
	R_wW_wS	never	21	5
	Control	–	0	0
2016	R_wW_wS	2 weeks	74	–
	R_wW_wS	3 weeks	50	–
	R_wW_wS	never	100	–
	R_wW_wS	over	32	–

Table 4. Summary of plausible models (ΔAIC < 2) obtained by model selection statistics for *Cerambyx cerdo*. Detection/non-detection data were recorded during the two years' study carried out at Bosco della Fontana in 2014 and 2016.

Year	Analysis	Model	*K*	-2Log(*L*)	ΔAIC	*w*
2014	*Closed vs open models*	ψ, e_c, d_c, p_c	6	89.74	0.00	0.53
	Multi method High	ψ, θ, p_s	5	91.37	0.00**	0.98
	Multi method Low	ψ, p	2	51.31	0.00**	0.49
		ψ, θ, p_s	5	38.77	0.76**	0.33
	Covariates effects	ψ_{TS*TD}, p	2	65.09	0.00	0.32
		ψ, p	3	63.73	0.64	0.23
		ψ_{TD}, p	3	64.33	1.24	0.17
		ψ_{TS+TD}, p	4	62.68	1.59	0.14
		ψ_{TS}, p	3	64.80	1.71	0.13
2016	*Closed vs open models*	$\psi, e_c, d_{c.}, p_c$	6	574.35	0.00	0.60
	Multi method	ψ, θ, p_s	5	603.68	0.00	0.50
		ψ, θ, p_{s+t}	16	581.94	0.26	0.44
	Covariate effect	ψ, p_t	13	209.96	0.00	0.8046

Large scale occupancy (ψ), small scale occupancy (θ), detection (*p*), entry (*e*) and departure (*d*) probabilities are considered. Constraints: $_c$ = constrained time dependence (hypothesis where *p* between the first and last capture is different from the *p* observed during the other weeks); $_s$ = methods, i.e. baits or ageing; $_t$ = full time dependence. Site covariates: TD = tree diameter; TS = Tree species; * = interactive effect; + = additive effect. Survey covariates: RH = relative humidity; TE = temperature. COVARIATE+COVARIATE^2 = second order polynomial relationship. *K* represents the number of parameters in the model; *w* the Akaike weight and −2Log (*L*) is twice the negative log-likelihood value. Akaike Information Criteria (ΔAIC) were calculated for each model. The ** indicate the use of relative Quasi-Akaike Information Criteria (ΔQAIC), i.e. the AIC modified for overdispersion (Burnham and Anderson 2002).

conditional entry probabilities (*e*) were found within the constrained sampling period, whereas the highest conditional departure probability (*d*) were found outside of the constrained sampling period (Table 5).

Multi method occupancy models

In 2014, bait (R_wW_wS) and height of the traps strongly influenced the detection probabilities of *C. cerdo* (Table 4, *Multi method* High and *Multi method* Low). Estimated detection probabilities were reported in Table 6. In 2016, the ageing of the bait strongly influenced the detection probability (Table 4, *Multi method*). The "never-changed" baits resulted in the highest value of detection probability: A_N0.26 ± 0.03, B_{3Ws} 0.16 ± 0.03, C_{2Ws}0.16 ± 0.03 (values obtained from the top model ψ, θ, p_s of Table 4, Year 2016, *Multi method*).

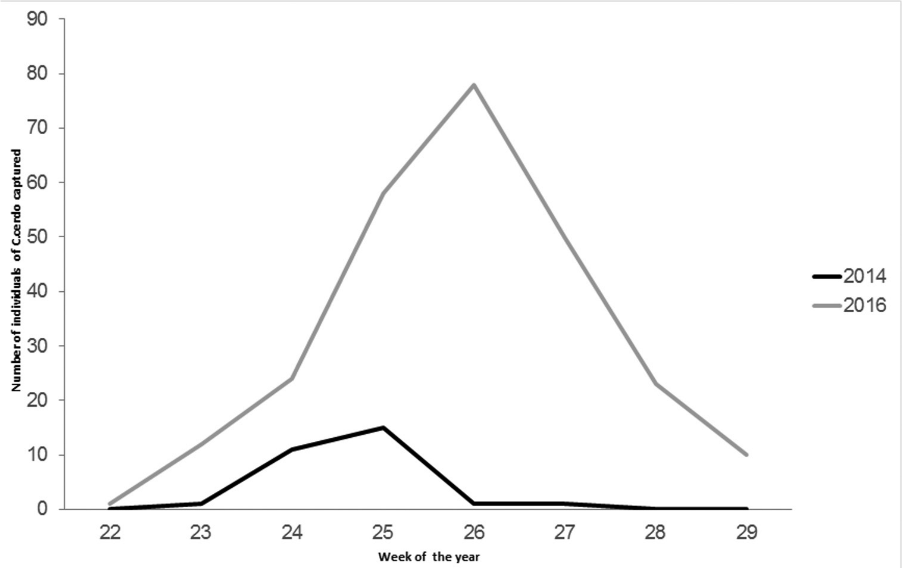

Figure 8. Phenology of *Cerambyx cerdo* during the sampling season of 2014 and 2016 at Bosco della Fontana.

Table 5. *Cerambyx cerdo* entry (*e*) and departure (*d*) probability estimates and associated standard errors (SE) are given for the top models. *w* is the Akaike's weight for each model. Detection/non-detection data were recorded during surveys carried out in 2014 and 2016.

Year	Model	w	Constrained time period (survey)	*e* (SE)	*d* (SE)
2014	ψ, e_c, d_c, p_c	0.53	1–4	0.00 (0.00)	1.00 (0.00)
			5–26	0.32 (0.13)	0.15 (0.07)
			27–31	0.00 (0.00)	1.00 (0.00)
2016	ψ, e_c, $d_.$, p_c	0.6	1–3	0.00 (0.00)	1.00 (0.00)
			4–32	0.14 (0.03)	0.03 (0.01)

Table 6. *Cerambyx cerdo* detection probability estimates ($\hat{p}$) and associated standard errors (SE) are given for the top models obtained for bottle traps (Con = control; $R_wB_eB_a$ = red wine, beer, banana; R_wW_wS = red wine, white wine, sugar) at different heights (High = 10–20 m; Low = 1.5–2 m). *w* is the Akaike's weight for each model. Detection/non-detection data were recorded during surveys of 48 bottle traps carried out at Bosco della Fontana between May and July 2014.

Bottle traps	Model	*W*	$\hat{p}_{Con}$ (SE)	$\hat{p}_{\mathrm{RwBeBa}}$ (SE)	$\hat{p}_{\mathrm{RwWwS}}$ (SE)
High	ψ, θ, p_s	0.98	0.00 (0.00)	0.05 (0.03)	0.31 (0.09)
Low	ψ, θ, p_s	0.49	0.03 (0.03)	0.03 (0.03)	0.03 (0.03)

* Parameter estimates reported are the mean values amongst the detection probability estimates for each daily check.

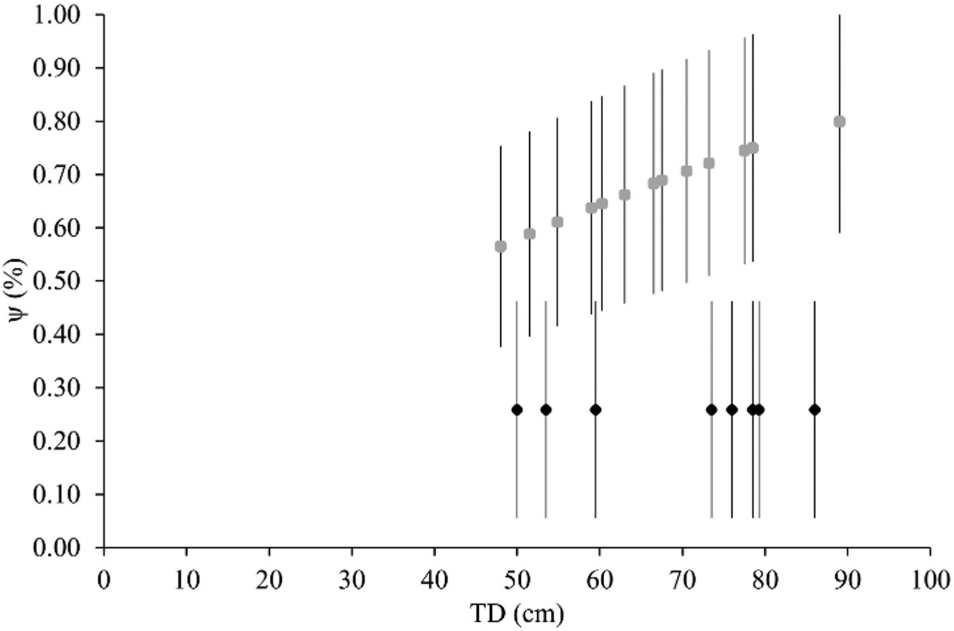

Figure 9. Estimated occupancy probability (ψ) in relation to tree species (dark grey = *Quercus cerris*; light grey = *Quercus robur*) and tree diameter (TD). Vertical bars show the standard errors (SE). Detection/non-detection data were recorded during surveys of baited bottle traps located in the Bosco della Fontana from May to July 2014.

Covariates effects

When *C. cerdo* was sampled in 2014 by baited traps with the best method (R_wW_wS) at the best position (High), between the first and last capture (Surveys 8-16), closed models were supported. For *C. cerdo*, statistical modelling reflects selection model uncertainty on species occupancy (Table 4, *Covariates effects*). Estimates of the relative importance for site covariates suggest an interactive effect between TS and TD (w_{TS*TD} = 0.32) (Table 4, *Covariates effects*; Figure 9). The occupancy of *C. cerdo*, tested in 2016, was not affected by NSN (Table 4, *Covariates effects*). To achieve a SE of 0.05 for the estimate of the parameter ψ, using the values obtained for p, with the traps set high, baited with R_wW_wS and in which the mixture was not changed during the season, it is sufficient to use 10 traps which are checked 15 times during the experimental period. In other words, 10 traps checked 15 times during the experimental period are sufficient to detect the species with a high degree of certainty (SE 0.05) if the species occurs in the area.

The standard method for the monitoring of *Cerambyx cerdo*

Description of the proposed monitoring method

From the results obtained, the use of baited traps was proposed with some practical considerations as the standard method for the monitoring of *C. cerdo*. Traps baited with red wine, white wine and sugar, positioned over 10 m high resulted in the best combination to detect the target species at Bosco della Fontana. The most important factors correlated with the capture of *C. cerdo* are the diameter of the tree (capture probability increasing with the increase in the DBH) and the tree species (*Q. robur*). At Bosco della Fontana, *Q.robur*, *Q.cerris* and *C. betulus* represent the co-dominant tree species, thus the choice of the tree for positioning of the trap should be undertaken by considering the preferences of *C. cerdo* for oak species and the native hardwood species occurring in the study area (Weckwerth 1954, Döhring 1955, Neuman 1985).

The choice of the tree species should be guided by the following considerations: (i) Searching for oak with greater DBH available (at least 50 cm if possible), (ii) Searching for oaks, living or partially dead with damage at the trunk or branches and a suffering but still vital canopy and (iii) Searching for colonised trees, with visible holes characterised by wood meal and red-coloured interior.

A partially dead tree, currently suitable for monitoring, will not be suitable after some years when degradation of the wood will progress and the tree will eventually die. Thus, for any long term monitoring programme, it is clear that the single tree initially selected will have to be replaced by other trees which will become suitable in future years. Any choice of trees to be surveyed should consider the long term monitoring of an area and hence the changes which the trees will face in the future in order to plan forest management and protect biodiversity in case of mandatory cuts. The statistical analysis of these data showed that, although the occupancy of *C. cerdo* was not affected by the number of suitable trees in the neighbourhoods, this could be influenced by the homogeneity of the forest at Bosco della Fontana, characterised by many oaks suitable for *C. cerdo*. Thus, in a forest characterised by a greater heterogeneity in terms of tree composition, the presence of large trees (DBH ≥ 50 cm) and partially dead trees, which are suitable for monitoring, it is probably advisable to add these trees to those selected for the standard monitoring programme.

As explained in sampling plan, the trap consists of two jars: the lower one containing the bait as liquid mixture, the upper one as the capture chamber. The two jars are separated by a wire net to ensure the survival of the specimens in the trap and avoiding any contact with the liquid. In practice, each litre of mixture was formed by 50% of red wine and 50% of white wine (500 cm^3 for each) with the addition of 220 g of sugar. The mixture should be prepared a week before the trap setting in order to obtain a bait with an initial degree of fermentation and to allow the sugar to dissolve completely in the mixture. As demonstrated, the bait, whose mixture was never changed, resulted in the highest value of detection probability, compared to the mixtures replaced every three and two weeks respectively. Thus, during the entire sampling period, the mixture should never be changed, except for topping up when the mixture falls below the level of 500 cm^3 due to evaporation. It is recommended to carefully attend to the traps exposed to sun, indicate the correct level of the liquid with a marking pen on the jar and to quickly check the amount of the mixture during each daily control. Traps should be positioned in each study area, at least for the first time, at two height positions: on the trunk at about 1.5–2 m high and on branches over 10 m. According to the sampling plan, in 2014, traps were positioned at two heights above ground to evaluate the presence of the species both at the underground level and canopy lower level. After the extremely low detection probability of *C. cerdo* in the underground level of Bosco della Fontana was found, only the higher traps were set in 2016. The low number of *C. cerdo* sightings at the underground level of Bosco della Fontana could be due to the highly shaded condition of the understory. This hypothesis could be corroborated by the very low number of trees with exit holes at the base of the trunk detected in this study area, rather than on the highest branches which had fallen on the ground. (Author's personal observation).

The locations of each pair of traps (low and high) should be chosen to facilitate their setting, mostly the higher ones and also to make them easy to check. It is therefore recommended to set the traps on suitable trees along forest roads avoiding steep terrain. As explained in the sampling plan section, for a baited trap positioned at a lower canopy level, a tree-climb slingshot (BigShot by Sherrill tree) is used for the launch of the rope, to which the trap is then tied. During the launch of the rope, there should be enough space around. Thus in a dense forest, the only suitable trees could be located along the forest road. The standard monitoring protocol (Table 7) needs to be repeated at the same site in future years without any changes in methodology, to ensure that the data gathered provide reliable information on eventual changes in local population abundance and can be compared chronologically as well as with other areas investigated. In each area, 10 trees should be selected; a distance of at least 100 m between them is suggested. A pair of baited traps (one low and one high) per tree must be set for monitoring. In case of very small woodlands with few suitable trees (n < 10), the number of paired traps and/or the distance between the trees should be reduced. Traps should be checked daily, three times a week for five weeks. It is recommended to check the traps in the morning (from 08:00h to 11:00h). Traps should be activated on Monday and remain active until Thursday being checked in three consecutive mornings. After the last check, traps must be temporarily deactivated by closing the jar with a lid and removing the one modified with the funnel. The duration of each survey (checking ten traps, high and low) depends on the number of captured individuals and on the distribution of the traps in the study area; as an indication, two hours should be sufficient for each survey.

Our results demonstrate that the application of the proposed protocol in terms of number of traps, frequency checks and the number of monitoring weeks allows a SE of 0.05. Furthermore, our results suggest that starting should be at the 23rd and ending

Table 7. Summary of the monitoring protocol for *Cerambyx cerdo*.

Monitoring protocol	
Method	**Baited trap**
Number of trees	10
Number of baited traps	20 traps for each site
Position on tree	One trap on the trunk (1.5–2 m high); the other on branches (over 10 m high)
Placement of baited traps	On trees along forest roads or pathways
Distance between trees with baited trap	At least 100 m
Monitoring period	June-July
Number of weeks	5
Number of surveys	15
Frequency of surveys	Three a week
Time of the day	08:00–11:00h
Number of operators	2
Hours per person	40
Equipment	A clipboard, a field sheet, a pencil, GPS, a rope, two replacement jars, bottles with mixture

at the 27th week of the year (June and early July) but this period should be adjusted according to previous knowledge about the population phenology of *C. cerdo* observed at local level. If the local phenology of the species is unknown (or cannot be reasonably inferred from available data), exclusively for the first year of monitoring, it is recommended to begin the sampling earlier from the 21st week of the year.

The standard method, described here, is based exclusively on counts of *C. cerdo* individuals captured. If additional aspects of the local population are to be investigated (e.g. population size, life expectancy etc.), the monitoring protocol proposed can be extended using the capture-recapture protocol. During MIPP fieldwork, this technique was successfully applied using tags for queen bees (http://www.enolapi.net/wordpress/prodotti/bollini-segnare-le-regine/) glued to the elytra of the adults by Loctite Super Attack Power Flex Gel) (Figure 10).

Protocol, materials and equipment

The first step involves the selection of the tree on which the baited trap will be set up, after identifying a suitable tree according to the characteristics explained above i.e. DBH ≥ 50 cm, living or partially dead and with signs of the presence of *C. cerdo*; the selected tree must be identifiable by a unique numerical code and its geographical position registered with a GPS in order to locate each single tree. During the selection of the trees, it is important to set the ropes in place on branches over 10 m for the higher traps using the sling shot, as described in the sampling plan.

Figure 10. An individual of *Cerambyx cerdo* captured with a baited trap and bearing the glued tag (Photo by M. Bardiani).

The second step involves the preparation of the bait. The mixture should be prepared a week before the trap setting in order to obtain a bait with an initial degree of fermentation and to allow the sugar to dissolve completely in the mixture. Each litre of mixture is formed by 50% of red wine and 50% of white wine (500 cm^3 of each), with the addition of 220 g of sugar. On the day established for starting the sampling activity, the mixture should be distributed inside the lower jar of each baited trap.

The third step involves the setting up of the ten baited traps: on each tree, one baited trap low and one baited trap high should be set. The lower trap must be positioned at 1.5–2 m on the trunk. Once the traps are positioned, the lid of the upper jar should be removed and replaced by the lid modified with the funnel.

The fourth and last step involves the checking of the traps. The traps should be checked three times a week, during the period of maximum activity of *C. cerdo*, when weather conditions are favourable; if weather conditions are not favourable on a pre-selected day, it is advisable to carry out the fieldwork on another day as soon as possible to prevent the death of the beetle inside the trap. Once the checking of the traps has been completed, the number of individuals collected should be counted, specifying the number of males and females. After the compilation of the fieldsheet

(See Suppl. material 2: Field sheet 2), the individuals must be released on to trees around the traps.

Spatial validity, constraints and possible interferences

In the present study, the capture-recapture protocol applied in 2016, has been used to calculate the distances covered by recaptured individuals. This calculation showed that adults of *C. cerdo* can move on average 750 m ± 309 m standard deviation (SD). Therefore, it is assumed that the validity of the results of the monitoring extends to an area surrounding the tree selected for baited traps to a maximum of 1000 m. If the average distance between the 10 selected trees investigated is 100 m and if one calculates the area which extends to a maximum of 1000 m from these trees, an area of about 300 ha is obtained. This area represents the forest surface for which the results of the monitoring are assumed to be valid. If the monitored area is located within a homogeneous forest (for tree composition, tree age, tree management, dead wood amount etc.), the validity extends to the whole of this area.

The major constraints of this method involve the obligatory daily check of the traps in order to avoid any injury or death of the individuals collected and dangerous diurnal temperatures inside the plastic traps during the day.

A possible interference on the use of baited traps is related to the presence of the dormouse (*Glis glis*). In fact, as reported by Bardiani et al. (2017a) this rodent could inhabit the trap, preventing the collection of the beetles or killing the individuals captured.

In areas which are accessible by people, the trap set low on trunks and the points where the ropes of the high trap are tied (e.g. small shrub branches), are easily visible and approachable by visitors. For the tied points of ropes, it is suggested that they be hidden as far as possible but in general, the use of explanatory signs about the monitoring and the function of the trap might be the best way to inform people and to try to avoid any possible interferences.

In addition, the use of baited traps could influence the monitoring of other beetles (e.g. stag beetles, flower chafers) which are attracted by this kind of bait (Bardiani et al. 2017b).

Counting, quantification and data sharing

In order to assess the conservation status of populations of *C. cerdo* for a given season and for a given area, a reference value is calculated as follows (Table 8):

1) For each week, calculate the total number of individuals (males + females) by adding up the number of individuals found in each baited trap. It is recommended to separately report the number of individuals captured by low and high traps.

2) Calculate the mean values of individuals captured in each week and for each type of trap (H and L).

Table 8. An example of calculation of the total and mean value of the individuals counted. The mean value obtained is the reference number to compare the long-term data and to identify a population trend. The range of values obtained during the MIPP project varied between 29 captures with 8 baited traps (Bosco Fontana 2014) and 256 captures with 54 baited traps (Bosco Fontana 2016). (BT = baited trap, H= high, L=low)

	BT1		**BT2**		**BT3**		**BT4**		**BT5**		**BT6**		**BT7**		**BT8**		**BT9**		**BT10**		**Total per week**	**Mean value per week**
	H	**L**	**H**	**L**	**H**	**L**	**H**	**L**	**H**	**L**	**H**	**L**	**H**	**L**	**H**	**L**	**H**	**L**	**H**	**L**		
Week1	0	0	1	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	3	0.15
Week 2	1	1	1	1	0	0	2	0	1	0	2	0	0	1	0	0	1	0	1	0	12	0.6
Week 3	1	3	0	1	1	0	1	1	4	1	5	4	3	1	4	1	3	0	4	0	38	1.9
Week 4	2	0	3	1	0	0	3	0	1	2	2	1	0	0	2	2	1	0	1	1	22	1.1
Week 5	1	1	0	1	1	0	0	0	2	0	0	0	1	0	1	0	0	0	0	0	8	0.4
Total per H/L	5	5	5	4	2	0	6	2	8	3	10	5	4	2	7	3	5	0	6	1	83	4.15
	Mean number of captures per trap and week																					0.83

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References

Albert J, Platek M, Cizek L (2012) Vertical stratification and microhabitat selection by the Great Capricorn Beetle (*Cerambyx cerdo*) (Coleoptera: Cerambycidae) in open-grown, veteran oaks. European Journal of Entomology 109: 553–559. https://doi.org/10.14411/eje.2012.069

Alexander KNA (2002) The invertebrates of living and decaying timber in Britain and Ireland. Research report 467. English Nature, Peterborough, Cambridgeshire, 1–142.

Allemand R, Aberlenc H-P (1991) Une méthode efficace d'echantillonage de l'entomofaune des frondaisons: le piège attractif aérien. Bulletin de la Société Entomologique Suisse 64: 293–305.

Amstrup SC, McDonald TL, Manly BFJ (2005) Handbook of capture-recapture analysis. Princeton University Press, Princeton, New Jersey, 1–296.

Bardiani M, Chiari S, Maurizi E, Tini M, Toni I, Zauli A, Campanaro A, Carpaneto GM, Audisio P (2017) Guidelines for the monitoring of *Lucanus cervus*. In: Carpaneto GM, Audisio P, Bologna MA, Roversi PF, Mason F (Eds) Guidelines for the Monitoring of the Saproxylic Beetles protected in Europe. Nature Conservation 20: 37–78. https://doi.org/10.3897/natureconservation.20.12687

Bardiani M, Tini M, Carpaneto GM, Audisio P, Bussola E, Campanaro A, Cini A, Maurizi E, Mason F, Sabbatini Peverieri G, Roversi PF, Toni I, Chiari S (2017b) Effects of trap baits and height on stag beetles and flower chafers monitoring: ecological and conservation implications. Journal of Insect Conservation. https://doi.org/10.1007/s10841-017-9965-3

Bense U (1995) Longhorn Beetles: Illustrated Key to the Cerambycidae and Vesperidae of Europe. Margaf Verlag, Weikersheim, 1–512.

Bensetti F, Gaudillat V (2002) Connaissance et gestion des habitats et des espèces d'intérêt communautaire – Tome 7: Espèces animales. La documentation Française, Muséum national d'histoire naturelle, Paris, 1–353.

Burnham KP, Anderson DR (2002) Model selection and multimodel inference: a practical information-theoretic approach. Springer, Berlin, 1–48.

Buse J, Schröder T, Assmann B (2007) Modelling habitat and spatial distribution of an endangered longhorn beetle – A case study for saproxylic insect conservation. Biological Conservation 137: 372–381. https://doi.org/10.1016/j.biocon.2007.02.025

Buse J, Ranius T, Assmann T (2008b) An endangered longhorn beetle associated with old oaks and its possible role as an ecosystem engineer. Conservation Biology 22: 329–337. https://doi.org/10.1111/j.1523-1739.2007.00880.x

Buse J, Zábranský P, Assmann T (2008a) The xylobiontic beetle fauna of old oaks colonised by the endangered longhorn beetle *Cerambyx cerdo* Linnaeus, 1758 (Coleoptera: Cerambycidae). Mitteilungen der Deutschen Gesellschaft für allgemeine und angewandte Entomologie 16: 109–112.

Campanaro A, Bardiani M, Spada L, Carnevali L, Montalto F, Antonini G, Mason F, Audisio P (2011a) Linee guida per il monitoraggio e la conservazione dell'entomofauna saproxilica. Quaderni Conservazione Habitat 6, Cierre Grafica, Verona, 1–8 + CD-ROM.

Campanaro A, Toni I, Hardersen S, Grasso DA (2011b) Monitoring of *Lucanus cervus* by means of remains of predation (Coleoptera: Lucanidae). Entomologia generalis 33: 79–89. https://doi.org/10.1127/entom.gen/33/2011/79

Carpaneto GM, Baviera C, Biscaccianti AB, Brandmayr P, Mazzei A, Mason F, Battistoni A, Teofili C, Rondinini C, Fattorini S, Audisio P (2015) A Red List of Italian Saproxylic Beetles: taxonomic overview, ecological features and conservation issues (Coleoptera). Fragmenta entomologica 47(2): 53–126.

Carpaneto GM, Campanaro A, Hardersen S, Audisio P, Bologna MA, Roversi PF, Sabbatini Peverieri G, Mason F (2017) The LIFE Project "Monitoring of insects with public participation" (MIPP): aims, methods and conclusions. In: Carpaneto GM, Audisio P, Bologna MA, Roversi PF, Mason F (Eds) Guidelines for the Monitoring of the Saproxylic Beetles protected in Europe. Nature Conservation 20: 1–35. https://doi.org/10.3897/natureconservation.35.12761

Corezzola S, Bardiani M, Hardersen S, Spada L, Toni I (2012) Progetto di attività di monitoraggio della fauna invertebrata in attuazione del piano di gestione del Sito ITB20B0011 Bosco Fontana e definizione di protocolli di monitoraggio. Relazione finale, Mantova, 1–84.

Döhring E (1955) Zur Biologie des Großen Eichenbockkäfers (*Cerambyx cerdo* L) unter besonderer Berücksichtigung der Populationsbewegungen im Areal. Zeitschrift für angewandte Zoologie 42: 251–373.

Dupom P, Zagatti P (2005) *Cerambyx cerdo* Linnè, 1758. http://www.inra.fr/opie-insects/observatoire/coleos/cerambyx/c-cerdo.htm

Ehnström B, Axelsson R (2002) Insektsgnag i bark och ved. Art- Databanken SLU, Uppsala, Sweden, 1–512.

El Antry S (1999) Biologie et dégâts de *Cerambyx cerdo mirbecki* Lucas (Coléoptere, Cerambycidae) en subéraie de la Mamora. IOBC/WPRS Bulletin 22: 59–64.

Harde KW (1996) Fam. Cerambycidae. In: Freude H, Harde KW, Lohse GA (Eds) Die Käfer Mitteleuropas band 9, Cerambycidae, Chrysomelidae. Goecke & Evers Verlag, Krefeld, Germany, 1–299.

Harvey DJ, Hawes CJ, Gange AC, Finch P, Chesmore D, Farr I (2011) Development of non-invasive monitoring methods for larvae and adults of the stag beetle, *Lucanus cervus*. Insect Conservation and Diversity 4: 4–14. https://doi.org/10.1111/j.1752-4598.2009.00072.x

Hill D, Fasham M, Tucker G, Shewry M, Shaw P (2005) Handbook of Biodiversity Methods – Survey, Evaluation and Monitoring. University Press. Cambridge, 1–573. https://doi.org/10.1017/CBO9780511542084

Hines JE, MacKenzie DI (2004) PRESENCE, version 8.3. http://www.mbr-pwrc.usgs.gov/software.html

Horák J, Büche B, Dodelin B, Alexander K, Schlaghamersky J, Mason F, Istrate P, Méndez M (2010). *Cerambyx cerdo*. The IUCN Red List of Threatened Species 2010: e.T4166A10502932 [Downloaded on 22 September 2016]

Jansson N (2011) Attraction of stag beetles with artificial sap in Sweden. Bulletin of the Amateur Entomologist's Society 70: 51–56.

Kendall WL, Hines J, Nichols J, Campbell GA (2013) Relaxing the closure assumption in occupancy models: staggered arrival and departure times. Ecology 94: 610–617. https://doi.org/10.1890/12-1720.1

Keszthelyi S (2015) Diversity and seasonal patterns of longhorn beetles (Coleoptera: Cerambycidae) in the Zselic region, Hungary. North-Western Journal of Zoology 11: 62–69.

Klausnitzer B, Bense U, Neumann V (2003). *Cerambyx cerdo*. In: Petersen B, Ellwanger G, Biewald G, Hauke U, Ludwig G, Pretscher P, Schröder E, Ssymank A (Eds) Das europäische Schutzgebietssystem Natura 2000. Ökologie und Verbreitung von Arten der FFH-Richtlinie in Deutschland. Band 1: Pflanzen und Wirbellose, Schriftenreihe für Landschaftspflege und Naturschutz Heft 69/Band 1, Bonn-Bad Godesberg, 362–369.

López-Pantoja G, Nevado LD, Sánchez-Osorio I (2008) Mark-recapture estimates of the survival and recapture rates of *Cerambyx welensii* Küster (Coleoptera Cerambycidae) in a cork oak dehesa in Huelva (Spain).Central European Journal of Biology 3: 431–441. https://doi.org/10.2478/s11535-008-0044-3

Luce JM (1997) *Cerambyx cerdo* Linneaus,1758. In: Helsdingen, PJ, Willemse L, Speight MCD (Eds) Background Information on Invertebrates of the Habitats Directive and the Bern Convention. Part I – Crustacea, Coleoptera and Lepidoptera, Nature and Environment, vol. 79. European Commission, Strasbourg, 22–26.

MacKenzie DI, Nichols JD, Royle JA, Pollock KH, Bailey LL, Hines JE (2006) Occupancy estimation and modeling: inferring patterns and dynamics of species occurrence. Academic Press, New York, 1-344

MacKenzie DI, Royle JA (2005) Designing occupancy studies: general advice and allocating survey effort. Journal of Applied Ecology 42: 1105–1114. https://doi.org/10.1111/j.1365-2664.2005.01098.x.

Marianelli L, Marziali L, Squarcini M, Benassai D, Parri F, Nardella L, Pennacchio F, Roversi PF (2011) *Cerambyx cerdo*: da xilofago di piante senescenti a nuova emergenza fitosanitaria forestale. Atti del XXIII Congresso Nazionale Italiano di Entomologia, Genova 13-16 giugno 2011: 135.

Marović R (1973) The development of Cerambyx cerdo L. under laboratory conditions. Acta entomologica Jugoslavica 9: 1–2.

Mason F, Cerretti P, Tagliapietra A, Speight MCD, Zapparoli M (2002) Invertebrati di una foresta della Pianura Padana, Bosco della Fontana, Primo contributo. Conservazione Habitat Invertebrati 1. Gianluigi Arcari Editore, Mantova, 1–176.

Müller G (1950) I coleotteri della Venezia Giulia, Vol. II Coleoptera Phytophaga (Cerambycidae, Chrysomelidae, Bruchidae). Centro Sperimentale Agrario e Forestale Trieste, pubblicazione n. 4 (1949–1953). La Editoriale Libraria, Trieste, Italy, 1–685.

Müller T (2001) Heldbock (*Cerambyx cerdo*). Angewandte Landschaftsökologie 42: 287–295.

Nenadović V, Prolić Z, Lazarević J, Al Arid L, Ivanović J (1999) Royal jelly as a food additive and its possible biostimulating effects. Acta Veterinaria 49: 105–116.

Nessing R (1988) Eichenbock (*Cerambyx cerdo*) frißt an Trauben-Eiche (*Quercus petraea*). Entomologische Nachrichten und Berichte 32: 95–99.

Neumann V (1985). Der Heldbock (*Cerambyx cerdo*). Neue Brehm Bücherei Ziemsen, Wittenberg, Germany.

Neumann V, Schmidt V (2001) New ecological-faunistic aspects of *Cerambyx cerdo* L. (Col.: Cerambycidae). Hercynia NF 34: 286–288.

Nichols JD, Bailey LL, O'Connell AF Jr, Talancy NW, Grant EHC, Gilbert AT, Annand EM, Husband TP, Hines JE (2008) Multiscale occupancy estimation and modelling using multiple detection methods. Journal of Applied Ecology 45: 1321–1329. https://doi.org/10.1111/j.1365-2664.2008.01509.x

Özdikmen H, Turgut S (2009) On Turkish *Cerambyx* Linnaeus, 1758 with zoogeogrephical remarks (Coleoptera: Cerambycidae: Cerambycinae). Munis Entomology & Zoology Journal 4: 301–319.

Palm T (1959) Die Holz- und Rindenkäfer der süd- und mittelschwedischen Laubbäume. Opuscula Entomologica Supplement XVI Lund Sweden.

Pavlović R, Grujić M, Dojnov B, Vujćić M, Nenadović V, Ivanović J, Vujćić Z (2012) Influence of nutrient substrates on the expression of cellulases in *Cerambyx cerdo* L. (Coleoptera: Cerambycidae) larvae. Archives of Biological Sciences 64: 757–765. https://doi.org/10.2298/ABS1202757P

Peris-Felipo FJ, Jiménez-Peydró R (2011) The diversity of Cerambycidae in the protected Mediterranean landscape of the Natural Park of Carrascal de La Font Roja, Spain. Bulletin of Insectology 64: 87–92.

Pesarini C, Sabbadini A (1994) Insetti della Fauna Europea. Coleotteri Cerambicidi. Natura 85: 1–132.

Ranius T, Nilsson SG (1997) Habitat of *Osmoderma eremita* Scop (Coleoptera: Scarabaeidae), a beetle living in hollow trees. Journal of Insect Conservation 1: 193–204. https://doi.org/10.1023/A:1018416000766

Rudnew DF (1936) Der große Eichenbock, *Cerambyx cerdo* L., seine Lebensweise, wirtschaftliche Bedeutung und Bekämpfung. Zeitschrift für angewandte Entomologie 22: 61–96. https://doi.org/10.1111/j.1439-0418.1936.tb00413.x

Sama G (1988) Coleoptera Cerambycidae. Catalogo topografico sinonimico. Fauna d'Italia, Vol. XXV. Calderini, Bologna, 1–216.

Sama G (2002) Atlas of the Cerambycidae of Europe and the Mediterranean Area, vol 1 Northern, Western, Central and Eastern Europe, British Isles and Continental Europe from France (Excl. Corsica) to Scandinavia and Urals. Nakladatelství Kabourek Editions, Zlín, 1–173.

Sama G, Rapuzzi P (2011) Una nuova Checklist dei Cerambycidae d'Italia (Insecta Coleoptera Cerambycidae) Quaderno di Studi e Notizie di Storia Naturale della Romagna 32: 121–164.

Schnitter P (2006) Käfer (Coleoptera). In:Schnitter P, Eichen C, Ellwanger G, Neukirchen M, Schröder E (Eds) Empfehlungen für die Erfassung und Bewertung von Arten als Basis für das Monitoring nach Artikel 11 und 17 der FFH Richtlinie in Deutschland. Berichte des Landesamtes für Umweltschutz Sachsen-Anhalt (Halle), Sonderheft 2: 145–146.

Švácha P, Danilevsky ML (1987) Cerambycoid larvae of Europe and Soviet Union (Coleoptera, Cerambycoidea), Part II - Acta Universitatis Carolinae - Biologica 31: 121–284.

Tavakilian G, Chevillotte H (2017) TITAN: Cerambycidae database (version Apr 2015). In: Roskov Y, Abucay L, Orrell T, Nicolson D, Bailly N, Kirk P, Bourgoin T, DeWalt RE, Decock W, De Wever A, Nieukerken E van, Zarucchi J, Penev L (Eds) Species 2000 & ITIS Catalogue of Life, 27th February 2017. Digital resource at www.catalogueoflife.org/col. Species 2000: Naturalis, Leiden, the Netherlands.

Torres-Vila LM (2017) Reproductive biology of the great capricorn beetle, *Cerambyx cerdo* (Coleoptera: Cerambycidae): a protected but occasionally harmful species. Bulletin of Entomological Research, 1–13. https://doi.org/10.1017/s0007485317000323

Torres-Vila LM, Sánchez-González Á, Merino-Martínez J, Ponce-Escudero F, Conejo-Rodríguez Y, Martín-Vertedor D, Ferrero-García JJ (2013) Mark–recapture of *Cerambyx* *welensii* in dehesa woodlands: dispersal behaviour, population density, and mass trapping efficiency with low trap densities. Entomologia Experimentalis et Applicata 149: 273–281. https://doi.org/10.1111/eea.12133

Torres-Vila LM, Sanchez-González Á, Ponce-Escudero F, Martín-Vertedor D, Ferrero-García JJ (2012) Assessing mass trapping efficiency and population density of *Cerambyx welensii* Küster by mark-recapture in dehesa open woodlands. European Journal of Forest Research 131: 1103–1116. https://doi.org/10.1007/s10342-011-0579-0

Trizzino M, Audisio P, Bisi F, Bottacci A, Campanaro A, Carpaneto GM, Chiari S, Hardersen S, Mason F, Nardi G, Preatoni DG, Vigna Taglianti A, Zauli A, Zilli A, Cerretti P (2013) Gli artropodi italiani in Direttiva Habitat: biologia, ecologia, riconoscimento e monitoraggio. Quaderni Conservazione Habitat, 7. CFS-CNBFVR, Centro Nazionale Biodiversità Forestale. Cierre Grafica, Sommacampagna, Verona,1–256.

Villiers A (1978) Faune des Coléoptères de France. 1. Cerambycidae. Editions Lechavalier, Paris, France, XXVII + 1–611.

Vitali F (2001) Description des œufs des espèces françaises du genre *Cerambyx* (première contribution à l'étude des œufs des longicornes) (Coleoptera, Cerambycidae, Cerambycinae). Les Cahiers Magellanes 4: 1–14.

Vrezec A, Kapla A (2007) Quantitative beetle (Coleoptera) sampling in Slovenia: a reference study. Acta entomologicaslovenica 15(2): 131–160 [In Slovenian with English summary]

Vrezec A, Polak S, Kapla A, Pirnat A, Grobelnik V, Šalamun A (2007). Monitoring populacij izbranih ciljnih vrst hrošøev – *Carabus variolosus*, *Leptodirus hochenwartii*, *Lucanus cervus*, *Morinus funereus*, *Rosalia alpina*. Nacionalni inštitut za biologijo, Ljubljana, 1–145.

Weckwerth W (1954) Unsere bekanntesten Bockkäfer Neue Brehm Bücherei. Ziemsen Verlag, Wittenberg.

Supplementary material 1

Field sheet 1

Authors: Lara Redolfi De Zan, Marco Bardiani, Gloria Antonini, Alessandro Campanaro, Stefano Chiari, Emiliano Mancini, Michela Maura, Simone Sabatelli, Emanuela Solano, Agnese Zauli, Giuseppino Sabbatini Peverieri, Pio Federico Roversi

Data type: field sheet

Explanation note: Field sheet for choosing the most suitable trees for the baited traps. The operator should mark with an "x" the corresponding box for the status of the canopy and the bark, the presence or not of sap or exit holes. It is also useful to write the presence of suitable trees around those selected.

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Supplementary material 2

Field sheet 2

Authors: Lara Redolfi De Zan, Marco Bardiani, Gloria Antonini, Alessandro Campanaro, Stefano Chiari, Emiliano Mancini, Michela Maura, Simone Sabatelli, Emanuela Solano, Agnese Zauli, Giuseppino Sabbatini Peverieri, Pio Federico Roversi

Data type: field sheet

Explanation note: Field sheet to be compiled during each survey (three a week for five weeks, 15 on the whole). For each trap checked, the operator must write the number of individuals captured, divided by sex and for trap height.

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Guidelines for the monitoring of *Rosalia alpina*

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Abstract

Rosalia alpina (Linnaeus, 1758) is a large longhorn beetle (Coleoptera: Cerambycidae) which is protected by the Habitats Directive and which typically inhabits beech forests characterised by the presence of mature, dead (or moribund) and sun-exposed trees. A revision of the current knowledge on systematics, ecology and conservation of *R. alpina* is reported. The research was carried out as part of the LIFE MIPP project which aims to find a standard monitoring method for saproxylic beetles protected in Europe. For monitoring this species, different methods were tested and compared in two areas of the Apennines, utilising wild trees, logs and tripods (artificially built with beech woods), all potentially suitable for the reproduction of the species. Even if all methods succeeded in the survey of the target species, these results showed that the use of wild trees outperformed other methods. Indeed, the use of wild trees allowed more adults to be observed and required less intensive labour. However, monitoring the rosalia longicorn on wild trees has the main disadvantage that they can hardly be considered "standard sampling units", as each tree may be differently attractive to adults. Our results demonstrated that the most important factors influencing the attraction of single trunks were wood volume, sun-exposure and decay stage. Based on the results obtained during the project LIFE MIPP, as well as on a literature review, a standard monitoring method for *R. alpina* was developed.

Keywords
Habitats Directive, Saproxylic beetles, Monitoring methods, Transects, Logs

Introduction

The rosalia longicorn, *Rosalia alpina* (Linnaeus, 1758), is a large longhorn beetle (Coleoptera: Cerambycidae), generally associated with beech forests with the presence of mature, dead (or moribund) and sun-exposed trees occurring in open sites. It is listed in Annexes II and IV of the Habitats Directive (Council Directive 92/43/EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora). The Habitats Directive provides that Member States prepare, every six years, a report on the conservation status of the species listed in the Annexes. In order to address this obligation, the Life Project "Monitoring of insects with public participation" (LIFE11 NAT/IT/000252) (hereafter, MIPP) conducted experimental fieldwork to develop a standardised method for the monitoring of the saproxylic beetle species of the project: *Osmoderma eremita* (hermit beetle, Scarabaeidae), *Lucanus cervus* (European stag beetle, Lucanidae), *Cerambyx cerdo* (great capricorn beetle, Cerambycidae), *Rosalia alpina* (rosalia longicorn, Cerambycidae) and *Morimus asper/funereus* (morimus longicorn, Cerambycidae).

The present paper is part of a special issue on the monitoring of saproxylic beetles which are protected in Europe and is focused on *R. alpina*. Firstly, a comprehensive revision of the current knowledge on systematics, distribution, ecology, ethology and conservation of *R. alpina* has been provided. A detailed account of the fieldwork carried out during the MIPP project has then been reported. The statistical analyses investigated habitat preference of *R. alpina* and compared different monitoring methods in order to develop a quick and reliable protocol for the monitoring of this species. Finally, this paper concludes with a description of the proposed monitoring method.

Systematics and distribution

R. alpina belongs to the family Cerambycidae, subfamily Cerambycinae. The species was described on the basis of a specimen collected in the Swiss Alps by Scheuchzer in 1703 (Duelli and Wermelinger 2010). Although the genus *Rosalia* (sensu stricto) includes five species in the Holarctic Region (Tavakilian and Chevillotte 2017), only one occurs in Europe.

The populations of *R. alpina* live mainly in the mountain regions of central and southern Europe from the Cantabrian range to the southern Urals and the Caucasus (Müller 1950, Von Demelt 1956, Sama 1988, 2002, Bense et al. 2003, Binner and

Bussler 2006, Özdikmen 2007, Krasnobayeva 2008, Shapovalov 2016, Danilevsky 2017). Apparently, the geographic range of the species mainly tracks the distribution of the beech (*Fagus sylvatica* and *F. orientalis*) but also extends to areas where these trees are absent, especially in central Europe and southern Russia. All records from North Africa or Levant countries (except for Turkey) are doubtful because of errors or misinterpretation of collection localities (Sama 2002, 2010, Alì and Rapuzzi 2016). Two subspecies of *R. alpina* were reported in the literature (Sama and Löbl 2010): *R. alpina alpina* (Linnaeus 1758) widespread in Europe and Northern Turkey (Pontus range) and *R. a. syriaca* Pic, 1895 (Müller 1950, Von Demelt 1956, Sama 2002). The latter was described from Akbes (=Akbez), a village in southern Turkey, close to the border with Syria. This relic population was considered extinct by Sama (2002) but recently confirmed on the western slopes of the Nur mountains (Topaktas village, Dortyol area) (Sama et al. 2012, Alì and Rapuzzi 2016). Although the range of *R. alpina* appears relatively extended, its populations are today highly fragmented because of the rarefaction of suitable habitats (see "Conservation status and threats" section). In fact, in many parts of its range, the species is now restricted to small patches of broadleaf mountain forests, rich in senescent trees (mainly beech) and with abundant decaying wood (Sama 1988, Duelli and Wermelinger 2010). The species is occasionally found in coastal woodlands, e.g. in southern Italy and Bulgaria. Of the few known isolated Italian coastal populations of *R. alpina*, associated with hygrophilous woodlands, without beech trees, one has become extinct (e.g. Maccarese, Rome) and one is still present (Policoro, Matera) (Rapuzzi P. pers. com.).

Genetic analysis (Drag et al. 2015) pointed out a high genetic diversity in populations of *R. alpina* from north-western Greece and a significant decline in diversity related to latitude. In the northern hemisphere, there is a common genetic pattern of a post-glacial colonisation from a glacial refugium (Hewitt 2000). In fact, populations from central Europe are genetically less variable than those from the south, where distinct refugia in the four Mediterranean peninsulas occurred. In this context, Italian populations of *R. alpina* seem to be genetically unique and, thus, may represent relevant conservation units in Europe (Molfini M. pers. com.).

Morphology

Adults of *R. alpina* (Figure 1) are approximately 14-40 mm long (antennae excluded) and have a peculiar colour pattern with a velvety bluish-grey to light blue body and some symmetrically arranged black spots of variable shape on the elytra (Bense 1995, Bense et al. 2003). The black spots (usually three on each elytron) can be more or less large and variously shaped, sometimes partially/totally fused, rarely extended to the whole elytra, sometimes completely absent and they can be replaced by transverse black stripes (Demelt 1956, Bense 1995, Luce 1996, Harde 1996, Bense et al. 2003). The most common dorsal pattern shows six almost symmetrical spots: the hind spots are the smallest while the mid spots are the largest. A black spot can also be present

Figure 1. Photograph of an adult specimen of *R. alpina* (photograph by P. Buonpane, taken in the locality Piana delle Sécine, Letino (CE), on date 12.07.2015, record n. 2229, citizen science database of the LIFE MIPP Project.

on the pronotum, usually adjacent to its anterior margin (Villiers 1978). The high variability of this dorsal pattern, especially in shape and size of the elytral spots, was recently used for individual identification and also to carry out capture-remark studies (Luce 1996, Caci et al. 2013, Rossi de Gasperis et al. 2017). The antennae are long and show a clear sexual dimorphism: they are a little longer than the body in females, while they are up to twice the body length in males (Harde 1996). The colour of the antennal segment varies according to their anatomic position: the first two antennal segments are wholly black, from the 3rd to the 6-7th are blue, each with a conspicuous tuft of black hairs on the apex, while the last three or four segments are blue with a dark and smooth apex (Villiers 1978, Bense 1995). These morphological features make the adults of *R. alpina* unique and make them difficult to confuse with any other longhorn beetle of the European fauna (Luce 1996).

The larva of *R. alpina* shows the typical traits shared by many wood-boring longhorn beetles: body elongate, subcylindrical with dorsal and ventral side slightly flattened, lightly sclerotised surface, almost glabrous with small and scattered setae; head typically retracted into prothorax, with mouthparts well-sclerotised and dark; prothorax enlarged with areas of distinct asperities; legs reduced. In particular, the larva of *R. alpina* has a body of creamy-white colour, yellowish thoracic segments and pitchy-brown mouth parts; the pronotum has antero-dorsal bright orange areas with asperities; the small legs are well distinct; body is up to 40 mm long and 9 mm wide (cf. Švácha and Danilevsky 1987, Duelli and Wermelinger 2010).

Ecology

R. alpina is an obligate saproxylic, xylophagous, xerothermophilic species. The habitat selection and host plants' preference across Europe have been thoroughly investigated (Sama 2002, Duelli and Wermelinger 2005, Ciach et al. 2007, Horák et al. 2009, Čížek et al. 2009, Russo et al. 2011, 2015, Trizzino et al. 2013, Michalcewicz et al. 2013, Di Santo and Biscaccianti 2014, Castro et al. 2016). In contrast, only a few studies have been published on adult and larval ecology and behaviour (Drag et al. 2011, Russo et al. 2011, 2015) and many gaps in knowledge still exist.

R. alpina has a plastic ecology in Europe. It is considered a montane species, associated with beech forests but the species is also able to colonise a variety of other deciduous tree species (i.e. Aceraceae, Betulaceae, Fagaceae, Oleaceae, Tiliaceae, Ulmaceae), from the coastline to about 2000 m a.s.l. (Duelli and Wermelinger 2005, Ciach et al. 2007, Cizek et al. 2009a, Bosso et al. 2013, Lachat et al. 2013, Di Santo and Biscaccianti 2014). At the landscape level, *R. alpina* prefers open and semi-open woodlands rather than forests (Russo et al. 2011). On a smaller scale, it can reproduce in a wide range of trees, but shows a preference for mature, dead (or moribund) and sun-exposed trees occurring in open sites and/or in sites with a low percentage of canopy closure. Finally, the species prefers trees not surrounded by tall undergrowth which might impede flight. Additionally, trees occupied by *R. alpina* had, on average, thicker bark when compared to trees not occupied (Russo et al. 2011).

These specific requirements are responsible for the fact that forest management practices are predicted to be drivers for population trends of *R. alpina* and thus also drivers for local extinction. In general, the relatively limited dispersal capacity of adults clearly exposes this species to risks imposed by habitat fragmentation (Drag et al. 2011, Russo et al. 2011, Bosso et al. 2013) (see "Conservation status and threats" section for further details).

Generally, adults of *R. alpina* are active and mobile. Under sunny and warm weather conditions, they can be active from 10:00h-11:00h until 16:00-18:00h with peaks at around 12:00h and 14:00h while no differences in daily activity patterns have been found between males and females (Drag et al. 2011).

Although adults usually move within a habitat patch, they are also able to fly long distances between patches. Mark-recapture studies showed that local movements are quite common within patches, in the range of dozens to hundreds of metres and no difference between sexes was found (Drag et al. 2011). The longest registered dispersion is 1.5 km (Drag et al. 2011, Rossi de Gasperis 2016). However, mark-recapture techniques might significantly underestimate dispersion distances.

The maximum lifespan in the wild, estimated by means of a mark-recapture study in the Czech Republic, was found to be 24 and 15 days for males and females respectively (Drag et al. 2011). Adults seem not to depend on flowers with pollen (Lachat et al. 2013) and might not feed at all, as has been observed in species of several subfamilies of Cerambycinae. Data published by Drag et al. (2011) support this view, as no feeding was observed in more than 1500 capture events.

Adult phenology depends on latitude, altitude and local climatic conditions. Although the emergence of adults can start in May, the most likely encounter period is between July and August (Duelli and Wermelinger 2005, Drag et al. 2011) and the first and last recorded captures are between the third decade of June and the second decade of September (Di Santo and Biscaccianti 2014). The period of greatest activity in Italy is from mid-July to mid-August (Duelli and Wermelinger 2005) as has also been confirmed by the LIFE-MIPP monitoring of *R. alpina* in beech forests of Foreste Casentinesi (Tuscany-Emilia Romagna) and National Park of Abruzzo, Lazio and Molise (Rossi de Gasperis et al. 2017). Citizen Science data from Italy showed a longer period: *R. alpina* was active from mid-May to mid-September, but most observations were reported between early July and late August. Data also show the peak of activity might occur even later with increasing altitude (Campanaro et al. 2017).

Males emerge almost a week before females and remain on the cracked bark of a dry trunk exposed to sunlight defending their territory against other male competitors (Duelli and Wermelinger 2005). Soon after copulation, females lay their fertilised eggs in crevices of dry wood of old, standing (partially vital) trees exposed to the sun. To identify suitable sites for oviposition, females probe the bark with their sensory organs and ovipositor (Duelli and Wermelinger 2005); they usually prefer trunks or branches at least 20 cm thick (Castro et al. 2012) with dry or decomposing wood (Bense 1995). Females of *R. alpina* prefer trunks rather than branches (Castro et al. 2012) but occasionally also lay eggs on stumps or large branches which have fallen on the ground (Duelli and Wermelinger 2005, Campanaro et al. 2011, Castro et al. 2012). Such preference for standing trunks of large diameter might be explained by: (i) a greater food availability in larger trunks, (ii) greater isolation from the humid decay conditions typical of dead/fallen woods and (iii) larger trees representing more durable habitat in terms of both food and humidity conditions (Castro et al. 2012).

Females clearly prefer bare wood for oviposition (even though they do not seem to mind wood with bark) and lay their eggs in deep crevices (Čížek L. pers. com.). Larvae hatch ca. 1-1.5cm under the wood surface and they move deeper as they grow. As a consequence, most galleries occur at a depth of 4–10 cm (Čížek L. pers. com.). Larval development is complete after two-three years depending on weather conditions and wood quality (Sama 1988, 2002). In Europe, larvae have often been found in the wood of beech (*Fagus* spp.) but in many cases they develop in the wood of other broadleaf species, such as maple, elm, ash, chestnut, alder, willow, hazel, linden and hornbeam trees (Sama 2002, Duelli and Wermelinger 2005, Ciach et al. 2007, Čížek et al. 2009, Horák et al. 2009, Simandl 2012, Michalcewicz et al. 2013, Trizzino et al. 2013). In keeping with this observation, numerous populations exist which do not rely on beech, such as in lowlands of central and south-eastern Europe, as well as in Russia (except for Caucasus); even in beech forests, the beetle regularly exploits maples and elms (Čížek L. pers. com.). Before their last winter, larvae move towards the surface (cortex) and, in spring or early summer, they build a pupation cell and a vertical and elliptical exit-tunnel. The exit holes span from 4.9 mm to 12 mm in length and from 3 mm to 8 mm in width (Campanaro et al. 2011, Ciach and Michalcewicz 2013). The shape of the tunnel is usually elongated along the longitudinal axis, which is parallel to the direction of the wood fibres of the trunk or branches (Campanaro et al. 2011). Width and height of the exit holes are positively correlated with adult size, in particular with adult pronotal width (Ciach and Michalcewicz 2013).

Conservation status and threats

Rosalia alpina is listed in Annex II of the European Habitats Directive and considered "Nearly Threatened" (NT) in Italy (Carpaneto et al. 2015) and "Least Concern" (LC) at the European (Nieto and Alexander 2010) and Mediterranean scale (Verdugo et al. 2016). On a global scale, the species has been classed as "Vulnerable" (VU) (World Conservation Monitoring Centre 1996). As mentioned before, the present geographical distribution of *R. alpina* appears very fragmented (Sama 2002; Jurc et al. 2008) due to the loss of suitable habitats (Čížek et al. 2009, Drag et al. 2011, Russo et al. 2011, Michalcewicz and Ciach 2015). Habitat fragmentation leads to isolated populations, this being a considerable threat for species, as *R. alpina* and other saproxylic beetles are characterised by their limited dispersion capabilities (Drag et al. 2011, Bosso et al. 2013).

The main drivers for population trends, including local extinctions, of *R. alpina* are: (i) the abandonment of traditional forest management (such as pollarding and the management of wooded pastures) and/or their conversions to high forests, which reduces the availability of sun-exposed trees (Drag et al. 2011, Lachat et al. 2013), (ii) the removal of dead wood or veteran trees, which causes a marked decrease in dead wood availability and negatively affects the survival of *R. alpina* (Duelli and Wermelinger 2005, Čížek et al. 2009, Russo et al. 2010); the senescent or dead standing trees can, in fact, accommodate a large number of larvae and adults and thus represent "key trees" for a given population or saproxylic community (Audisio et al. 2014), (iii) the practice of stacking felled trees in forests, which attract egg-laying females and which represent an ecological trap, if this wood is removed and used by man before adult emergence (Duelli and Wermelinger 2005, Adamski et al. 2016) and (iv) forest fires (Duelli and Wermelinger 2005, Trizzino et al. 2013).

Review of monitoring experience in European countries

The methods so far proposed for the monitoring of *R. alpina* can generally be assigned to one of the two main strategies: (i) counting of new emergence holes and (ii) searching for adults along transects or in reference plots. However, it seems important to additionally mention that Ray et al. (2009) reported the identification, synthesis and field bioassays of a volatile, male-produced aggregation pheromone of *Rosalia funebris* Motschulsky, 1845 [(Z)-3-decenyl (E)-2-hexenoate]. The authors collected significant numbers of adults in field bioassays in the USA by using traps baited with this compound. If the aggregation pheromone of *R. alpina* is identified, this might open up new possibilities for monitoring.

In the following paragraphs, an overview of the monitoring methods published for the different European countries is reported.

Austria

Paill andMairhuber (2010) searched for exit holes of *R. alpina* in standing and lying beech trees. Friess et al. (2014) investigated a total of 79 “standard sites” (each measuring 141 m × 141 m) in the Nationalpark Kalkalpen and searched for exit holes in beech trees, inspecting the higher parts of trunks with binoculars. Additionally, adults of *R. alpina* observed during July and August were also noted. This work was carried out by two persons within a total of 20 field days.

Bulgaria

The monitoring protocol used in Bulgaria (Anonymous 2015) is based on transects with a total length of 1 km, carried out by two people walking side by side and detecting the presence of *R. alpina* on each side. Within the transect, participants focused on finding micro-habitats suitable for the species and then searched for adults of the species within the micro-habitats. Operators moved in parallel at a relatively short distance from each other (1–1.5 m) and visually searched for appropriate types of micro-habitats to about 10 metres from the imaginary middle of the transect (i.e., the transect has a width of 20 metres). Suitable micro-habitats are large, standing, declining or dead trees, felled or fallen trees or large branches or piles of cut wood. Monitoring of *R. alpina* was carried out on sunny days, without wind and during the hottest hours of the day, between 11:00h and 17:00h and preferably between 13:00h and 15:00h.

Czech Republic

The standard method for the Czech Republic are transect walks 20 m wide and monitoring has to be carried out four times in intervals of about one week; preferably between mid-July and mid-August (Vávra and Drozd 2006). The one-way transect walks were conducted on very warm and windless days and the number of adults, both flying and perching on trees, were recorded. Each site should be visited every three years. The authors suggested that it might be helpful to also apply the mark and recapture method as this allows the estimation of population size. It is also recommended to mark fresh emergence holes, as this allows gathering additional information on the populations. Čížek et al. (2009b) monitored the last population of *R. alpina* in Bohemia, using the mark-recapture approach and marked 598 individuals. Drag et al. (2011) selected sites of old beech forests using aerial photos and here searched for adults and exit holes between 7 and 25 July, from 10:00h to 17:00h under suitable weather conditions. Based on these data, they were able to divide the sites into three categories according to the estimated volume of available dead-wood (low, medium, high) and according to the status of the local population of *R. alpina*: (i) large population – adults and exit holes commonly found, (ii) small population – exit holes and/or adults rare and (iii) no evidence – neither exit holes nor beetles observed. Subsequently, they conducted mark-recapture studies in those sites which had been classed as containing large populations. Firstly, suitable trees (old, dead or with dead parts), coarse woody debris and other trees (live, rotten, stumps etc.) were selected. The selected trees and coarse woody debris were searched for adult beetles in suitable weather (>15°C, no rain) between 10:00h and 18:00h. Individuals were marked on the elytra using black permanent marker and the tip of the elytra was cut to distinguish marked individuals even if the marker had washed off. The beetles were also photographed.

France

Bensettiti and Gaudillat (2004) stated that the observation of this species in the field is often accidental and that it is currently difficult to establish a quantitative monitoring programme for populations. In recent years, a national inventory of saproxylic beetles was set up, aiming to establish the current distribution of each species of saproxylic beetles in France (http://saprox.mnhn.fr). The first results for *R. alpina* have been recently published (de Flores and Sueur 2015); about 400 contributors responded to the call for the survey in 2014, providing 979 data points.

Germany

In Bavaria, the standard monitoring method for *R. alpina* is searching for the characteristic emergence holes. During the first months after emergence, the bright colour of the wood on the inside allows the separation of holes created in the current year from older ones. This method was first tested in 2004 and, from 2006, it has been used as a standard method for monitoring in Natura 2000 sites in Bavaria (Binner and Bußler 2006, Bußler et al. 2016). Prior to the fieldwork, a digital map was produced which identified potential habitats for *R. alpina* (Binner and Bußler 2006). Every 100 ha of forest site were investigated and, in each site, 5-10 dead wood structures suitable for *R. alpina* were studied. The area of the single sites was not standardised but was defined based on the availability of suitable structures (Binner and Bußler 2006). In 2006, the same method was also indicated as the standard method for Germany (Binner and Bußler 2006), the authors specifying that it was not obligatory to search for adults and that the observation of adults was only informative and might be considered an indication of dispersal. Additionally for Baden-Würtemberg (Biewald et al. 2014), monitoring of *R. alpina* was based on counts of emergence holes and fresh holes were to be separated from older ones. More than 50 fresh emergence holes were considered to be indicative of large populations, 6-50 emergence holes indicated middle-sized populations and less than 6 holes were interpreted as small populations.

Poland

A monitoring protocol has been proposed by Ciach (2015). The monitoring of the conservation status of the populations was based on the observation of adults (imagos and their remains) and on searches for emergence holes. Naturally occurring dead wood structures of deciduous species, mainly beech, were searched. No structures which attract *R. alpina* were used, such as log piles and stacks of wood, as these might lead to misinterpretations on the sites where populations were present. A forest area was chosen that included habitats which were currently breeding sites of *R. alpina* or were potentially suitable for reproduction. An area of 1 km^2 was selected and it was divided into four quarters. A transect, measuring 500 m, was placed in each quarter. On each transect, five circles were placed, with a diameter of 10 m, evenly spaced every 100 m. Here searches for *R. alpina* were carried out by carefully checking each tree which was potentially suitable for breeding (mainly dead beech trees and other deciduous species). Adults and exit holes were searched, by scanning the surface of trunks and branches using binoculars if required. The number of individuals was recorded.

Romania

Fusu et al. (2015) indicated the selection of an area of approx. 1 ha (e.g. a length of 500 m and a width of 20 m) and to search characteristic host plants and microhabitats for *R. alpina,* these being dead wood or old living trees, often *Fagus*, but sometimes also *Acer* ssp. or other hardwood species. Adults can also be found on piles of recently felled logs. The number of host trees, their geographical coordinates and number of individuals of beetles were recorded. If characteristic habitats were present in sufficient numbers, three searches were performed per 1ha. The number of observers recommended was three.

Slovenia

Vrezec et al. (2012) applied a daytime survey of freshly cut *Fagus sylvatica* and suggested the survey of at least 33 wood units to estimate abundance and also to use 78 wood units to confirm species absence in an area. The period of the year indicated for the field work is late July – early August. In parallel with the application of the monitoring method, records of *R. alpina* were collected from the public. This Citizen Science approach was an integral part of the national monitoring. Each year, different media (TV, local papers, national newspapers, websites etc) were used to reach the public, for example posters were exhibited in schools, shelters, protected areas etc. All records were validated by experts (Vernik M. pers. com.).

Spain

Pagola Carte (2007) monitored *R. alpina* in plots by selecting seven sampling units (stumps, logs, snags etc.) in each. The plots were visited nine times in the months of July and August. Monitoring of *R. alpina* was carried out by searching for live individuals and remains (elytra, legs, antennae etc.) of either sex between 13:00h and 16:00h. The seven sampling units were investigated during a total of 10 minutes (not counting the time employed for moving from one unit to another). The authors suggested that individuals should be photographed for future identification. Castro et al. (2012) surveyed beech trees located in 81 1×1 km UTM grids by placing routes through accessible potential habitats (woodlands and forests with old or dead trees). All sun-exposed dying and dead beech trees (snags and logs) were surveyed for adults of *R. alpina* and emergence holes were counted mainly from June to September. Castro and Fernández (2016) monitored a population of *R. alpina* in the following way: (i) firstly, they identified trees available as habitats in the study area and (ii) these trees either showed emergence holes or hard and dry dead wood, which could also be part of a tree with a minimum volume equivalent to a trunk with a diameter of 25 cm and 2 m long. Each tree was visually inspected for living adults and remains eight times during July and August 2010, between 11:00h and 18:00h. Finally, the number of emergence holes per tree was recorded from September to November.

Switzerland

Duelli and Wermelinger (2005, 2010) placed trunks of *Fagus sylvatica* in three stations where *R. alpina* was known to be present. In each of the stations, dead trunks of various types and sizes were exposed: long (2 m) and short (1 m) trunks, lying and vertical, large (>25 cm) and small (<20 cm). *R. alpina* (males and females) preferred large and long, standing trunks.

Italy

Russo et al. (2010) searched the study area for trees used by *R. alpina* by walking 49 strip transects (surveying a 50 m buffer around each transect) in July and August 2007.

Average length of transects was 366 m ±134 m standard deviation (SD). They examined all suitable trees, including large live trees with decaying parts, snags, stumps (i.e., standing dead wood) and fallen trunks for adults. The authors pointed out that *R. alpina* creates emergence holes of potentially diagnostic shape, but that there is a risk of confusion with holes made by other beetles or woodpeckers. These holes might indicate trees which had been occupied in the past, but which may not be at the time of data collection, providing suitable conditions and microhabitats for the species. However, the authors found that adult beetles as well as emergence holes showed that the open forest was used more by *R. alpina*. Mazzei et al. (2013) investigated forests in Calabria in the late summer of 2013, through careful control of large senescent trunks presenting obvious signs of the presence of saproxylics, searching for dead and alive adults as well as for emergence holes. The monitoring proposed by Trizzino et al. (2013) was based on mark-recapture methods and *R. alpina* was searched on wood elements which are suitable for their development, such as old trees with clear signs of debility, dead standing trunks, uprooted trees, stumps, branches (>20 cm) on the ground and wood piles. A minimum of five stations were searched between 11:00h and 17:30h, eight times during July and August. Each time, every station was inspected for a minimum of five minutes. Rossi de Gasperis et al. (2017), in the context of the MIPP project, tested a computer-aided photographic identification of natural markings which could be implemented in a capture-mark-recapture population study of *R. alpina*. In addition, Bologna et al. (2016) suggested applying the mark-recapture method which uses photographs. In a study, site monitoring stations were selected and these were defined as: old trees with clear symptoms of decay (fruiting bodies of fungi, dead wood present in the trunk or in branches), standing dead trees, uprooted trees, stumps with roots, large branches (diameter >20 cm) on the ground and woodpiles. Subsequently a transect was defined which connected at least five stations and individuals of captured *R. alpina* were photographed and released. Surveys were to be carried out twice a week, for 4 weeks. It was recommended to carry out the field work in July-August, on sunny days and between 13:00h and 17:30h.

Summary of the monitoring experiences

Monitoring methods from eleven European countries have been reported. In six countries, the monitoring of *R. alpina* was focused on searching for adults (Bulgaria, Romania, Slovenia, Spain, Switzerland and Italy) while, in two countries the monitoring was based on searches for emergence holes (Austria and Germany). In the Czech Republic and in Poland, the monitoring was based on searches for emergence holes and adults. Finally, in France there was no national monitoring programme for *R. alpina*.

Analysing the monitoring methods amongst the six countries which searched for adults of *R. alpina* along transects: three countries conducted the searches along transects which varied in length from an average of 366 m (Italy) to 1 km (Bulgaria, Romania and Italy) with a buffer zone of 20 m (Bulgaria and Romania) or 50 m (Italy), three countries selecting wood stations (stumps, logs, snags etc.) for the surveys. The wood pieces varied in length from 1 m to 2 m (Spain and Switzerland) or were trunks with a minimum diameter of 25 cm (Spain).

Finally, amongst countries which searched for exit holes, only Germany provided threshold values to define the size of the populations: 50 fresh emergence holes were indicative of large populations, 6–50 of middle sized populations while less than 6 holes indicated small populations.

Methods

The three methods tested for monitoring of presence and abundance of *R. alpina* during the LIFE MIPP project were: visual encounter surveys (VES) applied on wild trees, beech tripods (see below) and logs. The VES method consisted of counting individuals on the entire surface of a sampling station with the aid of binoculars for higher levels.

Potentially suitable wild trees were identified by the presence of the following characteristics: presence of dead wood on the trunk and tree exposed to direct sunlight for at least 1–2 hours during the day (Figure 2 and Figure 3).

Tripods consisted of 3 beech logs (diameters 20–25 cm), with debarked bands and positioned as an “Indian tent” (Figure 4). They were placed in open areas within easy access (along forest roads or clearings) at a minimum distance of 30 m between each. Logs consisted of beech trunks, with diameter of 28–75 cm, placed as single log or in groups of logs (Figure 5).

The research on *R. alpina* was carried out in two study areas: the Foreste Casentinesi (FC) and the Abruzzo, Lazio and Molise National Park (PA) in the years 2014, 2015 and 2016 (see Carpaneto et al. in this issue for the description of the study areas). Table 1 provides a summary of the monitoring schemes which are described in detail in the next section. The field sheet used for monitoring is reported in the Supplementary Files. Maps of the study areas are shown in Figure 6 and Figure 7.

Sampling plan

Foreste Casentinesi 2014

In this study area, 3 transects were established (one for each sub-area) which covered all wild trees selected (N=23) and all tripods (N=10, N=13, N=7 respectively for the three sub-areas). The wood used for building the tripods was cut in the study area during the winter 2013–2014. The study period lasted from 14 July to 27 August and consisted of seven sessions (once a week). Each session consisted of three surveys and these were preferably checked on Monday, Wednesday and Friday. For each transect, the direction of the walks was inverted between sessions to avoid checking a given tree or tripod always at the same time of the day. Each survey started at 12:00h to ensure that controls

Figure 2. A wild tree used as sampling unit for the monitoring of *R. alpina* in the Foreste Casentinesi during 2014–2016.

were carried out during the warmest hours of the day, the best time for contacting the species (Drag et al. 2011) and this procedure was maintained for subsequent years as well as in PA.

Figure 3. A wild tree used as sampling unit for the monitoring of *R. alpina* in the Abruzzo, Lazio and Molise National Park during 2014–2016.

Abruzzo, Lazio and Molise National Park 2014

Within this study area, three monitoring transects were selected to cover all wild trees (N= 30, N=30, N=28). The study period lasted from 14 July to 27 August and con-

Figure 4. A tripod made with beech woods, used as sampling unit for the monitoring of *R. alpina* in the Foreste Casentinesi during 2014–2016.

Table 1. Main characteristics of the survey schemes applied for the monitoring of *R. alpina*.

Study area	Year	Number of sub-areas	Sampling sessions	Sampling frequency	No. sampling units		
					Wild trees	Beech tripods	Logs
FC	2014	3	7	3/week	23, 23, 23	13, 10, 7	-
	2015	2	16	4/week	24, 24	15, 15	-
	2016	2	10	4/week	15, 15	15, 15	-
PA	2014	3	7	3/week	30, 30, 28	-	-
	2015	2	14	4/week	15, 15	15, 15	-
	2016	2	10	4/week	15, 15	-	15

sisted of seven sessions (once a week). During each session, surveys were carried out on three consecutive days (one for each sub-area, preferably on Monday, Tuesday and Wednesday).

Foreste Casentinesi 2015

In 2015, only two transects were selected to cover all wild trees (N=24) and all tripods (N=15). The tripods used were the same as in 2014 and thus were one year old. The

Figure 5. A group of logs used as sampling unit for monitoring the presence of *R. alpina* in the Abruzzo, Lazio and Molise National Park in 2016.

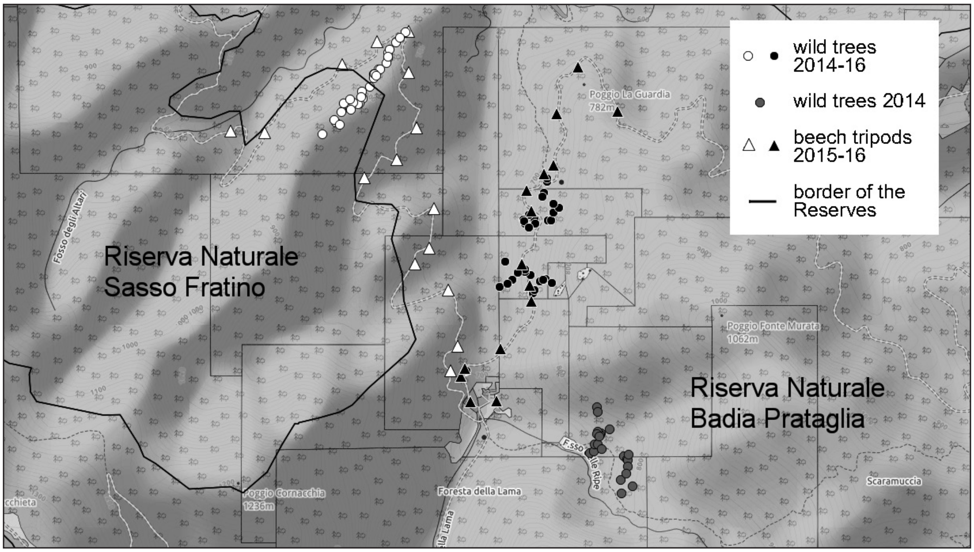

Figure 6. Map of the study area Foreste Casentinesi. White symbols refer to the sub-area "Poggio Ghiaccione", black symbols refer to the sub-area "Strada per Badia", grey symbols refer to the sub-area "La Vetreria".

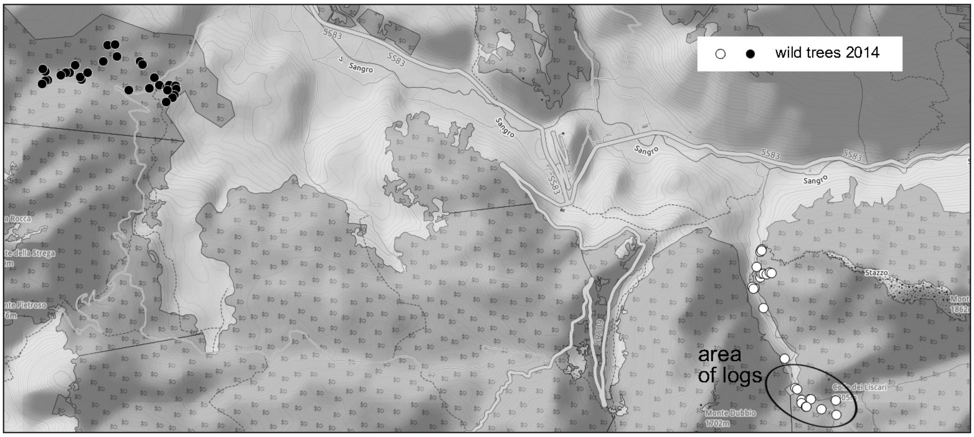

Figure 7. Map of the study area Abruzzo, Lazio and Molise National Park. White symbols refer to the sub-area "Val Fondillo", black symbols refer to the sub-area "Difesa di Pescasseroli".

study period lasted from 15 July to 30 August (16 sessions). Each session consisted of two surveys (one per day) and two sessions were repeated consecutively. After two sessions, there was a pause of two days.

Abruzzo, Lazio and Molise National Park 2015

In 2015, in this study area, two monitoring transects were placed in each sub-area selected. One transect covered wild trees (N=15) and one covered the tripods (N=15). The wood used for the tripods was cut in 2015. The study period lasted from 21 July to 28 August (14 sessions). Surveys were carried out in the same manner as in FC 2015.

Foreste Casentinesi 2016

In this study area, two monitoring transects were checked, but the number of wild trees was reduced from 24 to 15; only those trees which had yielded the highest number of *R. alpina* in 2014 and 2015 were retained. The same number of tripods monitored was maintained as in 2015. The study period lasted from 4 July to 5 August (10 sessions). Each session consisted of two consecutive surveys (one per day) and, in each week, two sessions were carried out.

Abruzzo, Lazio and Molise National Park 2016

The sampling units consisted of wild trees and beech logs. Two transects covering the 15 wild trees from the previous year were maintained, while the tripods were discarded

Table 2. Sampling units made by beech logs for the survey of *R. alpina*.

Sampling Unit	No. logs	Volume of wood (m^3)	Mean diameter (cm)
1	6	3.768	54
2	5	5.505	55
3	4	2.114	50
4	6	4.401	50
5	1	0.428	48
6	2	1.256	47
7	1	0.622	45
8	14	7.877	54
9	1	0.181	62
10	2	0.514	52
11	3	0.466	44
12	1	0.198	47
13	3	0.679	46
14	1	0.217	49
15	1	0.298	54

because in the previous year, these had been damaged or destroyed by cattle or by human activities. Additionally, in one sub-area, 70 logs which had been cut during the summer of 2015, were surveyed. The logs represented 15 sampling units, i.e., distinct groups of logs (see Table 2). The study period lasted from 18 July to 19 August (10 sessions). Each session consisted of two surveys carried out in consecutive days. Surveys were carried out as in FC 2015.

Collection of environmental variables

Wild trees, tripods and logs were mapped using Global Positioning System (GPS) receivers (Garmin 60CSX, 62st and 64st). For wild tree and logs, six environmental variables were measured (cfr. Ranius and Jansson 2000) and these are summarised in Table 3. This analysis was not performed for tripods which had similar physical and biological characteristics.

Table 3. Environmental variables collected on wild trees and logs.

Environmental variable	Type of variable
DNC = Distance from the Nearest Colonised tree	Continuous (m)
DBH = Tree Diameter at Breast Height	Continuous (cm)
CC = Canopy Closure	Categorical (%)
WA = Woodpecker Activity	Binary
TS = Tree Status	Categorical (three categories)
WDC = Wood Decay Class	Categorical (five categories)

Garmin MAP Source software was used to measure DNC; DBH was calculated at about 1.30 m from the ground (wild trees); diameters of logs on the ground were measured at their centre; TS was assessed by coding each tree with one of the three categories: dead, dying or living tree; CC was measured by visual assessment in an area of 5–10 m of radius around each tree. Presence or absence of WA was recorded visually (i.e., foraging holes made by woodpeckers). Finally, WDC was measured according to Hunter 1990, based upon the degree of penetration of a knife blade (Opinel n°8), attributing each tree to one of the five decay classes: I) recently dead, no evidence of decay, intact bark; II) solid wood, less than 10% of decaying wood, knife blade penetrates less than 1cm, intact bark; III) 10% to 25% of decaying wood, knife blade penetrates up to 1 cm and soft wood on surface with some detached bark; IV) more than 26% decaying wood, knife blade penetrates more than 1cm with wood soft to the touch and in depth, bark missing; and V) 76% to 100% decaying wood, very soft wood throughout its entire thickness. The knife was used in four different positions of the tree trunk and then the average value was calculated.

Statistical analysis

Comparison amongst study areas

In order to compare the study areas in terms of wild trees characteristics (TS, DBH, WDC and CC), a Multivariate Analysis of Variance (MANOVA; Anderson 2001) with 1000 permutations was computed. Principal component analysis (PCA) was performed and visualised using a clustering algorithm.

Habitat preferences of *R. alpina*

To investigate the habitat preference of *R. alpina*, Generalised Linear Models (GLMs) were used (family = poisson, link = log, “glm” function in stats R-package) between observed individuals (response variables) and the six environmental variables collected (explanatory variables). GLMs were performed for two types of wood surveyed (i.e. wild trees and logs), separately for each study area and for each year. For those years in which count data of *R. alpina* were zero-inflated, the function glm.nb (MASS R-package) was used. The models were compared on the basis of their “goodness of fit” using Akaike’s information criterion (AIC). Models that differed by less than 2 in AIC scores were considered to be indistinguishable from each other in their explanatory power (Burnham and Anderson 2002). The Variance Inflation Factor (VIF) was computed to highlight collinearity amongst the explanatory variables; all the variables with VIF values below 4.0 were considered without a serious collinearity and retained for building the GLMs models.

Comparison of wild trees, tripods and logs

The Krukall-Wallis test was used to compare the number of observed individuals on wild trees, tripods and logs in the study areas. The habitat preference analyses and non-parametric tests were performed using R 3.3.2 software (R Development Core Team 2010); the alpha set for all the analyses was 0.05.

Results

Difference between study areas

PCA performed on the four tree characteristics (TS, DBH, WDC and CC) in both study areas, showed important differences between FC and PA. Of the four principal components' axes, PC1:PC3 explained 90% of the variance. All environmental variables analysed, differed significantly amongst sites: CC = F: 19.54 $p<0.001$; DBH = F: 217.59 $p<0.001$; TS = F: 11.09 $p<0.002$; WDC = F: 9.98 $p<0.003$ (Figure 8). In particular, trees with smaller diameters were surveyed in FC (Mean and SD: 41.90 cm ± 23.77cm) when compared with PA (Mean and SD: 104.35 cm ± 35.71 cm). A larger number of trees were dead and in the early stages of decay in FC (TSdead = 25, TSdying = 4, TSliving = 0; WDCclass I = 0, WDCclass II = 20, WDCclass III = 6, WDCclass IV = 1) with respect to PA. Here a larger number of trees at a medium or advanced decay status were investigated (TSdead = 16, TSdying = 7, TSliving = 6; WDCclass I = 0, WDCclass II = 5, WDCclass III = 20, WDCclass IV = 4).

Number of sightings and habitat preferences for wild trees

The total number of individuals of *R. alpina* observed on wild trees in FC in 2014, 2015 and 2016 was respectively: 9, 15 and 52. Due to the low number of individuals recorded in 2014 and 2015, it was decided to report only the results of the GLMs performed on the count data of 2016. The GLMs performed on the count data of 2016, considering as explanatory variables DBH, TS, CC and WDC, showed that the best predictors were: DBH ($p<0.01$) and CC ($p<0.01$). Starting from the full model ($M1_{2016}$FC, AIC = 93.31), the best model selected by AIC resulted as follows: $M2_{2016}$FC, AIC = 89.32 (Table 4). Figure 9 shows the relationship between the number of observed *R. alpina* and DBH, the number of individuals observed increasing with increasing DBH.

The numbers of individuals observed on wild trees in PA in 2014, 2015 and 2016 were respectively: 140, 172 and 136. The GLMs performed on the count data of *R. alpina* from PA for 2014 (three sub-areas), showed that the final best model obtained by AIC selection, resulted in $M3_{2014}$PA (AIC = 707.1) with four significant explanatory variables (DBH: $p<0.01$; TSdying: $p<0.01$; WDC: $p<0.01$; CC: $p<0.01$) (Table 5). In

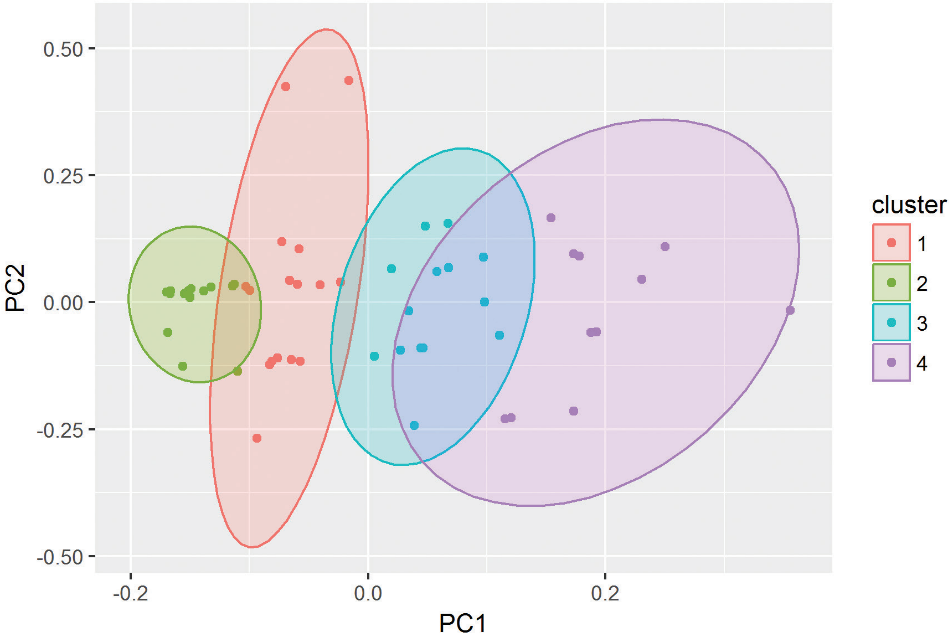

Figure 8. Ordination plot obtained by PCA on the environmental variables collected on the selected trees for the two sub-areas for each study area: FC and PA. The MANOVA test confirmed the dissimilarities between the two study areas FC and PA, as shown by the limited overlap. Clusters correspond to different sub-areas: cluster 1 “Strada per Badia”, cluster 2 “Poggio Ghiaccione”, cluster 3 “Difesa di Pescasseroli” and cluster 4 “Val Fondillo.

Table 4. Generalised Linear Models results. **A**) Explanatory variables selection and final best model for count data of *R. alpina* in FC in 2016. For each model are reported the function used to build the model, AIC value and Delta AIC for selecting the best model, **B**) Estimates and standard error (S.E.) of the two explanatory variables of the best model; significant codes: 0 ‘***’, 0.001 ‘**’, 0.01‘*’, 0.05 ‘.’.

A

Model	Function	AIC	Delta AIC
M2$_{2016}$FC	glm (count data ~ DBH + CC, family = poisson (link=log))	89.32	0
M1$_{2016}$FC	glm (count data ~ DBH + TS + WDC + CC, family = poisson (link=log))	93.31	3.99

B

Best Model M2$_{2016}$FC

Explanatory Variables	Estimates	S.E.	z value	pr (>|z|)	
Intercept	-1.07E+00	7.86E-01	-1.35	0.17	
DBH	2.96E-02	6.54E-03	4.52	0.00000060	***
CC	-1.57E+00	5.63E-01	-2.78	0.0053	**

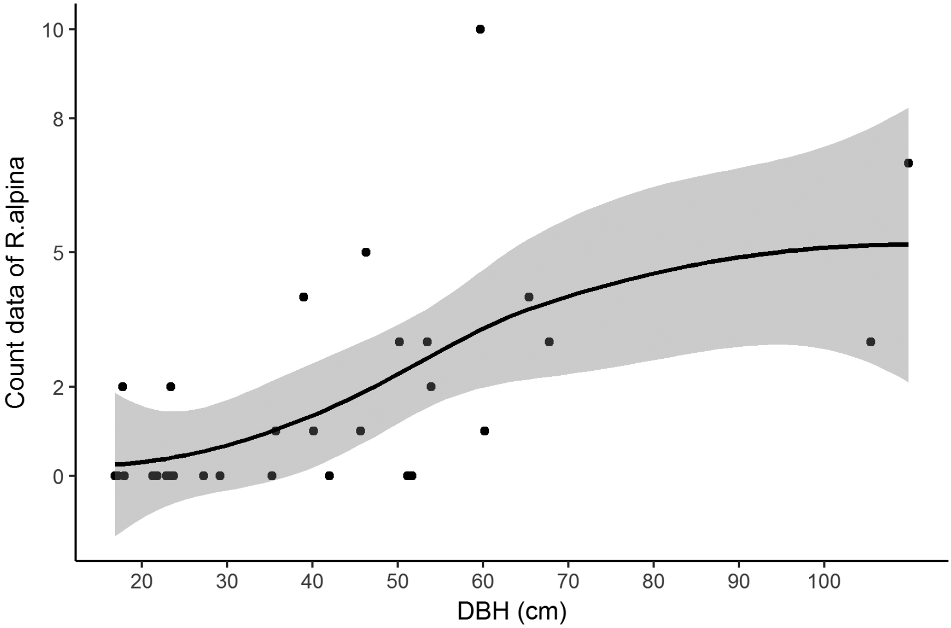

Figure 9. Visualisation of the fitted GLM model using LOESS (locally weighted scatter plot smoothing) function. The figure shows the relationship between DBH and count data of *R. alpina* in FC during 2016. A larger number of individuals were observed on trees with DBH greater than 60 cm.

Table 5. Generalised Linear Models results. **A)** Explanatory variables selection and final best model for count data for *R. alpina* in PA in 2014. For each model are reported the function used to build the model, AIC value and Delta AIC for selecting the best model, **B)** Estimates and standard error (S.E.) of the five explanatory variables of the best model; significant codes: 0 '***', 0.001 '**', 0.01'*', 0.05 '.'.

A

Model	Function	AIC	Delta AIC
M3$_{2014}$PA	glm (count data ~ DBH + TS + WDC + CC, family = poisson (link=log))	707.1	0
M2$_{2014}$PA	glm (count data ~ DBH + TS + WDC + CC + WA, family = poisson (link=log))	707.3	0.2
M1$_{2014}$PA	glm (count data ~ DBH + TS + WDC + CC + WA + DNC, family = poisson (link=log))	709.3	2.2

B

Explanatory Variables	Estimates	S.E.	z value	pr (>|z|)	
Intercept	-5.194	0.613	-8.47	2.00E-16	***
DBH	0.014	0.002	5.99	2.03E-09	***
TSdying	1.706	0.288	5.917	3.28E-09	***
TSliving	-0.611	0.319	-1.911	0.056	.
WDC	0.882	0.164	5.366	8.04E-08	***
CC	-0.024	0.004	-5.405	6.50E-08	***

Table 6. Generalised Linear Models results. **A**) Explanatory variables selection and final best model for count data for *R. alpina* in PA in 2015. For each model are reported the function used to build the model, AIC value and Delta AIC for selecting the best model, **B**) Estimates and standard error (S.E.) of the four explanatory variables of the best model; significant codes: 0 '***', 0.001 '**', 0.01'*', 0.05 '.'.

A

Model	Function	AIC	Delta AIC
M3$_{2015}$PA	glm.nb (count data ~ DBH + TS + WDC + DNC, family = poisson (link=log))	647.6	0
M2$_{2015}$PA	glm (count data ~ DBH + TS + WDC + CC + DNC, family = poisson (link=log))	726.9	79.3
M1$_{2015}$PA	glm (count data ~ DBH + TS + WDC + CC + WA + DNC, family = poisson (link=log))	728.5	80.9

B

Best Model M3$_{2015}$PA

Explanatory Variables	Estimates	S.E.	z value	pr (>|z|)	
Intercept	-5.156	0.926	-5.56	2.66E-08	***
DBH	0.011	0.003	3.29	0.00098	***
TSdying	0.998	0.333	2.99	0.00278	**
TSliving	-0.374	0.352	-1.065	0.2870	
WDC	0.829	0.255	3.244	0.0011	**
DNC	0.001	0.0006	2.207	0.0273	*

2015 and 2016 (two sub-areas), the final best models resulted in respectively: M3$_{2015}$PA, AIC = 647.66 (DBH: $p<0.01$; TSdying: $p<0.01$; WDC: $p<0.01$; DNC: $p<0.05$) and M3$_{2016}$PA, AIC = 496.22 (TSdying: $p<0.01$; WDC: $p<0.01$; DNC: $p<0.05$) (Table 6 and Table 7). Figure 10 shows the relationship between the number of observed individuals and the significant explanatory variables for 2015 in PA. Based on the results of GLMs for PA, it is possible to describe the wild tree with the best characteristics for monitoring *R. alpina*: a larger number of individuals was observed on the tree with larger DBH, dying, belonging to the medium-to-advanced decay status and with a distance of less than 300 m from colonised trees.

Number of sightings and habitat preferences for logs

The total number of individuals observed on the logs surveyed in 2016 in PA was 122. In order to evaluate the preferences of *R. alpina* for these logs, GLMs were performed. For each sampling unit (cfr. Table 2), the mean diameter and volume of logs were calculated. The explanatory variables considered in the model were: the number of logs (SU_NL), average diameter (SU_DIAM), average volume (SU_VOL) and canopy closure (SU_CC) for each sampling unit. Starting with the full model (M1$_{logs}$PA, AIC = 191.67), the final best model resulted in: M2$_{logs}$PA, AIC = 189.67 with two significant

Table 7. Generalised Linear Models results. **A)** Explanatory variables selection and final best model for count data for *R. alpina* PA in 2016. For each model are reported the function used to build the model, AIC value and Delta AIC for selecting the best model, **B)** Estimates and standard error (S.E.) of the three explanatory variables of the best model; significant codes: 0 '***', 0.001 '**', 0.01'*', 0.05 '.'.

A

Model	Function	AIC	Delta AIC
M3_{2016}PA	glm.nb (count data ~ TS + WDC + DNC, family = poisson (link=log))	496.2	0
M2_{2016}PA	glm.nb (count data ~ DBH + TS + WDC + CC + DNC, family = poisson (link=log))	497.9	1.7
M1_{2016}PA	glm.nb (count data ~ DBH + TS + WDC + CC + WA + DNC, family = poisson (link=log))	498.2	2

B

Best Model M3_{2016}PA

Explanatory Variables	Estimates	S.E.	z value	pr (>|z|)	
Intercept	-0.599	0.215	-2.78	0.00536	**
TSdying	1.307	0.433	3.01	0.00259	**
TSliving	-0.048	0.356	-0.136	0.8916	
WDC	0.829	0.255	3.244	0.0011	***
DNC	0.001	0.0006	2.004	0.04505	*

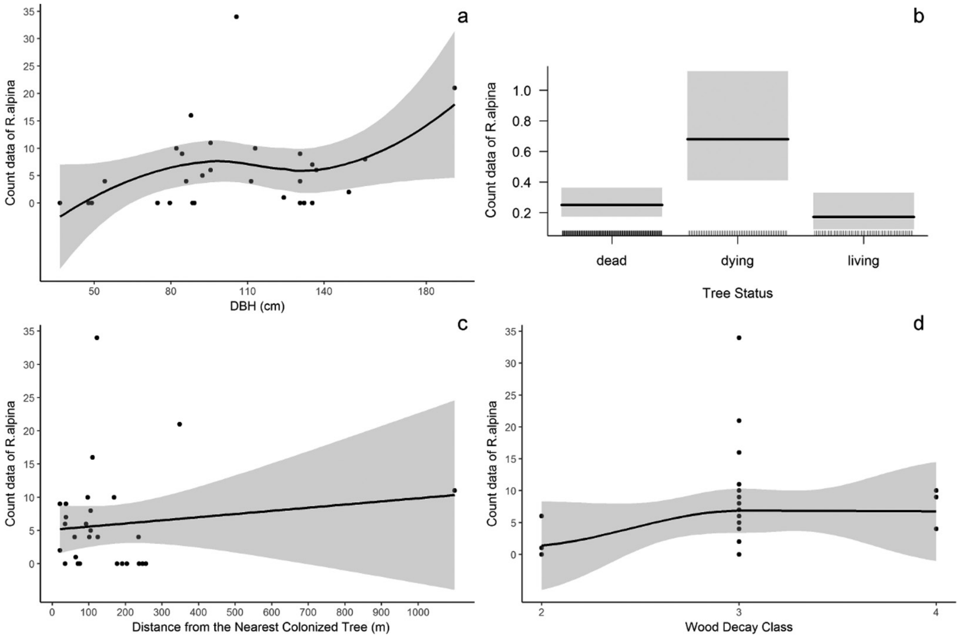

Figure 10. Figure shows the significant environmental variables of the fitted GLM models, on count data of *R. alpina* in PA during 2015. In figures **A), B)** and **D)** LOESS (locally weighted scatter plot smoothing) function has been used. A larger number of individuals was observed on trees with DBH greater than 80 cm, dying, belonging to the middle-advanced decay status and distance less than 300 m from other colonised trees.

Table 8. Generalised Linear Models results. **A)** Explanatory variables selection and final best model for count data of R. alpina on logs in PA. For each model are reported the function used to build the model, AIC value and Delta AIC for selecting the best model, **B)** Estimates and standard error (S.E.) of the two explanatory variables of the best model; significant codes: 0 '***', 0.001 '**', 0.01'*', 0.05 '.'.

A

Model	Function	AIC	Delta AIC
M2$_{logs}$PA	glm (count data ~ SU_DIAM + SU_VOL + SU_CC, family = poisson (link=log))	191.3	0
M1$_{logs}$PA	glm (count data ~ SU_NL + SU_DIAM + SU_VOL + SU_CC, family = poisson (link=log))	193.2	1.9

B

Best Model M2$_{logs}$PA

Explanatory Variables	Estimates	S.E.	z value	pr (>|z|)	
Intercept	4.529	3.650	1.241	0.215	
SU_DIAM	-0.120	0.074	-1.621	0.060	.
SU_VOL	1.153	0.166	6.910	4.83E-12	***
SU_CC	-0.109	0.019	-5.621	189E-08	***

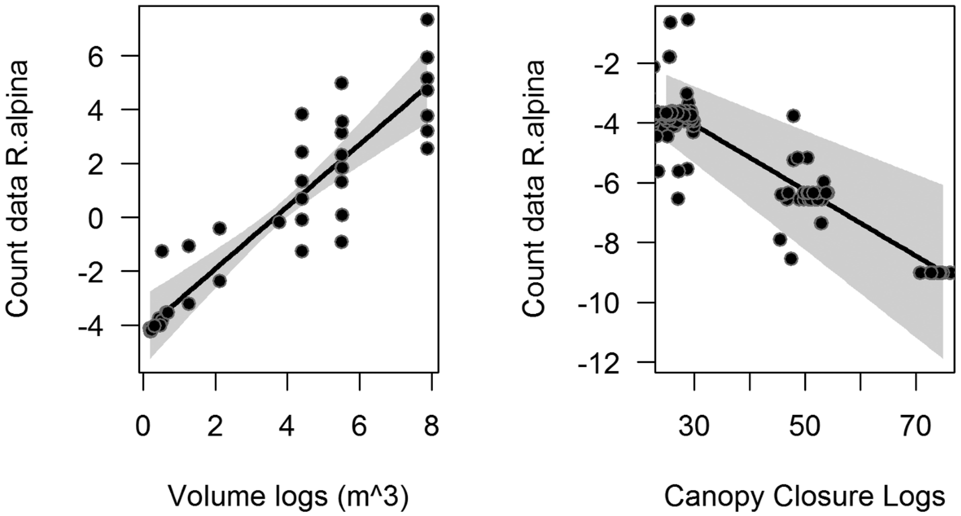

Figure 11. Significant environmental variables of the GLM models performed, on count data of *R. alpina* on logs in PA 2016. The number of individuals is positively correlated with log volume and negatively with canopy closure.

explanatory variables (SU_VOL: *p*<0.01; SU_CC: *p*<0.01) (Table 8). The relationships between the significant variables and the number of observed individuals are shown in Figure 11.

Comparison of the number of observed individuals

In order to compare the number of observed individuals on wild trees, tripods and logs for each study area, a Kruskall-Wallis test was performed. The total number of observed individuals in FC during 2015 and 2016 was respectively, 15 and 52 for wild trees and 19 and 16 for tripods. The comparison of count data for 2015 and 2016 between tripods and wild trees showed significant differences for both years (Kruskal-Wallis test 2015: chi-squared = 6.32, DF = 1, p = 0.01; Kruskal-Wallis test 2016: chi-squared = 4.95, DF = 1, p = 0.02). The total number of individuals observed in PA during 2015 on wild trees and tripods was 172 and 0 respectively, thus no analysis has been performed. During 2016, the total number of observed individuals on wild trees and logs was 136 and 122 respectively. The Kruskall-Wallis test showed no significant differences in terms of observed individuals between logs and wild trees (chi-squared = 0.06, DF = 1, p = 0.79).

Phenology

The phenological data for three years (2014-2015-2016) in the two study areas showed that the adults of *R. alpina* were observed from the end of July to the end of August for PA (Figures 12a, b and d), while for FC, the season starts from early July to the first days of August (Figures 12a, c and e). The survey campaign did not cover the entire period of adult activity, as it can be easily extrapolated from the graph in Figure 12.

Discussion

Our results showed that wild trees best suited for monitoring *R. alpina* are those with large diameters, partially dead, belonging to the medium to advanced decay status and which are less than 300 m from other colonised trees. These results are in line with other works which showed that diameter seems to be the most important parameter positively selected by *R. alpina* (Duelli and Wermelinger 2005, Russo et al. 2010, Drag et al. 2011, Castro et al. 2012, Castro and Fernández 2016). It was found that the highest number of individuals was on beech trees with 110 cm of diameter in PA, while the minimum diameter in PA was considered to be 40 cm. In contrast in the study area FC, smaller trees were included and here *R. alpina* was found on trees with diameters of 20–40 cm. On rare occasions, *R. alpina* develops in trunks of less than 20 cm in diameter (Duelli and Wermelinger 2005, Castro et al. 2012).

Our research on wild trees employed standing trees which were alive, dying (partially dead) or dead. The analysis showed that standing dying trees are positively selected by *R. alpina* and this finding is in line with the results reported by Castro and Fernández (2016). In contrast, Russo et al. (2010) did not observe differences in the

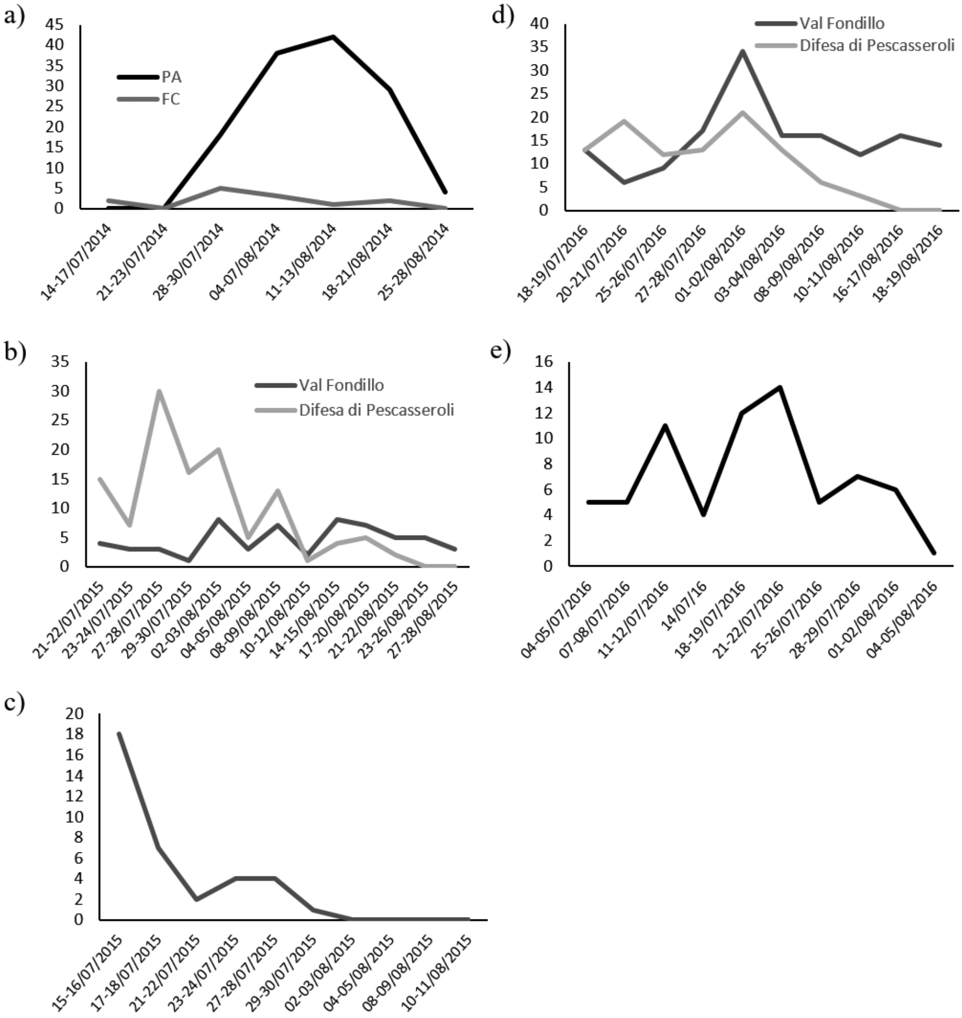

Figure 12. Phenology of *R. alpina* in the study areas. The dates of survey and the numbers of observed individuals are plotted. **A)** year 2014, study areas PA and FC (data of different sub-areas have been summed) **B)** year 2015, study area PA (the data of different sub-areas have been maintained separate) **C)** year 2015, study area FC (data of different sub-areas have been summed) **D)** year 2016, study area PA (the data of different sub-areas have been maintained separate) **E)** year 2015, study area FC (data of different sub-areas have been summed).

colonisation rate between living and standing dead trees. An important finding reported here is that, although standing dead wood is important for *R. alpina*, the closure of the forest canopy could render otherwise attractive trunks unsuitable. The fact that a tree was assigned to the category "standing dying tree" is correlated with its decay status and the medium decay status was positively selected by *R. alpina*. Medium decay status means trees with part of the wood decaying (around 20%), exposed soft wood and with some detached bark, characteristics also selected by another longicorn beetle, *Cerambyx cerdo* (Döhring 1955, Buse et al. 2007).

An interesting result is that trunks less than 300 m from other colonised trees were positively selected by *R. alpina* and this is in line with findings by Russo et al. (2010), these authors found that the distance to the next occupied tree was the most powerful predictor. The distances reported are variable; from <212 m (Castro and Fernández 2016) to <1000 m (Russo et al. 2010). However, these distances are all less than the observed maximum dispersal distances: 1628 m (Drag et al. 2011) and 1.5 km (Rossi de Gasperis 2016)

The GLMs on individuals observed on lying logs in 2016 in PA showed that the volume of dead wood and canopy openness were positively selected by *R. alpina*. The Kruskall-Wallis test showed no significant differences in terms of observed individuals between lying logs and wild trees. In contrast, Duelli and Wermelinger (2005), showed *R. alpina* to prefer standing over fallen dead wood, but Russo et al. (2010) found no support for the fact that fallen trees were less attractive. Our analyses showed that a high volume of dead wood was positively selected by *R. alpina*. A higher volume of dead wood means more food for the larvae which prefer sapwood (Duelli and Wermelinger 2005). In fact, on large logs characterised by a volume of 8 m^3, many female *R. alpina* were found to be laying eggs. However, the influence of tree condition and volume of dead wood needs to be investigated further.

Another explanatory variable which resulted positively selected by *R. alpina* was canopy openness, this variable resulting in an explanatory variable for wild trees in FC as well as for logs in PA. In both cases, *R. alpina* showed a preference for sun-exposed and semi-shaded conditions (Figure 11 for logs). This result is in line with findings by Russo et al. (2010); they found that sun exposure was one of the main determinants of habitat selection. In our study areas, the FC forest was characterised by a high canopy closure and this explained why sun-exposed sites were positively selected by *R. alpina*. In contrast, in PA, logs were positioned both in sun-exposed and shady conditions and the count data demonstrated that half-shade and fully exposed sites were preferred, whereas shady conditions attracted less individuals.

On the basis of the results discussed above, a monitoring method for *R. alpina* is proposed in the next paragraphs.

Description of the proposed monitoring method

As a standard method for the monitoring of *R. alpina*, it is proposed to use beech trees with large diameters which are dead or partially dead (see below for more details) and which are the natural breeding habitats where adults occurred in higher density. It is important to acknowledge that such trees cannot be considered “standard sampling units”, as each tree may be differently attractive to adults. The most important factors correlated with the numbers of *R. alpina* observed on single trunks are the volume of wood (i.e. trunk diameter) and exposure to the sun. Thus, it is obvious that the dead trunk of a relatively small tree (diameter 30 cm), which is partially shaded, attracts much less adults when compared with the dead trunk of a large veteran tree (diameter 120 cm) which is never shaded by other trees. Before describing how to select trees to be surveyed, some general principles and problems should be considered.

A dead (or partially dead) tree, currently suitable for monitoring, will not be suitable after some years when the degradation of the wood has progressed to the point which makes the tree no longer attractive to *R. alpina*. Thus, for any long-term monitoring programme, it is clear that the single trees initially selected will have to be replaced by other trees which will become suitable in future years. For example, if a forest contains five very large veteran beech-trees (>100 cm) which are still suitable for monitoring and the remaining forest consists exclusively of trees with a diameter of 20-40 cm, it might not be advisable to use the veteran trees as the principal sampling units for the long-term monitoring programme. This example was chosen to demonstrate the difficulties arising from selecting wild trees which are naturally available. It is also clear that a natural forest does not produce "standard" dead trees and choosing similar trees will always be a compromise.

Choosing trees

For monitoring, 15 trees (mainly *Fagus sylvatica*) which are dead or partially dead and have large diameters (a DBH of at least 30 cm) need to be chosen Additionally, the trunks need to be exposed to direct sunlight at least during the central hours of the day and, for this reason, standing trees are preferable with respect to fallen trees. Leaning or lying trees might also be used but it is very important that the wood of these trunks (especially those lying) is fairly dry. Figure 2 and Figure 3 show trees investigated during the MIPP project and which attracted adults of *R. alpina*. In many natural beech forests, sun-exposed trunks are rare and ridges often provide the best situation for findings trunks which respond to the above characteristics. A further important consideration is accessibility. The 15 trees chosen for monitoring should be relatively easily accessible on foot and relatively near to each other; ideally, they should all be within walking distance in order to allow a single transect connecting all selected trees to be placed. To choose the single trees for monitoring, it is necessary to intensively survey the local forest prior to the final selection with the aid of local personnel.

It is advisable not to use tripods or logs specifically created for monitoring (Figure 4 and Figure 5), as these are not considered practical alternatives. One important reason is that the volume of dead wood is positively correlated with the number of *R. alpina* detected. Given that breeding trees have a minimum diameter of 20 cm (Castro et al. 2012), they provide a large volume of dead wood. Our results showed that the probability of observing *R. alpina* is ever higher with increasing volumes of dead wood and the authors documented this up to a volume of 8 m^3. Man-made structures need to compete with wood-volumes found in wild trees. The tripods used in FC, which were built without mechanical aids, attracted low numbers of *R. alpina*. Larger volumes of dead wood were placed in PA in 2015 and surveyed in 2016. Although these volumes (up to 8 m^3) allowed the successful monitoring of *R. alpina*, manipulating such large volumes of dead wood requires specialised equipment which is normally not available to managers of forest reserves. Additionally, if the wood volumes, made available in 2015, were to be considered as the basis for a standard monitoring protocol, the volume of wood involved would result in considerable financial consequences. However, if large volumes of dead wood, specialised equipment and open sites, exposed to the sun are available, logs or tripods could be used for monitoring. Once a trunk has been chosen for the monitoring programme, the following characteristics need to be recorded:

- DBH (Diameter at breast height)
- Height of trunk
- Status (dead, partially alive)
- Decay class of wood (Hunter, 1990)
- Canopy openness (0–25%, 26–50%, 51–75%, 76–100%)
- Geographic coordinates

The distance range between trunks should be 50–300 m.

Checking the single trees

In Table 9, a summary of the monitoring protocol for *R. alpina* is provided. The wild trees should be checked once a week, during the period of maximum activity of *R. alpina* when weather conditions are favourable: without rain and with a mean daily temperature above 20°C. If weather conditions are not favourable, it is advisable to carry out the fieldwork on another day, as soon as possible afterwards. The interval between successive monitoring sessions should be between 5–9 days.

Table 9. Summary of the monitoring protocol for *R. alpina*.

Monitoring protocol	
Method	Wild trees (dead or partially dead)
Number of trees	15 for each site to be monitored
Position of trees	Along transects
Distance between trees	Between 50 m – 300 m
Monitoring period	July-August
Number repeats	5
Frequency	Once a week
Time of the day	11:00h–15:00h
Number of operators	2
Hours per person	10 hours/person
Equipment	A clipboard, a field sheet, a pencil, a clock, binoculars and GPS

The protocol requires the presence of two operators who simultaneously search for *R. alpina* by sight on the surface of the wild tree on opposite sides of the tree. They should communicate any sightings and particularly movements of adults observed to avoid counting the same individual twice. The upper part of the trunk should be checked with binoculars. It is also important to carefully check cavities and large cracks as the adults might hide there. The check of a single wild tree should last approximately 1–2 min. Only very large veteran trees might require more time.

Once the search of the wild tree has been completed, the number of individuals (the sum from both observers) is calculated, specifying the number of males and females and the field sheet is compiled (see Supplementary Files). The equipment required are a clipboard, field sheet, pencil, clock, binoculars and GPS.

Constraints, spatial validity and possible interferences

The mark-recapture studies carried out by Rossi de Gasperis (2016) showed that adults of *R. alpina* can move up to 1.5 km in 6 days. Therefore, at present, it is assumed that the validity of the results of the monitoring extends to an area surrounding the selected tree up to a maximum of 1000 m. In other words, if the average distance between the trees investigated is 150 m, the number of trees is 15 and if one calculates the area which extends to a maximum of 1000 m from these trees, an area of about 290 ha is obtained. This area represents the size of the forest for which the results of the monitoring are assumed to be valid. If the area monitored is located within a homogeneous forest (e.g. similar tree composition, age of trees, management, dead wood etc.), the validity extends to this area.

A possible interference in the areas to be monitored is represented by wood piles of trunks present along forest roads, as these might affect the number of *R. alpina* observed. This is particularly the case if the logs have been cut more than a year ago. A final aspect to be considered is the interactions with other monitoring activities. Methods employed for the monitoring of *M. asper/funereus* and *Cucujus cinnaberinus* (Scopoli, 1763) (Coleoptera, Cucujidae), might also affect the monitoring of *R. alpina*. It is recommended to allow for a distance of a least 1000 m between monitoring stations for the different species.

Counting, quantification and data sharing

In order to assess the conservation status of populations of *R. alpina* for a given season and for a given area, a reference value is calculated as follows:

1) For each session, calculate the total number of individuals (males + females) by adding up the number of individuals found on each wild tree.

2) Calculate the mean value of the total numbers of individuals counted in each session, excluding the session with the lowest count. Removing the lowest count, as

proposed for other insect species (Trizzino et al. 2013), allows the elimination of eventual outlier values due to adverse climatic conditions (e.g. low temperature and/or rainfall) or other factors which do not represent the local population and it is meant to reduce the variability of the final value.

Table 10. Example of calculation of the reference value for the monitoring of *R. alpina* in one site in one year (Wt: wild tree, S: Session).

	Wt1	Wt2	Wt3	Wt4	Wt5	Wt6	Wt7	Wt8	Wt9	Wt10	Wt11	Wt12	Wt13	Wt14	Wt15	Total
S1	0	1	2	1	4	0	1	0	0	1	1	1	2	1	3	18
S2	1	2	2	1	2	3	0	1	1	2	0	4	3	2	0	24
S3	2	0	1	3	2	2	1	2	3	0	4	2	1	2	3	28
S4	1	0	0	2	1	2	3	1	3	1	2	2	1	1	2	22
S5	0	0	0	1	2	3	1	0	2	1	3	1	2	0	1	17
Average value for the four counts with the highest average total																23

Table 10 reports an example of calculation of the mean value of the individuals counted. The mean value obtained is the reference value for the assessment of the conservation status of the species in a given year. This value allows comparison of the long-term data and the identification of a population trend. The values obtained during the MIPP project, for the sub-area Difesa di Pescasseroli where the higher number of individuals have been observed, are: 12 (2014), 19 (2015) and 17 (2016).

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LIFE 11 NAT/IT/000252

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References

Adamski P, Bohdan A, Michalcewicz J, Ciach M, Witkowski Z (2016) Timber stacks: potential ecological traps for an endangered saproxylic beetle, the Rosalia longicorn *Rosalia alpina*. Journal of Insect Conservation 20: 1099–1105. https://doi.org/10.1007/s10841-016-9932-4

Alì K, Rapuzzi P (2016) Second contribution to the knowledge of Longhorn Beetles of the Syrian Coastal Region (Coleoptera Cerambycidae). Biodiversity Journal 7: 261–272.

Anonymous (2015) Operativna Programa “OKOLNA Sreda 2007–2013 G.” Proekt “Terenni prouchvaniya na razprostranenie na vidove / otsenka na sŭstoyanieto na vidove i khabitati na teritoriyata na tsyalata strana – I faza”, 1–14.

Audisio P, Baviera C, Carpaneto GM, Biscaccianti AB, Battistoni A, Teofili C, Rondinini C (2014) Lista Rossa IUCN dei Coleotteri saproxilici Italiani. Comitato Italiano IUCN e Ministero dell’Ambiente e della Tutela del Territorio e del Mare, Roma, 1–132.

Bense U (1995) Longhorn Beetles: Illustrated key to the Cerambycidae and Vesperidae of Europe. Margraf Verlag, Weikersheim, 1–512.

Bense U, Klausnitzer B, Bussler H, Schmidl J (2003) *Rosalia alpina* (Linnaeus, 1758) Alpenbock. In: Petersen B, Ellwanger G, Biewald G, Hauke U, Ludwig G, Pretscher P, Schröder E, Ssymank A (Eds) Das europäische Schutzgebietssystem Natura 2000. Ökologie und Verbreitung von Arten der FFH-Richtlinie in Deutschland. Band 1: Pflanzen und Wirbellose. Schr.R. f. Landschaftspfl. u. Natursch. 69/1: 426–432.

Biewald G, Marx J, Dümas J (2014) Handbuchzur Erstellung von Management-Plänenfür die Natura 2000 - Gebiete in Baden-Württemberg, Version 1.3. LUBW, Landesanstaltfür Umwelt, Messungen und Naturschutz, Baden-Württemberg, 1–461.

Binner V, Bußler H (2006) Erfassung und Bewertung von Alpenbock-Vorkommen - Umsetzung von Natura 2000 in Bayern am Beispiel von *Rosalia alpina* (L.,1758). Naturschutz und Landschaftsplanung 38: 378–382.

Bologna MA, Rovelli V, Zapparoli M (2016) *Morimus asper funereus* (Sulzer, 1776) (Morimo funereo). In: Stoch F, Genovesi P (Eds) Manuali per il monitoraggio di specie e habitat di interesse comunitario (Direttiva 92/43/CEE) in Italia: specie animali. ISPRA, Serie Manuali e linee guida 141/2016: 76–77.

Bosso L, Rebelo H, Garonna AP, Russo D (2013) Modelling geographic distribution and detecting conservation gaps in Italy for the threatened beetle *Rosalia alpina*. Journal for Nature Conservation 21: 72–80. https://doi.org/10.1016/j.jnc.2012.10.003

Bußler H, Schmidl J, Blaschke M (2016) Die FFH-Art Alpenbock (*Rosalia alpina* Linnaeus, 1758) (Coleoptera, Cerambycidae) in Bayern. Naturschutz und Landschaftsplanung 48: 273–280.

Burnham KP, Anderson DR (2002). Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach, New York, 1–455.

Caci G, Biscaccianti AB, Cistrone L, Bosso L, Garonna AP, Russo D (2013) Spotting the right spot: computer-aided individual identification of the threatened cerambycid beetle *Rosalia alpina*. Journal of Insect Conservation 17: 787–795. https://doi.org/10.1007/s10841-013-9561-0

Campanaro A, Bardiani M, Spada L, Carnevali L, Montalto F, Antonini G, Mason F, Audisio P (2011) Linee guida per il monitoraggio e la conservazione dell'entomofauna saproxilica. Quaderni Conservazione Habitat, 6. Cierre Grafica, Verona, 1–8.

Carpaneto GM, Baviera C, Biscaccianti AB, Brandmayr P, Mazzei A, Mason F, Battistoni A, Teofili, C, Rondinini C, Fattorini S, Audisio P (2015) A Red List of Italian Saproxylic Beetles: taxonomic overview, ecological features and conservation issues (Coleoptera). Fragmenta Entomologica 47: 53–126. https://doi.org/10.4081/fe.2015.138

Carpaneto GM, Campanaro A, Hardersen S, Audisio P, Bologna MA, Roversi PF, Sabbatini Peverieri G, Mason F (2017a) The LIFE Project "Monitoring of insects with public participation" (MIPP): aims, methods and conclusions. In: Carpaneto GM, Audisio P, Bologna MA, Roversi PF, Mason F (Eds) Guidelines for the Monitoring of the Saproxylic Beetles protected in Europe. Nature Conservation 20: 1–35. https://doi.org/10.3897/natureconservation.35.12761

Campanaro A, Hardersen S, Redolfi De Zan L, Antonini G, Bardiani M, Maura M, Maurizi E, Mosconi F, Zauli A, Bologna MA, Roversi PF, Sabbatini Peverieri G, Mason F (2017b) Analyses of occurrence data of protected insect species collected by citizens in Italy. In: Carpaneto GM, Audisio P, Bologna MA, Roversi PF, Mason F (Eds) Guidelines for the Monitoring of the Saproxylic Beetles protected in Europe. Nature Conservation 20: 265–297. https://doi.org/10.3897/natureconservation.20.12704

Castro A, Martinez de Murgia L, Fernandez J, Casis A, Molino-Olmedo F (2012) Size and quality of wood used by *Rosalia alpina* (Linnaeus, 1758) (Coleoptera: Cerambycidae) in beech woodlands of Gipuzkoa (northern Spain). Munibe, Ciencias Naturales-Natur Zientziak, 60: 77–100.

Castro A, Fernández J (2016) Tree selection by the endangered beetle *Rosalia alpina* in a lapsed pollard beech forest. Journal of Insect Conservation 20: 201–214. https://doi.org/10.1007/s10841-016-9854-1

Ciach M, Michalcewicz J, Fluda M (2007) The first report on development of *Rosalia alpina* (Linneaus, 1758) (Coleoptera: Cerambycidae) in wood of Ulmus L. in Poland. Polish Journal of Entomology 76: 101–105.

Ciach M, Michalcewicz J (2013) Correlation between selected biometric traits of adult *Rosalia alpina* (L.) (Coleoptera: Cerambycidae) and size of their exit holes: New perspectives on insect studies? Polish Journal of Entomology 61: 349–355.

Ciach M (2015) 1087 Nadobnica alpejska - *Rosalia alpina* (Linnaeus,1758). In: Makomaska-Juchiewicz M, Bonk M (Eds) Monitoring gatunków zwierząt. Przewodnik metodyczny. Część IV. GIOŚ, Warszawa, 125–145.

Čížek L, Schlaghamersky J, Borucky J, Hauck D, Helesic J (2009a) Range expansion of an endangered beetle: Alpine Longhorn *Rosalia alpina* (Coleoptera: Cerambycidae) spreads to the lowlands of Central Europe. Entomologica Fennica 20: 200–206.

Čížek L, Pokluda P, Hauck D, Roztočil O, Honců M, (2009b) Monitoring Tesarika Alpskeho v Ralské Pahorkatine. Bezděz 18: 125–140.

Danilevsky ML (2013) A check-list of Longicorn Beetles (Coleoptera, Cerambycoidea) of Europe. A.N. Severtsov Institute, Moscow, Russia, http://www.cerambycidae.net/europe.pdf

de Flores M, Sueur A (2015) La Rosalie des Alpes - premiers résultats d'enquête. Insectes 176: 37–38.

Di Santo D, Biscaccianti AB (2014) Coleotteri saproxilici in Direttiva Habitat del Parco Nazionale del Gran Sasso e Monti della Laga. Bollettino della Società Entomologica Italiana 146: 99–110. https://doi.org/10.4081/BollettinoSEI.2014.99

Drag L, Hauck D, Pokluda P, Zimmermann K, Cizek L (2011) Demography and dispersal ability of a threatened saproxylic beetle: a mark-recapture study of the rosalia longicorn (*Rosalia alpina*). PLoS ONE 6: e21345. https://doi.org/10.1371/journal.pone.0021345

Drag L, Hauck D, Bérces S, Michalcewicz J, Jelaska LS, Aurenhammer S, Cizek L (2015) Genetic differentiation of populations of the threatened saproxylic beetle Rosalia longicorn, *Rosalia alpina* (Coleoptera: Cerambycidae) in Central and South-east Europe. Biological Journal of the Linnean Society 116: 911–925. https://doi.org/10.1111/bij.12624

Duelli P, Wermelinger B (2005) *Rosalia alpina* L.: Un Cerambicide raro ed emblematico. Sherwood 114: 19–23.

Duelli P, Wermelinger B (2010) Der Alpenbock (*Rosalia alpina*) Ein seltener Bockkäferals Flaggschiff-Art. WSL Merkbl. Prax. 39: 1–8.

Friess T, Holzinger WE, Mairhuber C, Mehlmauer P, Pill W, Zimmermann P (2014) Der Alpenbockkäfer, *Rosalia alpina* (Linnaeus, 1758) (Coleoptera, Cerambycidae), im Nationalpark Kalkalpen (Österreich). Entomologische Nachrichten und Berichte 58: 113–119.

Fusu L, Stan M, Dascălu MM (2015) Coleoptera. In: Iorgu IS (Ed.) Ghid Sintetic pentru Monitorizarea Speciilor de Nevertebrate de Interes Comunitar din România. Bucharest, 44–61.

Harde KW (1996) Fam. Cerambycidae. In: Freude H, Harde KW, Lohse GA, Verlag G, Vergal E (Eds) Krefeld Die Käfer Mitteleuropas, Bd. 9: Cerambycidae - Chrysomelidae, Einbeck.

Hewitt G (2000) The genetic legacy of the Quaternary ice ages. Nature 405: 907–913. https://doi.org/10.1038/35016000

Horák J, Tezcan S, Mico E, Schmidl J, Petrakis P (2009) *Rosalia alpina*. In: IUCN, Red List of Threatened Species. http://www.iucnredlist.org

Hunter ML (1990) Wildlife, Forests, and Forestry. Principles of Managing Forests for Biological Diversity. Prentice Hall, Englewood Cliffs, 1–370.

Jurc M, Ogris N, Pavlin R, Borkovic D (2008) Forest as a habitat of saproxylic beetles on Natura 2000 sites in Slovenia. Revue d'Ecologie 63: 53–66.

Krasnobayeva TP (2008) List of the Longicorn Beetles (Coleoptera: Cerambycidae) from Samarskaya Luka. Russian Entomological Journal 17(3): 295–298.

Lachat T, Ecker K, Duelli P, Wermelinger B (2013) Population trends of *Rosalia alpina* (L.) in Switzerland: a lasting turnaround? Journal of Insect Conservation 17: 653–662. https://doi.org/10.1007/s10841-013-9549-9

Luce JM (1996) *Rosalia alpina* (Linnaeus 1758). In: Van Helsdingen PJ, Willemese L, Speight MCD (Eds) Background information on invertebrates of the Habitats Directive and the Bern Convention: Part I – Crustacea, Coleoptera and Lepidoptera. Council of Europe Publishing – Nature and Environment Series 79: 70–73.

Mazzei A, Bonacci T, Gangale C, Pizzolotto R, Brandmayr P (2013) Nuovi dati faunistici ed ecologici di *Rosalia alpina* (Linnaeus, 1758) in Calabria. Quaderno di Studi e Notizie di Storia Naturale della Romagna 38: 181–190.

Michalcewicz J, Bodziarczyk J, Ciach M (2013) Development of the rosalia longicorn *Rosalia alpina* (L.) (Coleoptera: Cerambycidae) in the sycamore maple Acer pseudoplatanus L.–the first report from Poland. Polish Journal of Entomology/Polskie Pismo Entomologiczne 82: 19–24. https://doi.org/10.2478/v10200-012-0019-6

Michalcewicz J, Ciach M (2015) Current distribution of the Rosalia longicorn *Rosalia alpina* (Linnaeus, 1758) (Coleoptera: Cerambycidae) in Poland. Polish Journal of Entomology 84: 9–20. https://doi.org/10.1515/pjen-2015-0002

Müller G (1950) I coleotteri della Venezia Giulia, Vol. II Coleoptera Phytophaga (Cerambycidae, Chrysomelidae, Bruchidae). Centro Sperimentale Agrario e Forestale Trieste, pubblicazione n. 4 (1949–1953). La Editoriale Libraria Trieste, Trieste, 1–685.

Nieto A, Alexander KNA (2010) European Red List of saproxylic beetles. Publications office of the European Union, Luxenbourg, 1–46.

Özdikmen H (2007) The Longicorn beetles of Turkey (Coleoptera: Cerambycidae) Part I – Black Sea Region. Munis Entomology & Zoology 2(2): 179–422.

Pagola Carte S (2007) Inventario y seguimiento de la entomofauna del hayedo de Oieleku (Oiartzun, Parque Natural de Aiako Harria) - Campaña, 1–100. http://www.lifeaiakoharria.net/datos/documentos/insectos%20Oieleku2007.pdf

Pail W, Mairhuber C (2010) Der Alpenbock (*Rosalia alpina*) im Lainzer Tiergarten – Erste Lokalisierung, Erhaltungszustand und Empfehlungen für Maßnahmen. Report for the Magistrat der Stadt Wien, Graz, 1–48.

R Development Core Team (2010) R: a language and environment for statistical computing. http://www.Rproject.org

Ranius T, Jansson N (2002) A comparison of three methods to survey saproxylic beetles in hollow oaks. Biodiversity and Conservation 11: 1759–1771. https://doi.org/10.1023/A:1020343030085

Ray AM, Millar JG, McElfresh JS, Swift IP, Barbour JD, Hanks LM (2009) Male-produced aggregation pheromone of the cerambycid beetle *Rosalia funebris*. Journal of Chemical Ecology 35: 96–103. https://doi.org/10.1007/s10886-008-9576-2

Rossi de Gasperis S (2016) Distribution patterns and population analysis of threatened longhorn beetles in forest habitats of Central Italy. PhD Thesis. Università degli Studi Roma Tre, Rome.

Rossi de Gasperis S, Carpaneto GM, Nigro G, Antonini G, Chiari S, Cini A, Mancini E, Mason F, Mosconi F, Redolfi De Zan L, Roversi PF, Sabbatini Peverieri G, Solano E, Campanaro A (2017) Computer-aided photographic identification of *Rosalia alpina* (Coleoptera: Cerambycidae) applied to a mark-recapture study. Insect Conservation and Diversity 10: 54–63. https://doi.org/10.1111/icad.12199

Russo D, Cistrone L, Garonna AP (2010) Habitat selection by the highly endangered longhorned beetle *Rosalia alpina* in Southern Europe: a multiple spatial scale assessment. Journal of Insect Conservation 15: 685–693. https://doi.org/10.1007/s10841-010-9366-3

Russo D, Cistrone L, Garonna AP (2011) Habitat selection in the highly endangered beetle *Rosalia alpina*: a multiple spatial scale assessment. Journal of Insect Conservation 15: 685–693. https://doi.org/10.1007/s10841-010-9366-3

Sama G (1988) Coleoptera, Cerambycidae. Catalogo Topografico e Sinonimico. Fauna d'Italia, 36. Ed. Calderini, Bologna, 1–216.

Sama G (2002) Atlas of the Cerambycidae of Europe and Mediterranean area. 1: northern, western, central and eastern Europe. British Isles and continental Europe from France (excl. Corsica) to Scandinavia and Urals. V. Kabourek, Zlin, 1–173.

Sama G, Löbl I (2010) Cerambycidae, Western Palaearctic taxa, eastward to Afghanistan excluding Oman and Yemen and the countries of the former Soviet Union. In: Lobl I, Smetana A (Eds) Catalogue of Palaearctic Coleoptera. 6. Chrysomeloidea. Apollo Books, Stenstrup, 84–334

Sama G, Rapuzzi P, Özdikmen H (2012) Preliminary report of the entomological surveys (2010, 2011) of G. Sama and P. Rapuzzi to Turkey (Coleoptera Cerambycidae). Munis Entomology & Zoology 7(1): 22–45.

Shapovalov AM (2016) New data on the fauna of longicorn beetles (Coleoptera, Cerambycidae) of the Southern Urals. Entomological Review 96(1): 131–133.

Švácha P, Danilevsky ML (1987) Cerambycoid larvae of Europe and Soviet Union (Coleoptera, Cerambycoidea), Part II. Acta Universitatis Carolinae Biologica 31: 121–284.

Tavakilian G, Chevillotte H (2017) TITAN: Cerambycidae database (version Apr 2015). In: Roskov Y, Abucay L, Orrell T, Nicolson D, Bailly N, Kirk P, Bourgoin T, DeWalt RE, Decock W, De Wever A, Nieukerken E van, Zarucchi J, Penev L (Eds) Species 2000 & IT IS Catalogue of Life, 27th February 2017. Species 2000: Naturalis, Leiden. http://www.catalogueoflife.org/col

Trizzino M, Audisio P, Bisi F, Bottacci A, Campanaro A, Carpaneto GM, Chiari S, Hardersen S, Mason F, Nardi G, Preatoni DG, Vigna Taglianti A, Zauli A, Zilli A, Cerretti P (2013) Gli artropodi italiani in Direttiva Habitat: biologia, ecologia, riconoscimento e monitoraggio. Quaderni Conservazione Habitat, 7. CFS-CNBFVR, Centro Nazionale Biodiversità Forestale. Cierre Grafica, Sommacampagna, VR, 1–256.

Vávra J, Drozd P (2006) Metodika monitoringu evropsky významného druhu – tesarík alpský (*Rosalia alpina*). Agentura Ochrany Prírody A Krajiny Cr, 1–9.

Verdugo A, Buse J, Bartolozzi L, Galante E, Mendez M (2016) *Rosalia alpina*. The IUCN Red List of Threatened Species 2016: e.T19743A21424012.

Villiers A (1978). Faune des Coléoptères de France. 1. Cerambycidae. Editions Lechavalier, Paris, 1–611.

Von Demelt C (1956) Beobachtungen und Bemerkungen über *Rosalia alpina* L. Entomologische Blätter 52: 170–175.

Vrezec A, Ambrozic S, Kapla A (2012) An overview of sampling methods tests for monitoring schemes of saproxylic beetles in the scope of Natura 2000 in Slovenia. Maja Jurc and others (Eds) In: Saproxilic beetles in Europe: monitoring, biology and conservation. Slovenian Forestry Institute, Silva Slovenica, Ljubljana, 73–90.

World Conservation Monitoring Centre (1996) *Rosalia alpina*. The IUCN Red List of Threatened Species 1996: e.T19743A9009447.

Supplementary material 1

Monitoring of *Rosalia alpina* – field sheet

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Data type: Field sheet

Explanation note: Field sheet for surveys of *Rosalia alpina*. The number of observed individuals should be written in the grey cells. Additional information to be reported: Study area, year, date, operator, start time and end time.

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Guidelines for the monitoring of *Morimus asper funereus* and *Morimus asper asper*

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Abstract

Morimus asper is a morphologically variable longhorn beetle that occurs in large parts of southern and central Europe. Although this saproxylic beetle is widespread in old-growth forests or well-structured woodlands, its populations are currently threatened by forest practices, such as the removal of wood (branches and logs). *Morimus funereus* is considered a valid species by some authors and is included in Annex II of the Habitats Directive. However, a recent molecular study found that all European and Turkish populations should be referred to a single species, *M. asper*. In this paper, the monitoring methods proposed for *M. asper* (*sensu lato*) in the various European countries are reviewed and the research carried out in Italy, which was aimed at developing guidelines for its monitoring, is presented. The experiments conducted, mainly with log piles built from freshly cut wood, investigated, amongst other things, the importance of wood type, diameter of logs and age of wood for the number of individuals observed. Based on these results and on a literature review, a detailed monitoring method for *M. asper* is proposed here, together with a discussion on its constraints, spatial validity and possible interferences. In order to facilitate the assessment of the conservation status of populations of *M. asper* and to allow for comparisons between populations and over time, a method for the calculation of a reference value, based on the monitoring method, is also presented.

Keywords
Habitats Directive, saproxylic beetles, Coleoptera, Cerambycidae, monitoring methods, forest biodiversity

Introduction

Morimus Brullé, 1832 is a genus of the family Cerambycidae with a complex taxonomic situation. *Morimus funereus* Mulsant, 1863 and *M. asper* (Sulzer, 1776), once considered distinct species, are now believed to belong to a single, genetically and morphologically variable species, *M. asper* (e.g. Sama and Löbl 2010, Sama and Rapuzzi 2011, Solano et al. 2013, Danilevsky 2015). Populations of this species typically live in old-growth forests or well-structured woodlands. *Morimus funereus* is included in Annex II of the Habitats Directive (Council Directive 92/43/EEC). The Habitats Directive provides that Member States prepare, every six years, a report on the conservation status of the species listed in the Annexes. In order to address this obligation, the Life Project "Monitoring of insects with public participation" (LIFE11 NAT/IT/000252) (hereafter, MIPP) conducted experimental fieldwork to develop a standardised method for monitoring of the saproxylic beetle species of the project: *Osmoderma eremita* (hermit beetle, Scarabaeidae), *Lucanus cervus* (European stag beetle, Lucanidae), *Cerambyx cerdo* (great capricorn beetle, Cerambycidae), *Rosalia alpina* (rosalia longicorn, Cerambycidae), *Morimus asper/funereus* (morimus longicorn, Cerambycidae).

The present paper is part of a special issue on the monitoring of saproxylic beetles protected in Europe and is dedicated to *Morimus asper/funereus*. Therefore, it starts with a comprehensive revision of the current knowledge on systematics, distribution, ecology, ethology and conservation. The review is followed by a detailed account of the fieldwork carried out during the project and concludes with a description of the proposed monitoring method.

Systematics and Distribution

Morimus Brullé, 1832 is a genus of the large beetle family Cerambycidae, subfamily Lamiinae. The genera *Morimus* and *Herophila* are the only European representatives of the tribe Phrissomini (Breuning, 1942), whose main distinguishing feature is the fact that they have reduced hindwings and are unable to fly. Until some years ago, the European populations of the genus *Morimus* were divided into five species, *M. asper* (Sulzer, 1776), *M. funereus* Mulsant, 1863, *M. orientalis* Reitter, 1894, *M. verecundus* (Faldermann, 1836) and *M. ganglbaueri* Reitter, 1894, based exclusively on morphological characters. However, many transitional forms exist amongst these taxa, are difficult to ascribe to one of the above mentioned species and their taxonomic position was often unclear. The different taxa, which are mostly geographical forms, differ from one another in certain morphological traits such as the size and distribution of the granules on the elytra and the colour pattern of the elytra, in particular the background colour and the shape and size of the black spots (Stanić et al. 1985, Sama 1988, 2002, Koren 2010, Sama and Löbl 2010, Danilevsky 2013). Recently, several authors have started considering *M. asper* and *M. funereus* as two subspecies of the same species (e.g. Sama and Löbl 2010, Sama and Rapuzzi 2011, Solano et al. 2013, Danilevsky 2015, Danilevsky et al. 2016). In particular, a molecular study based on COI and ITS2 gene sequences (Solano et al. 2013), found that all European and Turkish populations should be referred to a single, genetically and morphologically variable species which, according to the rules of the International Code of Zoological Nomenclature (http://www.iczn.org/iczn/index.jsp) should be named *M. asper* (Sulzer, 1776). All other taxa merit an intraspecific rank and are simple colour morphs or subspecies. Subsequent studies (Danilevsky 2015, Danilevsky et al. 2016) further complicated the taxonomic situation of the genus by describing a new species from Slovakia (*M. gabzdili* Danilevsky, 2015), a new subspecies of *M. asper* from Greece and a new subspecies of *M. verecundus* from Bulgaria (Danilevsky et al. 2016). Further molecular studies are needed to clarify the complicated taxonomic situation of the genus *Morimus*.

Morimus funereus was included in Annex II of the Habitats Directive with the taxonomic rank of a species, according to the literature of the time (Dajoz 1976). In light of new systematic findings, this protection should also be extended to *M. asper* (*sensu lato*).

When evaluating the systematic position of the taxon *funereus*, it is important to consider that it was described from south-eastern France (Mulsant 1863) and consequently it cannot be a valid name for the eastern European form which probably needs a different name. The taxonomic problems of the genus *Morimus* cannot be resolved in this paper, but need a systematic revision which will also have important implications for the conservation of this taxon.

Here, we consider the two taxa *asper* and *funereus* as subspecies of *M. asper*, as described by many authors (Sama and Löbl 2010, Sama and Rapuzzi 2011, Solano et al. 2013, Danilevsky 2015, Danilevsky et al. 2016). These occur respectively in western (*M. asper asper*) and eastern (*M. asper funereus*) Europe, while *M. asper ganglbaueri* is interpreted as a transitional form between these subspecies due to genetic introgression (Rapuzzi, in preparation). *Morimus asper* (*sensu lato*) is widely distributed in southern and central Europe, from the Iberian Peninsula to European Turkey and occurs in: Albania, Austria, Bosnia and Herzegovina, Bulgaria, Corsica, Croatia, Czech Republic, European part of Turkey, French mainland, Greek mainland, Hungary, Italian mainland, Montenegro, Republic of Macedonia, Republic of Moldova, Romania, San Marino, Sardinia, Serbia, Sicily, Slovakia, Slovenia, Spanish mainland and Switzerland (http://www.fauna-eu.org, Bringmann 1996, Gnjatović and Žikić 2010, Vrezec et al. 2012). The exact distribution of the two major subspecies (*M. asper asper* and *M. asper funereus*) is not clear because of the unreliability of many literature data. However, the distribution of the two subspecies in Italy is quite clear: *M. asper asper* occurs in all regions, including the major islands (only a few known sites in Sardinia). *Morimus asper funereus* was recorded only for the karst area in Friuli-Venezia Giulia (Trieste and southern Gorizia

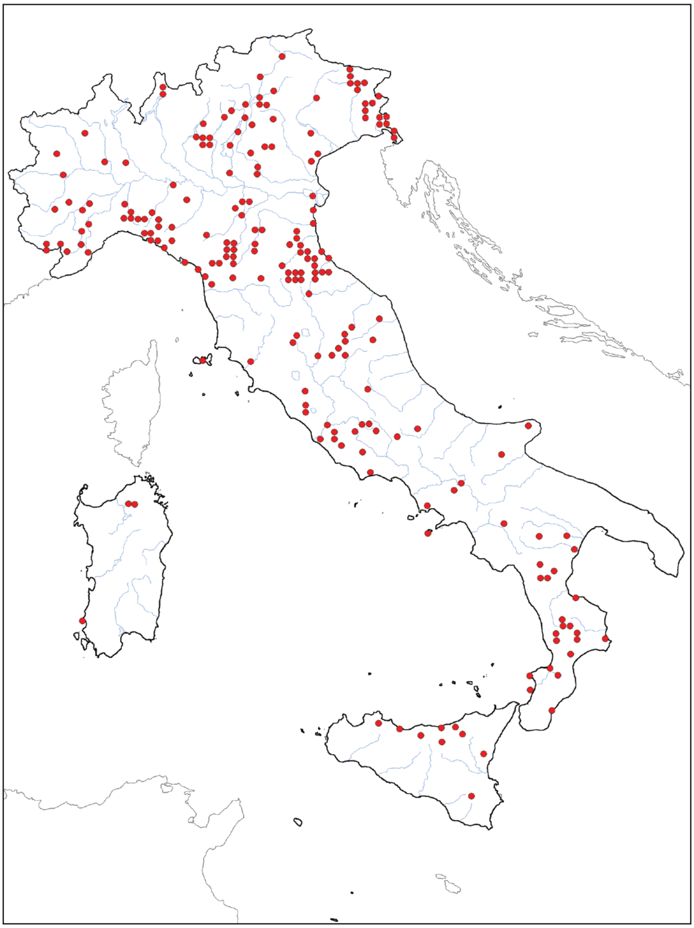

Figure 1. Distribution map of *M. asper* in Italy (Sama 2007).

provinces). The transitional form *ganglbaueri* occurs in Italy from the Tagliamento River to the Karst area of Friuli-Venezia Giulia (P. Rapuzzi, pers. com. 2016). The distribution of *M. asper* in Italy is given in Figure 1 (Sama 2007).

Morphology

The egg of *M. asper* has an ivory colour and measures about 4.5 × 1.2–1.6 mm, with the surface of the chorion consisting of a relief with stellate structures (Romero-Samper and Bahillo 1993). The development of the larval stages is typical for holometabolous insects, as the various instars do not vary greatly in shape, but only differ in body size. The larva has a typical morphology for saproxylophagous longhorn beetles: the colour is white and fleshy, the head is sclerified, with very small antennae and the body is legless (Pavan 1948a, 1948b, Romero-Samper and Bahillo 1993) (Figure 2). The newly hatched larvae measure about 5 mm in length but, at the last larval stage, can reach up to 60 mm (Romero-Samper and Bahillo 1993). The pupa is exarate, i.e. has free appendages (Pavan 1948a, 1948b) and initially is milk white, but darkens as the metamorphosis progresses (Romero-Samper and Bahillo 1993).

Adults (Figure 3) measure 15–40 mm in length (Parisi and Busetto 1992) and have an elongated-oval body. The elytra are fused together, have a grainy look and are typically dark grey or opaque black. Each elytron bears two black spots but these can be almost invisible in individuals with a very dark background colour. As mentioned above, this background colour differs amongst the various taxa and forms throughout the range of the species.

A conspicuous sexual dimorphism exists in the antennal length of *M. asper*, as in many other longhorn beetles. Male antennae can measure up to 7.5 cm, exceeding the length of the body and are much longer than in females (Parisi and Busetto 1992) (Figure 4). In a recent study, Rossi de Gasperis (2015) showed that the length of the antennae varies greatly amongst males from the same population and that males with longer antennae mate more frequently. The same study also revealed a sexual dimorphism in the pronotum length as well as in the width and length of the elytra, with males allocating more resources to the development of the anterior portion of the body, while females invest more in the posterior part of the body, where the reproductive system is located.

Identification and comparison with similar taxa

Morimus asper is chromatically variable throughout its range and the two subspecies *asper* and *funereus* are distinguished by different background colours (Figures 3 and 4): darker, almost black in *M. asper asper* and light grey with four very obvious spots in *M. asper funereus*. This chromatic variability is accompanied by slight differences in the micro sculpture of the elytra, in particular in the shape and size of the granules that cover their surface. *Morimus asper* can be easily distinguished from all other Italian longhorn beetles, with the only exception being *Herophila tristis* (Linnaeus, 1767) (Figure 5A) which belongs to the Phrissomini tribe as *M. asper* and *Lamia textor* (Linnaeus, 1758) (Figure 5B), of the Lamiini tribe. Compared to *H. tristis*, *M. asper* is generally longer (*M. asper*: 16–38 mm; *H. tristis*: 13–26 mm). Another important feature is the background colour which, in *H. tristis*, is clearly brown rather than black or grey. Further distinguishing characters are antennae and legs which are thicker and shorter in *H. tristis*. In *M. asper*, the first antennal segment is shorter than the third whereas, in *H. tristis*, it is longer or as long as the third segment.

Lamia textor is relatively rare in Italy and can be distinguished from *M. asper* by some characters that can be difficult to observe in the field, particularly in females. One such character is the absence of wings in *M. asper* (well developed in *L. textor*) and another is

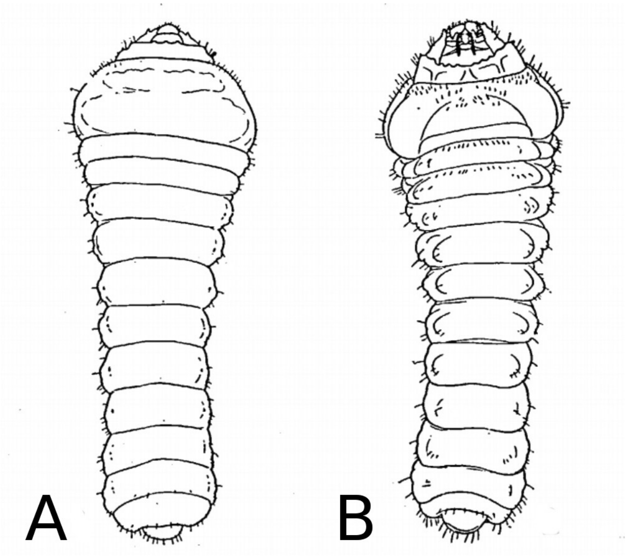

Figure 2. A freshly hatched larva of *Morimus asper asper*: **A** dorsal view **B** ventral view (Pavan 1948b).

that the first antennomere is shorter than the third in *M. asper*. In contrast, in *L. textor*, the first antennomere is as long or longer than the third and the circular keel of the first antennomere is well marked and complete whereas, in *M. asper*, this keel is less marked and incomplete. A lobe on the outer side of the mesotibia is well marked in *M. asper* but less visible in *L. textor* (Picard 1929, Porta 1932). Further differences relate to the form of the elytra; in *M. asper*, they are more humped and the humeral callus is more projecting (despite the fact that *M. asper* is wingless). Furthermore, the granules are more marked on the entire surface of the elytra of *M. asper* and smaller on the apical third (especially in the ssp. *funereus* where they are partly covered by bristles), but are always clearly visible. In contrast, in *L. textor*, granules are clearly visible only in the fore half of the elytra and tend to fade towards the rear and, in the apical area, form a slight and indistinct roughness. A final character concerns the surface of the elytra of *L. textor* which is much flatter, often showing a pattern of white specks that can be almost invisible.

Ecology

Habitat

Morimus asper is a silvicolous, xylophagous and saproxylic species, its main habitat being deciduous and mixed forests (Jurc et al. 2008, Carpaneto et al. 2015). The species lives mainly in old-growth forests or well-structured woodlands, with a medium-high density of dead wood (Trizzino et al. 2013, Bărbuceanu et al. 2015). In forests, rich in deadwood, such as in the well-studied site of Bosco della Fontana (Mantova, northern Italy), abundant populations can be present (Hardersen et al. 2017). According to the literature, *M. asper* mainly lives in deciduous forests, often dominated by oaks (*Quercus* spp.)

Figure 3. *Morimus asper funereus* (Photo by Kajetan Kravos).

or by beech (*Fagus sylvatica*) (López-Vaamonde et al. 1993, Romero-Samper and Bahillo 1993, Bărbuceanu et al. 2015, Rossi de Gasperis et al. 2016). Even though the preferred habitats are mature forests, populations of this beetle are often found in coppiced stands, characterised by the presence of old stumps and decaying wood on the ground (Cerretti 2008). For many areas of Europe, a preference for beech forests has been reported; for example in Slovenia (Jurc et al. 2008) and in the Iberian Peninsula (López-Vaamonde et al. 1993, Romero-Samper and Bahillo 1993). In Bulgaria, *M. asper* is much more common in the mountain beech forests than in the plains and hills, where it is mainly found in the more humid woodlands, such as riparian forests (Anonymous 2015).

Morimus asper is flightless and has a very limited dispersal capacity when compared to other longhorn beetles (Luce 1996, Bărbuceanu et al. 2015) and its populations are often fragmented. As a consequence, there are numerous habitats that are suitable for this species but are not colonised by it (Bărbuceanu et al. 2015). The research, carried out during the MIPP project, also confirmed this observation for some of the areas investigated in Italy. Rossi de Gasperis et al. (2016) marked 727 individuals in different areas of central Italy and only reported dispersal in 13 adults, with distances covered varying between 20 and 451 metres.

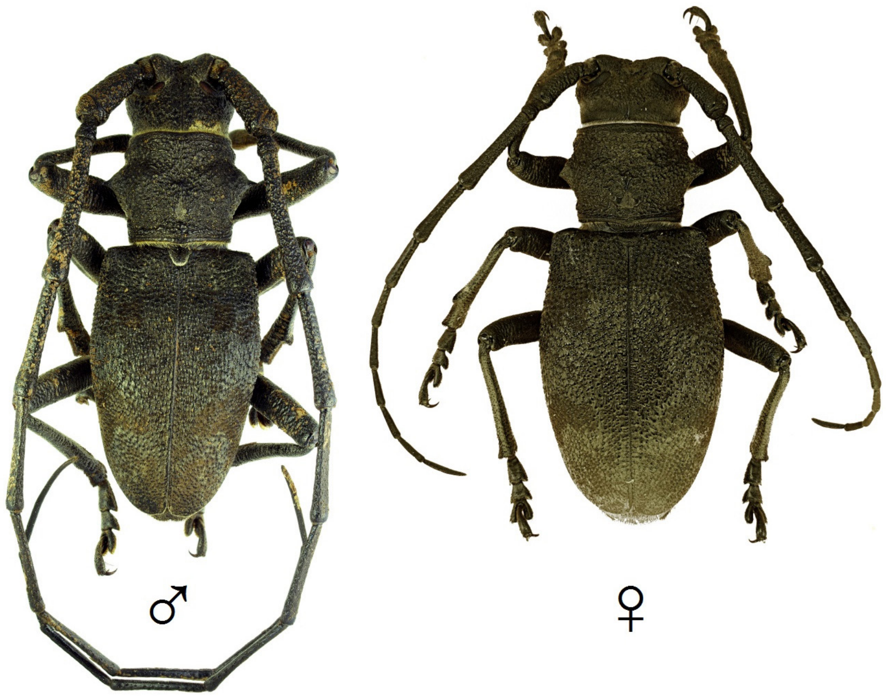

Figure 4. Sexual dimorphism in *Morimus asper asper* (photo by Federico Romiti).

Larval ecology

The larval development takes place in the wood of trunks and stumps and is believed to be completed in three or four years under natural conditions (Stanić 1985, Luce 1996), but may take less time in captivity (Pavan 1948a, Romero-Samper and Bahillo 1993, Dojnov et al. 2012). Generally, authors indicate tree species of several genera and families as larval food sources for *M. asper* (e.g. Sama 2002, Dojnov et al. 2012). However, it is often not clear if the host tree species, cited in the literature, were identified by observing adults or by having observed successful development and emergence of freshly metamorphosed individuals. In fact, observation of an adult on a tree does not prove that the species can reproduce in the wood of this plant species. For example, Romero-Samper and Bahillo (1993) reported, for the Iberian Peninsula, that adults were detected on eight tree species, but larvae were observed only in *Fagus sylvatica*, *Quercus robur*, *Q. faginea*, *Populus nigra* and *Pinus radiata*. Most authors concur that *M. asper* is a polyphagous species (e.g. Luce 1996, Sama 2002, Juillerat and Vögeli, 2004, Polak 2012). According to Sturani (1981), who reported the results obtained by collecting wood attacked by the larvae of longhorn beetles and keeping them alive up to adult emergence, *M. asper* is extremely polyphagous and has emerged from the wood of *Abies*, *Picea*, *Acer*, *Alnus*, *Castanea*, *Platanus*, *Juglans*, *Populus*, *Prunus*, *Quercus*, *Salix*, *Ulmus*, *Tilia* and *Fagus*. Subsequent authors confirmed the extreme polyphagy of this species (e.g. Luce 1996, Sama 2002, Juillerat and Vögeli 2004, Polak 2012) and

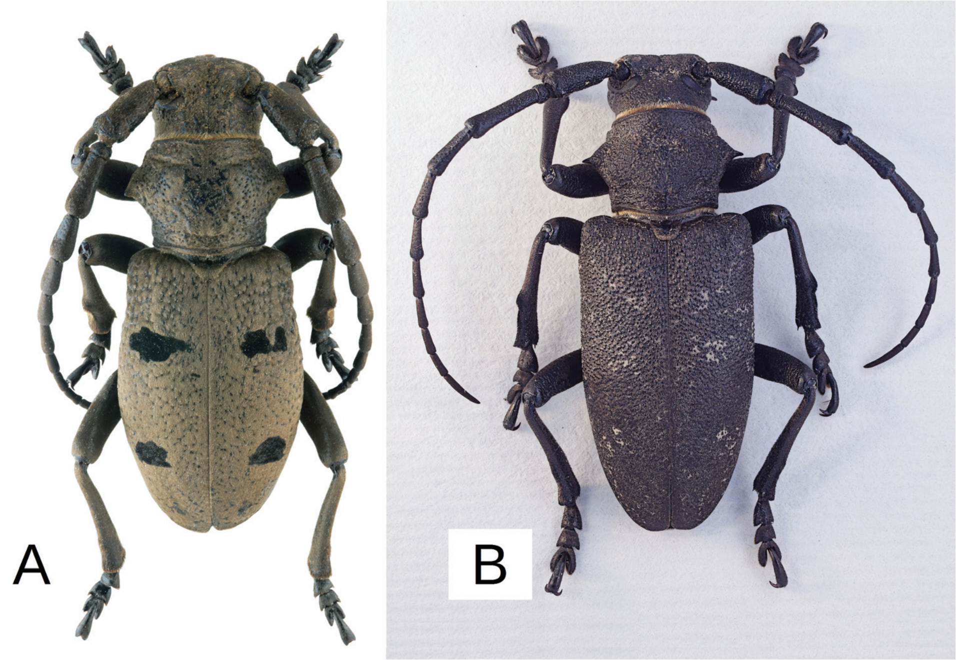

Figure 5. **A** *Herophila tristis* (Photo by Michal Hoskovec) **B** *Lamia textor* (Photo by Federico Romiti).

added new host plants to the lists. For Slovenia, Polak (2012) indicated as host plants *Fagus sylvatica*, *Quercus* spp., *Carpinus betulus*, *Acer* spp., *Castanea sativa*, *Abies alba* and others, while Vrezec et al. (2010) reported that the species selected *Quercus* sp. and *Abies alba*, but less so for *Fagus sylvatica* and *Picea abies*. In Romania, Bărbuceanu et al. (2015) observed adults of *M. asper* on *Quercus* sp., *Fagus sylvatica*, *Robinia pseudacacia* and *Prunus avium*, but they indicated that *Quercus petraea* seems to be the most important tree for larval development. Other Romanian authors more generally reported that this beetle prefers the dead wood of oak and beech trees (Fusu et al. 2015).

In the literature, no information was found on predators of *Morimus* adults.

Adult ecology

Morimus asper lives over a wide altitudinal range and the phenology of local populations is related to altitude. For example, in Slovenia, this species was observed between 150 and 1,240 m a.s.l. but is most common between 300 and 900 m a.s.l. (Vrezec et al. 2009). It was found up to an altitude of 1,500 and 1,800 m a.s.l. in Bulgaria (Bringmann 1996, Anonymous 2015) and between 10 and 1,000 m a.s.l. in the Iberian Peninsula, with single records up to 1,500 m a.s.l. (Romero-Samper and Bahillo 1993). In Italy, the altitudinal distribution of *M. asper* extends from sea level to 1,800 m a.s.l. (Trizzino et al. 2013, Bologna et al. 2016) and this has also been confirmed by a citizen science approach which collected data on this species between 2 m and 1,870 m a.s.l. (Campanaro et al. 2017).

It is generally believed that the adults of *M. asper* are long-living (Stanić et al. 1985, Polak 2012). In fact, overwintering adults are the first to emerge in spring and can also reproduce in the second year (Polak 2012, Rossi de Gasperis et al. 2016). Adults in captivity can live for up to 560 days (Stanić et al. 1985).

The period of the year when adults are active is quite long when compared to other insects but seems to be variable. For a site in Slovenia at 630 m a.s.l., Polak (2012) reported that adults were active from the beginning of May until the end of August, with males starting to appear slightly earlier than females. Drovenik and Pirnat (2003) observed adults from May to July, while Vrezec (2008), analysing data not collected systematically, found that *M. asper* was active from 2 February to 30 September, with most observations being made between 19 May and 11 July. In Romania, adults are active between April and the first half of August, with sporadic observations until late October (Bărbuceanu et al. 2015) and in Bulgaria between April and August (Bringmann 1996). In the Iberian Peninsula, adults are active from the second half of April until early or mid September with the peak of activity being between late July and early August (López-Vaamonde et al. 1993, Romer-Samper and Bahillo 1993). In contrast, Polak (2012) observed two peaks of activity in Slovenia, the first from the end of May to early June and the second between the end of July and early August and suggested that the second peak in August was the result of freshly emerged specimens. Adults enter diapause in August or September (Stanić et al. 1985, Luce 1996). In the plains of northern Italy (Bosco della Fontana, Mantova), *M. asper* was found to be active from late March to mid-July with the peak of activity in April (Hardersen et al. 2017), while in the Julian Alps (about 850 m a.s.l.) adults were observed from mid-May to mid-August (Leonarduzzi 2016). In the context of a citizen science approach carried out in Italy, *M. asper* was recorded from 8 January to 29 October, with large numbers observed from mid-April to mid-August (Campanaro et al. 2017). An important point of this study was the observation that peak activity was observed ever later during the year as altitude increased. Whereas peak activity of *M. asper* was observed at low altitudes (0–400 m) on 23 May, at an altitude of 1,201–1,600 m, the highest activity of *M. asper* resulted on 27 June (Campanaro et al. 2017).

Adults need to feed in order to survive for so many months. However indications on the food consumed in the wild are very scarce. Drovenik and Pirnat (2003) indicate that adults feed on sap from wounded trees and adults in captivity were found to feed on the bark of tree branches (Vrezec et al. 2010, R. Fabbri, pers. com.).

Morimus asper is mainly active in the evening and during the night (e.g. Romero-Samper and Bahillo 1993, Merkl and Hegyessy 2008, Polak 2012). At Bosco della Fontana, *M. asper* was observed with the highest abundance (and with the highest detectability) between 20:00h and 24:00h; however, the species was also detected during the day, but less than 30% of individuals were observed at 12:00h when compared to the peak of abundance (Hardersen et al. 2017). Similarly, Romero-Samper and Bahillo (1993) reported that *M. asper* is generally active during dusk/night but it is also not uncommon to observe adults during the day, even during the hottest hours. In contrast, Stanić et al. (1985) observed that adults are inactive from 12:00h to 15:00h, regardless of the temperature. According to Polak (2012), the activity of adults changes during the season: in spring the peak of activity was observed at midday whereas, during hot summer days with temperatures above 27°C, breeding activity shifted to late afternoon and night-time. Polak (2012) considered 17–27°C as the temperature limits of activity, whereas Stanić et al. (1985) observed active adults in a slightly more extended range of temperatures (12–30°C) and the data provided by Leonarduzzi (2016) showed a reduced activity below 17°C. Under inappropriate weather conditions, adults usually hide amongst loose bark or within holes in the earth (Polak 2012).

Adults of *M. asper* are attracted by damaged trees and recently cut wood (Drovenik and Pirnat 2003, Chiari et al. 2013) and the probability of observing adults increases with the volume of the wood (Chiari et al. 2013). Females oviposit in dead wood with bark still attached, with a preference for large standing trees, trunks on the ground and large stumps (Campanaro et al. 2011). They can also be found on log piles, provided that the bark is still present (Campanaro et al. 2011). According to Bărbuceanu et al. (2015), adults can be found on trunks and stumps, recently cut trees and trunks of old trees. Romero-Samper and Bahillo (1993), however, reported that females choose stumps, trunks of trees that had recently died or trees which are alive but weakened and recently cut trees (maximum two years previously). Polak (2012) noted that the stumps served as meeting points for mating and for breeding and that some fresh stumps were preferred by males. Stumps of trees cut more than one year before are hardly ever visited and old, dry and barkless trees are not attractive (Polak 2012).

Males select stumps that are most attractive for females and here usually stand in a typical "displaying posture" whereas other males, which are unable to defend a territory, permanently wander around (Polak 2012). In contrast, females showed no preference for a particular stump, were frequently observed away from dead wood and mated repeatedly with different males (Polak 2012). Males are observed more frequently than females and also have a higher probability of being recaptured (Chiari et al. 2013, Bărbuceanu et al. 2015), in accordance with the sex-specific behaviour described above.

Life cycle

The female starts oviposition about 16 days after emergence, gnaws with her mandibles into tree bark and lays one egg in each pit created, while the male exhibits mate-guarding to discourage other males (Polak 2012). Females lay eggs in dead wood, preferring large dead trunks, standing or fallen and are less attracted by trunk diameters less than 13 cm (Hardersen et al. 2017). Females are also attracted by piles of wood (Anonymous 2015), also ovipositing in physiologically debilitated trees or stumps which contain developing larvae hatched in the previous year (Dojnov et al. 2012, Polak 2012). Each female can lay more than one hundred eggs during the entire season (Stanić et al. 1985, Dojnov et al. 2012). In the laboratory, overwintering females can lay eggs during a second oviposition cycle after a diapause of at least 4 months. This period of inactivity is likely to be longer in nature, since females do not lay eggs at temperatures lower than 21°C (Stanić et al. 1985). Egg development lasts about 9–12 days and ends with the hatching of the larvae which feed by excavating subcortical galleries (Romero-Samper and Bahillo 1993). Dojnov et al. (2012) observed 5 or 6 larval stages (in some cases up to 12) in the laboratory, with different relative average durations (8, 11, 16, 23, 31 and 23 days). These data refer to larvae obtained from eggs laid by individuals collected in the field. The average rate of larval mortality in the laboratory for the first and the second generation were 9.7% and 20.6% respectively, while mortality for completion of larval development was between 10.3% and 34.9% (Dojnov et al. 2012). At the end of the last larval stage, the larvae create long pupal cells in the wood, up to 8 cm in length (Romero-Samper and Bahillo 1993). The pupa matures in 18 to 23 days (Romero-Samper and Bahillo 1993, Dojnov et al. 2012). The adult remains inside the pupal cell for another 14–20 days prior to emergence, producing a circular exit hole of 8–12 mm in diameter (Romero-Samper and Bahillo 1993). Altogether, the duration of the life cycle of larvae observed in the laboratory varied between 218 and 313 days (Dojnov et al. 2012), while under natural conditions, the development lasts 3–4 years (Stanić et al. 1985). As discussed above, adult *M. asper* are able to overwinter and can survive for more than a year (Stanić et al. 1985, Romero-Samper and Bahillo 1993, Dojnov et al. 2012, Polak 2012, Rossi de Gasperis et al. 2016). The life span of females in the laboratory ranged from 72 to 560 days (Stanić et al. 1985) and in the wild can exceed 400 days (Rossi de Gasperis et al. 2016).

Threats and conservation

Morimus funereus (now considered a subspecies of *M. asper*) is included in Annex II of the Habitats Directive. In the third national report under the Article 17 of the Habitats Directive, the overall assessment of the conservation status for *M. funereus* was "favourable" (Genovesi et al. 2014). This taxon is listed as a species in the IUCN Red List of Italy (Audisio et al. 2014, Carpaneto et al. 2015). It is included in the category Vulnerable (VU) by the IUCN Red List of Italy with the same status being given to the species in 1996 on a global scale (World Conservation Monitoring Centre 1996). Nevertheless, World Conservation Monitoring Centre (1996) and Carpaneto et al. (2015) suggested that *M. funereus* should probably be treated as a subspecies of *M. asper* (*sensu lato*). A revision of its taxonomic status is all the more urgent given the recent results on the existence of a single species throughout Europe which should be named *M. asper* and which is supported by a recent molecular study (Solano et al. 2013). The need to clarify the taxonomic position of the different "forms" of the genus *Morimus* is also highlighted by the fact that the taxon *asper* is not listed in any of the above documents.

One of the main threats to *M. asper*, reported in the literature, is the loss of habitat, such as the removal of branches and logs from the forest floor, as well as of standing dead or dying trees (Cerretti 2008, Dojnov et al. 2012, Trizzino et al. 2013, Rossi de Gasperis et al. 2016). Another threat resulting from forest management is the felling and removal of large trees which are debilitated and which are important larval habitats for *M. asper* and other saproxylic beetles (Audisio et al. 2014, Carpaneto et al. 2015, Rossi de Gasperis et al. 2016). The loss of suitable habitat can lead to a substantial reduction in the abundance of *M. asper* and the continued decline of resources can diminish the number of occupied sites (Dojnov et al. 2012, Rossi de Gasperis et al. 2016). The loss of suitable habitats can also lead to an isolation of the occupied areas, a factor particularly important for *M. asper*, given its low mobility (Cerretti 2008, Trizzino et al. 2013).

A further threat for this longhorn beetle is caused by the forestry practice of leaving freshly cut wood in large piles along forest roads for extended periods and during the breeding season between April and August (Polak 2012). These wood piles can act as "ecological traps" (*sensu* Robertson and Hutto 2006) for *M. asper* and other saproxylic beetles, as they are attracted to the timber where females lay eggs but, usually, this timber is removed and used commercially before the larvae can complete their life cycle. The large wood piles created by commercial forestry operations are particularly attractive for *M. asper* as the probability of occupancy increases with increasing volume of wood (Chiari et al. 2013). This is all the more worrying as the rest of the forest is generally depleted of dead wood, particularly of large diameter trunks. The negative effects can be mitigated by removing the piles before they are colonised (Hedin et al. 2008). It is important to consider that the wood piles proposed for monitoring *M. asper* (see below) are used for oviposition by females of *M. asper* (and other saproxylics). Therefore, it is important to leave this wood in the forest in order to facilitate successful reproduction of the species (Chiari et al. 2013, Hardersen et al. 2017).

Review of monitoring methods in European countries

The methods so far proposed for the monitoring of *M. asper* can be assigned to one of two general strategies: (1) searching/capturing adults which are attracted by freshly cut wood and (2) searching for adults along transects. However, none of the methods proposed has been tested at several sites and/or for several years. In the following paragraphs, an overview of the monitoring methods published for different European countries is presented.

Slovenia

In this country a standard method for the monitoring of *M. asper* has been published by Vrezec et al. (2009). Surveys were carried out with pitfall traps, placed in groups of two or three around fresh stumps (up to one year from the felling of the tree). The traps were fitted with a funnel (Figure 6) to prevent the escape of captured individuals (Vrezec et al. 2009). During these visits, the stumps were also checked for the presence of adults. The combination of pitfall traps and checking stumps significantly increases the probability of detecting the species (Vrezec et al. 2009, 2012). The traps were checked every day after their installation for a total of five days. According to Vrezec (2008), the monitoring should be carried out between the second half of May and the end of June, which is considered the optimal period. Each selected area was monitored using 25 sampling units (a stump with 2 or 3 traps). The problem with this method is that it is based on stumps created by felling trees and therefore cannot be applied in forests where no trees have been cut (Vrezec et al. 2012).

Figure 6. Pitfall traps used by Vrezec et al. (2009).

In addition to the standard monitoring method described above, *M. asper* is recorded in the entire territory of Slovenia by collecting records from the public and this Citizen Science approach is an integral part of the national monitoring scheme. To engage the public, various ways of communication (television, local newspapers, national newspapers, websites, social media etc.) are used, as well as posters which are exhibited in schools, shelters, protected areas etc. (Vernik 2014).

In a further survey applied in Slovenia by Polak (2012), stumps were checked for the presence of adults from the first week of May until the last week of August. For this study, stumps of freshly cut trees were selected (avoiding old and dry ones) in areas where trees had been felled. A total of 14 stumps were selected and each checked 35 times. Polak (2012) found that females were less visible and therefore less detectable than males. The number of observed adults fluctuated daily and these fluctuations were probably influenced by climatic conditions and the time of day in which the surveys had been carried out.

Bulgaria

The monitoring protocol used in Bulgaria (Anonymous 2015) was based on transects of a total length of 1km, carried out by two people walking next to each other. Both observers searched for *M. asper* on either side. Monitoring should be carried out in the afternoon-evening (15:00h-19:00h) and should last one hour. Adults were searched for on trunks of old dead standing trees, large branches on the ground and piles of wood. Records were registered every hour. Best results were obtained when the monitoring was repeated 10 times between May and August.

Romania

In Romania, the monitoring protocol (Fusu et al. 2015) was based on transect walks with a total length of 500 m and a width of 20 m. The minimum time for a transect was 30 minutes and the distance between two transects was 100 m. If possible, five transects were carried out in each area and these were to be conducted by two persons.

Hungary

For Hungary, Merkl and Hegyessy (2008) recommended not to undertake monitoring for *M. asper* as it is difficult methodologically. The method they proposed is based on line transects with a width of 20 m to be carried out in forest areas measuring at least 1ha. Adults were to be searched by following the longest straight line which can be placed in a given area and were counted while walking the transect. Since *M. asper* is mainly active in the evening and at night, it was recommended to also search shaded parts of the logs, stumps and log piles and the underside of freshly cut wood from oaks which had been placed against trunks. The survey was to be carried out four times in consecutive weeks.

Italy

For Italy, the first method proposed for the monitoring of *M. asper* was published by Campanaro et al. (2011) and was based on searching for adults visually and marking them. Standing old dead trees, fallen tree trunks, large branches on the ground, large stumps, wood piles made of large and medium-sized wood and trunks of old and senescent trees were investigated. The surveys were carried out during the day and during the night, but the authors recommended carrying out the fieldwork preferably during the late afternoon (e.g. from 15:00h to 19:00h). Chiari et al. (2013) tested freshly cut log piles as bait for monitoring of *M. asper* in the Nature Reserve Bosco della Fontana. Altogether, they used 29 log piles and counted the number of observed individuals daily between 3 and 8 May 2010. Wood piles with volumes of 0.50 m^3 and 0.25 m^3 were occupied with a cumulative probability of 99% and 95% respectively and the probability of occupancy increased with the volume of wood piles. In summary, the freshly cut log piles were shown to be a good method for monitoring the presence and the abundance of *M. asper*. Chiari et al. (2013) suggested that, in a habitat similar to the ancient forest of Bosco della Fontana, the most efficient allocation of resources for standard monitoring of *M. asper* is by surveying 33 wood piles of at least 0.25 m^3 five times. Trizzino et al. (2013) recommended the use of freshly cut log piles which are built from a variable number of logs (e.g. 15–30), at least 30–60 cm long and with a diameter between 20 cm and 40 cm. These are to be arranged along linear transects, at a distance between 30 m and 50 m. The authors also suggested applying the mark-recapture method. A minimum of six monitoring sessions should be carried out at least every three days. Bologna et al. (2016) also recommended the use of freshly cut log piles for monitoring.

Methods

During this research, the log piles used had a standard volume of 0.3 m^3 and were employed to address a number of objectives, in different years and in different study areas. Research was carried out during the period 2014–2016 and in two forest reserves, Bosco della Fontana in northern Italy (Province of Mantova, Italy, 25 m a.s.l.) and the Parco Naturale Regionale delle Prealpi Giulie in the Julian Alps, north-eastern Italy. At Bosco della Fontana, the study sites were situated between 45.19961°N, 10.73476°E and 45.19848°N, 10.74199°E. In the Prealpi Giulie, the research was conducted in a forest of the municipality of Resia, in the locality Starmiza di Resia (between 46.343490°N, 13.299400°E and 46.341420°N, 13.307800°E). The statistical analyses were performed using the programme R version 3.3.1 (R Development Core Team 2015). Generalised Linear Models (GLMs) for overdispersed count data with a Poisson distribution to test for differences between the different age classes, followed by a post-hoc Wilcoxon pairwise test were used. The following methods were tested: pitfall traps baited with potentially attractive compounds, stumps, trunks and freshly cut log piles. The log piles were used to provide answers to the following questions: (1) Do season and time of the day influence the detection probability and/or abundance of the species; (2) Does the diameter affect the number of *M. asper* observed on the piles; (3) Is the number of adults observed affected by the age of wood piles and (4) Does the attractiveness of different tree species vary?

Bosco della Fontana 2014

In 2014, freshly cut log piles and pitfall traps baited with chemical compounds were used to address the following questions: (1) do season and time of the day influence the detection probability and/or abundance of the species? (2) do the site covariates affect the detection and/or the abundance? (3) do selected chemical substances attract the species? This research, which employed wood from *Quercus robur* and *Carpinus betulus* of three diameter classes, has already been published (Hardersen et al. 2017). Additionally, an experiment was carried out to investigate whether selected chemical substances attracted *M. asper*. The substances tested were Fuscumol, Fuscumol acetate, Ethanol and Isopropanol (Hardersen et al. 2017).

Bosco della Fontana 2015

In 2015, two experiments were carried to investigate the effect of the age of wood piles on the number of adults observed. The first experiment involved woodpiles built from trees of *C. betulus* which had been cut during the following periods: 19.02.-03.03.2014 (hereafter 03_14), 16–17.12.2014 (hereafter 12_14) and 16–23.03.2015 (hereafter 03_15). These piles were built from wood cut from trunks and branches with a diameter 13–50 cm. For each age-group, a total of 10 wood piles were built in random order at the sides of forest roads, spaced at intervals of 50 m. The adults were searched for once a week from 1 April to 27 May 2015 (i.e. a total of nine surveys) starting at 20:00h. The second experiment, which was carried out once the first had been completed, involved the same wood piles built from trees of *C. betulus* as above. However, the oldest piles (03_14) had been replaced by new ones, built on 27.05–03.06.2015 (hereafter 05_15) from wood with a diameter 13–50 cm. The aim of this second experiment was to test whether the wood piles built in December and March had already lost their attraction by June/July. The adults were searched for once a week from 10 June to 8 July 2015 (i.e. a total of five surveys). Again fieldwork was started at 20:00h. For both experiments the effect of the age of the wood piles on the number of observed *M. asper* was analysed employing the sums of all *M. asper* observed during the surveys.

Bosco della Fontana 2016

In 2016, an investigation was initiated to determine whether the attractive properties of different tree species for *M. asper* varied. At Bosco della Fontana, 28 woodpiles were built from four tree species: *C. betulus*, *Fraxinus ornus*, *Juglans nigra*, *Quercus rubra* which had been cut from 26.01 to 21.03.2016. These wood piles were built from wood cut from trunks and branches with a diameter 13–45 cm and were randomly distributed at the sides of forest roads, spaced at intervals of 50 m. The adults were searched for once a week from 29.03 to 17.05.2016 (i.e. a total of eight surveys) starting at 20:00h. This research has already been published (Leonarduzzi et al. 2017).

Parco Naturale Regionale delle Prealpi Giulie 2015

In the literature, the following structures are cited as attractive for *M. asper*: log piles (Campanaro et al. 2011, Chiari et al. 2013, Anonymous 2015), fallen trunks (Romero-Samper and Bahillo 1993, Polak 2012, Bărbuceanu et al. 2015) and stumps (Romero-Samper and Bahillo 1993, Dojnov et al. 2012, Polak 2012, Bărbuceanu et al. 2015). In order to test which of these structures is more attractive, on 11.05.2015 wood from freshly cut beech (*Fagus sylvatica*) was exposed in the locality Sella Starmiza in the Parco Naturale Regionale delle Prealpi Giulie (Udine province) in 6 sites (coordinates: 46.34344°N, 13.30040°E to 46.34063°N, 13.30829°E) at an altitude between 760 to 870 m a.s.l. Each station was built from two beech trees, which consisted of two trunks (cut to a standard volume of 0.3 m^3), two wood piles (standard volume of 0.3 m^3) and the two stumps created by the felling of the trees. In each station, one wood pile had been built from the lower part of the trunk and one from the upper part. The sites were checked for the presence of adults of *M. asper* according to the following timetable: 29.06.2015 (08:15h-13:10h and 18:20h-21:50h); 30.06.2015 (08:09h-12:10h and 18:07h-21:50h); 01.07.2015 (08:03h-11:18h and 18:15h-20:15h); 28.07.2015 (08:00h-10:57h and 18:00h-20:18h); 30.07.2015 (08:39h-11:12h and 17:37h-20:21h) and 31.07.2015 (08:50h-11:24h and 18:18h-20:56h).

Parco Naturale Regionale delle Prealpi Giulie 2016

In 2016, an investigation was initiated to determine whether the attractive properties of different tree species for *M. asper* varied. In the locality Starmiza, the experiment was initiated on 03.05.2016. As it was known that *M. asper* was not evenly distributed (unpublished data), a randomised block design was employed with a distance of 85–215 m between the various blocks. Each block consisted of three piles built from wood of *F. sylvatica*, *Fraxinus excelsior* and *Picea abies*, with diameters of 13–30 cm. Adults of *M. asper* were searched for approximately once a week from 17.05 to 12.08.2016 (i.e. a total of 13 surveys) starting at 18:00h. This research is currently being published (Leonarduzzi et al. 2017).

Results

Bosco della Fontana 2014

Morimus asper was observed with the highest detectability and abundance between 20:00h and 24:00h (Hardersen et al. 2017). Although adults were also detected during the day, at 12:00h the abundance was less than 30% when compared to the maximum values. Additionally, the number of *M. asper* observed was strongly influenced by the characteristics of the wood piles, with wood from *Q. robur* clearly preferred to *C. betulus* and small diameter wood (<12 cm) being least attractive. Hardersen et al. (2017) recommended building piles from wood with a diameter >13 cm. An experiment, which investigated whether selected chemical substances attracted *M. asper*, showed that Fuscumol, Fuscumol acetate, Ethanol and Isopropanol did not attract *M. asper* into pitfall traps (Hardersen et al. 2017).

Bosco della Fontana 2015

The investigation of the effect of the age of wood piles on the number of observed *M. asper* revealed that the age of the timber was an important factor to consider. In the first experiment, the number of individuals observed on the piles built from wood which had been cut more than a year ago (03_14) was approximately 1% of that observed on piles built during winter (Figure 7A), a highly significant difference (Wilcoxon signed rank test, 03_14 vs. 12_14: *p*=0.006; 03_14 vs. 03_15: *p*=0.012). Interestingly, the numbers of individuals observed on the wood piles built in December (12_14) and in

March (03_15) were very similar, with a non-significant difference (Wilcoxon signed rank test, 03_15 vs. 12_14: *p*=0.49).

For the second experiment, the GLM was significant (*z*=2.188, *p*=0.0287) and, on the wood piles built during the winter (12_14 and 03_15), less than 20% of *M. asper* were observed when compared with the new wood piles built between 27.5 – 3.6.2015 (Figure 7B). This difference was significant (Wilcoxon signed rank test, 03_15 vs. 05_15: *p*=0.017; 12_14 vs. 05_15: *p*=0.017). Thus, during the summer months, the wood piles became less attractive after two months, presumably because the wood was drying out. In contrast, no difference was found between the log piles built in December and March (Wilcoxon signed rank test, 12_14 vs. 03_15: *p*=1.0), as in the previous experiment. Both experiments showed that the age of the wood was important for the number of *M. asper* observed. Only fresh wood was highly attractive and the wood piles became less attractive much more quickly during summer than in winter.

Bosco della Fontana 2016

This experiment clearly showed that adults were observed more frequently on the wood of some tree species: most individuals were observed on *Juglans nigra* and the least attractive tree species was *Fraxinus ornus* (Figure 8A), with *Quercus rubra* and *Carpinus betulus* being intermediary (Leonarduzzi et al. 2017).

Parco Naturale Regionale delle Prealpi Giulie 2015

Log piles, trunks and stumps attracted *M. asper*, but log piles and trunks permitted the observation of a significantly higher number of individuals, when compared with stumps (Figure 9A). The GLM model was highly significant (*z*=9.570, *p*<0.001).

The number of adults observed on trunks and log piles was high and significantly different from that observed on stumps (log piles vs. stumps: *p*=0.028; trunks vs. stumps: *p*=0.027). The number of individuals observed on wood piles was lower when compared with trunks, but this difference was not significant (log piles vs. trunks: *p*=0.481). The phenological data from this experiment showed that the adults of *M. asper* were observed in high numbers exclusively in June and early July (Figure 9B).

Parco Naturale Regionale delle Prealpi Giulie 2016

Similar to the experiment carried out in Bosco della Fontana 2016, this experiment, carried out in the Parco Naturale Regionale delle Prealpi Giulie 2016, showed clear differences in the numbers of individuals observed on the various tree species. *Fagus sylvatica* was the most attractive wood, followed by *Fraxinus excelsior* and *Picea abies* (Figure 8B) (Leonarduzzi et al. 2017).

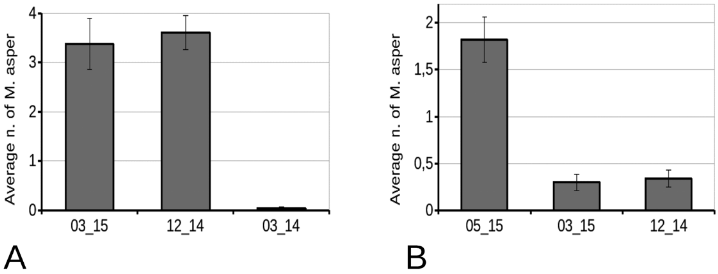

Figure 7. Average number of *M. asper* observed each week on single wood piles built with logs cut in different periods at Bosco della Fontana. **A** first experiment **B** second experiment (for details see text). Error bars represent standard errors.

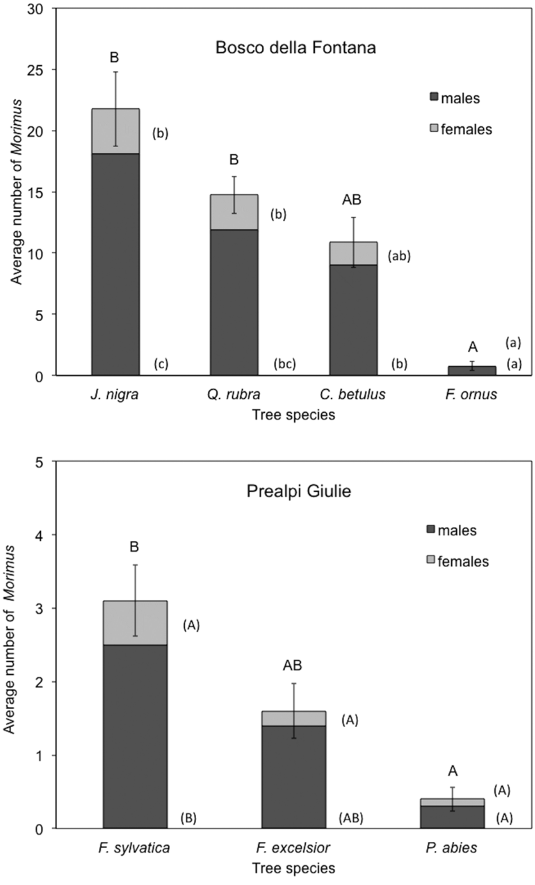

Figure 8. Average number (±SE) of adults of *M. asper* observed per day on single wood piles built from wood of different tree species. Different capital and small letters above columns (total adults) and on the right (males and females) indicate significant differences amongst wood types at the 0.01 and 0.05 levels respectively (Dunn's Multiple Comparisons Test) (see text for details). Figures from Leonarduzzi et al. (2017).

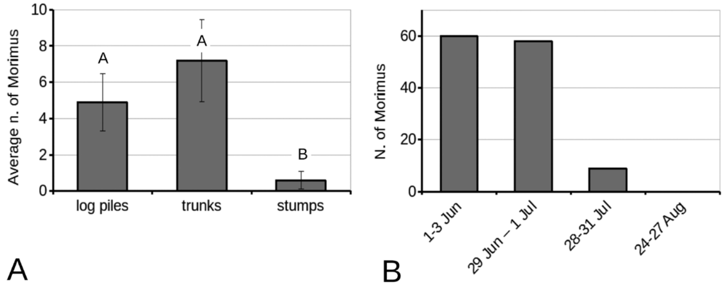

Figure 9. A Average numbers of *M. asper* observed on different structures in 2015 at Prealpi Giulie. Error bars represent standard errors **B** Total number of *M. asper* observed during different periods in 2015 at Prealpi Giulie.

Discussion

Description of the proposed monitoring method

As a standard method for the monitoring of *M. asper*, the use of freshly cut wood piles (Figure 10) is proposed, these attract adults of this species. The freshly cut wood piles, whose attraction was indistinguishable from that of a single log of the same volume, were suitable for detecting the presence of the species and for indicating the population size (Campanaro et al. 2011, Chiari et al. 2013). An important practical consideration, which favoured the choice of wood piles, is that a number of small logs can be transported with relative ease, whereas moving a single large tree trunk requires specialised equipment. Thus, using wood piles is much more versatile.

The wood piles can be made from trees (trunks or branches) which have recently fallen or from freshly cut trees. It is important that the tree had been alive at the beginning of winter and that monitoring starts as soon as the adults become active in spring, as the attraction of the timber will decrease after 1–2 months in summer. Log piles cut to a length of 60 cm lose their attraction faster than trunks and therefore it is recommended cutting the wood for the piles in the two-three weeks preceding the monitoring. The wood piles should be built from a single tree species in order to facilitate interpretations of the results and homogeneity of methods for future monitoring. The choice of the wood to be cut should be guided by the following considerations:

1. choose the dominant native hardwood timber present in the study area (if present, beech, oaks or European hornbeam);
2. choose a locally widespread tree species which will also be available in the future in order to avoid logistical problems during future years in which monitoring is envisaged;
3. consider whether the tree species will be available for cutting in the future (e.g. in nature reserves);
4. choose a tree species known to be attractive. So far, it seems that *F. ornus* and *P. abies* are not attractive for *M. asper*.

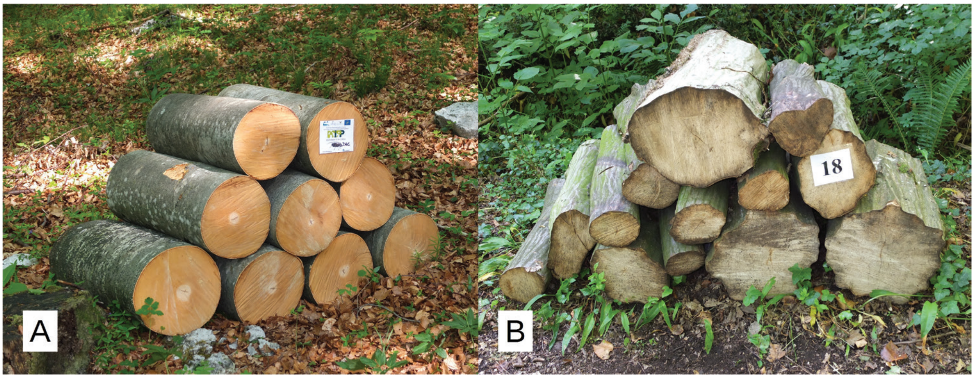

Figure 10. Two wood piles built from freshly cut wood. **A** *F. sylvatica*, freshly built **B** *C. betulus*, two months after being built.

The standard monitoring protocol (Table 1) needs to be repeated at the same site in future years, without any changes to the methodology, to ensure that the data gathered provide reliable information on local populations and can be compared chronologically as well as with other areas investigated.

In an attempt to optimise the use of resources and workforce, it is recommended that log piles be built with a volume of 0.3 m^3, which is the volume of the piles employed during the study and in accordance with the volume indicated by Chiari et al. (2013). It is suggested to cut trees and build the wood piles directly on the site. The excess timber (trunk and logs), which cannot be used to build other wood piles, must be removed from the area investigated as this material constitutes additional wood attractive to *M. asper* and could influence the number of individuals present on the piles built for monitoring. If it is not possible to use trees on site, it is recommended finding nearby sources in order to reduce transport time, to guarantee local eco-types and to facilitate access to the same wood for future monitoring.

The locations of the single wood piles should be chosen to facilitate building and also to make them easy to check during late afternoon/evening. It is therefore recommended to build the log piles along forest roads (with very little traffic) which cross suitable habitat and to avoid steep terrain on either side. It is important to regard the peculiarities of each study area and also to consider the morphology of the terrain, the type of habitats to be monitored and also practical considerations (e.g. distance to be travelled by personnel). In sloping areas, the trees can be cut higher up and the wood piles built further down, at least 50 m from the stumps, taking advantage of the slope when moving cut logs downhill.

In each area, seven wood piles have to be built for monitoring, arranged along linear transects with a distance of 100 m between them. The wood piles are to be checked once a week for five weeks, beginning at the time when *M. asper* starts to be active (April – May). It is recommended to check the wood piles after 20:00h, but if practical problems render this time of the day unfeasible, it is suggested to carry out the field work as late as possible. It is also important to maintain the same time of the day for

Table 1. Summary of the monitoring protocol.

Monitoring protocol	
Method	Freshly cut log piles
Number of log piles	7 for each site to be monitored
Placement of log piles	Along transects
Distance between log piles	100 m
Monitoring period	April-July
Number surveys	5
Frequency of repeats	Once a week
Time of the day	20:00h-24:00h
Number of operators	2
Hours per person	15 hours/person
Equipment	A clipboard, a field sheet, a head torch, a pencil, a clock, holding box for beetles and kneepads

future monitoring activities. The duration of each session (checking seven wood piles) depends on the number of observed individuals and on the distribution of the wood piles in the study area; indicatively, 45 minutes should be allowed for this activity. It is recommended to change the checking order of the wood piles for each session to avoid bias due to controlling the same wood pile always at the same time of the day. In this way, the variabilty of the results for the various wood piles can be reduced.

As stated above, the wood piles are to be checked once a week for five weeks. By controlling the piles once a week, operators are able to shift the day for controlling to avoid days with unsuitable weather conditions. The time-span of five weeks is long enough to guarantee that peak activity will fall in the monitoring period, even if unusual climatic conditions lead to shifts in the phenology of *M. asper*. However, if the local phenology of *M. asper* is not well known (or cannot be reasonably inferred from available data), exclusively for the first year of monitoring, it is recommended to extend the sampling period (e.g. to seven sessions) to better define the period of maximum activity in the study area. The five weeks identified as the period of maximum activity during the first year will be used for future monitoring in order to ensure that the data are comparable. It should also be considered that maximum activity occurs ever later in the year as altitude increases (Campanaro et al. 2017).

The standard method described here, which is based exclusively on visual counts, is the basic survey method. If additional aspects of the local population are to be investigated (e.g. population size, dispersion of individuals, life expectancy etc.), monitoring of the wood piles can be extended using the capture-mark technique. During the Project MIPP, this technique was successfully applied using tags for queen bees glued to the elytra of the adults by Loctite Super Attack Power Flex Gel.

Each year in which monitoring is to be carried out, new wood piles need to be built, as the attraction of the timber declines after a few months (in summer) and, after one year, the wood piles do not attract the species any more. Once the monitoring has been completed in a given year, the wood used for building the piles must be distributed in the forest to ensure the survival of the larvae of *M. asper* and of other saproxylic species.

Protocol, equipment and materials

Building the wood piles (Table 2)

Each log which is used for building a log pile, must be from the same tree species and the trees need to be alive when cut. If a recently fallen tree is used, it is important that it had been alive at the beginning of winter (e.g. trees uprooted by heavy snow). It is recommended to choose trees that allow building more than one wood pile in order to minimise the number of trees to be cut.

It is recommended to cut the wood for the log piles in the weeks preceding the monitoring activities, corresponding to the beginning of the activity by adults of *M. asper*. Altitude and latitude are probably the factors determining the beginning of the period of activity of the adults and this normally falls in the months of April or May. For example, in a mountain area of the Julian Alps of Italy (about 850 m a.s.l.), the species is active from mid-May until early August (Leonarduzzi 2016) and it is therefore recommended to instal the wood piles in early May. In contrast, in Bosco della Fontana (25 m a.s.l.), located in the lowlands of the Po Valley, the species is already active by the end of March/early April and therefore wood piles need to be built during early March (Hardersen et al. 2017). The exact shift in phenology for *M. asper* with increasing altitude is not well known, but Campanaro et al. (2017) found that peak activity of four insect species (also considering *M. asper*) was delayed by an average of 10 days at altitudes higher than 400 m.

The individual logs are to be cut from trunks/branches with a diameter of between 13–45 cm and to a standard length of 60 cm (Figure 10). Each log pile should contain at least one log with a diameter larger than 30 cm. It is recommended to carry out the measurements of the individual logs directly in the field during the installation of the log pile, using a ruler or a tree calliper. Logs must be positioned in 2–4 layers. Care should be taken to build a stable structure. Once completed, the log pile should cover an area of approximately 60 × 70–100 cm^2 (Figure 10). If the study area is on a slope, one side of the wood pile can be placed against a tree-trunk or against a rock, to prevent the logs rolling downhill.

The volume of each wood pile should be 0.30 m^3 and the desired volume can be most easily and accurately achieved by calculating the volumes of the single logs used for construction. It is therefore recommended to use a spreadsheet (Supplem. material 1: Excel sheet, Volume of log piles) to calculate the volumes of the single logs by entering the diameter (and length if a single trunk were not cut to 60 cm). Once the construction of a wood pile has been completed, it must be identifiable by a unique numerical code and its geographical position needs to be registered with a GPS. The geographical coordinates can also be important for locating the single wood piles for monitoring.

Checking the wood piles

The wood piles are to be checked once a week, during the period of maximum activity of *M. asper* when weather conditions are favourable i.e. without rain and a mean daily temperature between 15 and 26°C (Rossi de Gasperis et al. 2016). The adult activity will entirely stop at temperatures below 12°C (Stanic et al. 1985). If weather conditions are not favourable on a pre-selected day, it is advisable to carry out the fieldwork

Table 2. Building the wood piles (for details see the text).

Building the log piles	
When to build	March-May
Tree species tested: use	*Fagus sylvatica*, *Quercus* spp., *Carpinus betulus*, *Juglans nigra*
Tree species tested: avoid	*Fraxinus ornus*, *Picea abies*
Volume of wood	0.30 m^3
Diameter of trunks/branches	13–45 cm
Length of logs	60 cm
Work days	1–2 days
Number of operators	3, at least one forest worker (felling, chainsaw work)
Materials	Ruler or a tree calliper, GPS, numerical code

on another day, as soon as possible. The interval between successive monitoring sessions should be 5–9 days.

The protocol requires the presence of two operators who simultaneously search for *M. asper* by sight on the surface of the wood pile, amongst the logs and at the base of the pile. It is important to use a torch (e.g. head lamp) to carefully check the spaces between the logs as adults tend to hide, often in a resting position, in these relatively inaccessible places. The use of kneepads is recommended. Once an adult has been found, it is temporarily placed in a plastic container with a lid to prevent escape. After having thoroughly checked one side of the wood pile, the operators switch sides and thus each operator checks the entire wood pile. Once the search of the wood pile has been completed, the number of individuals collected is counted, specifying the number of males and females. After the compilation of the field sheet (see Supplem. material 2: Field sheet) the individuals must be released on to the same log pile. The required equipment includes: a clipboard, field sheet, head torch, pencil, clock, holding box for beetles and kneepads.

Constraints, spatial validity and possible interferences

The mark-recapture studies carried out by Rossi de Gasperis et al. (2016) and the research carried out during this project (unpublished data) showed that the large majority of adult *M. asper* moved less than 200 m during the adult stage. Therefore, at present, it can be reasonably assumed that the validity of the results of the monitoring extends to an area surrounding the wood piles up to a maximum of 200 m. In other words, the monitoring method proposed here provides valid data for an area of about 36.5 ha. If the area monitored is located within a homogeneous forest (e.g. tree composition, age of trees, management, dead wood etc.), the validity extends to this area.

A possible interference in the areas to be monitored is represented by freshly felled trees, as these might affect the number of *M. asper* observed. If logging has been carried out just prior to the period of monitoring (or during the monitoring) close to the selected forest area, it is likely that the logs created by the felling operation may have attracted the adults away from the monitoring stations, thus rendering the data invalid. Stumps and trunks older than two years are unlikely to attract *M. asper*. A further aspect to be considered is the fact that people might remove logs from the wood piles built for monitoring. This is more likely along roads that are accessible by car and in forests where the collection of timber for firewood is permitted or not prevented because of poor surveillance. The best strategy for avoiding the removal of logs is to place the log piles in areas which are under surveillance or where access is limited. A further problem might be the unauthorised collection of individuals by amateur entomologists, given that wood piles attract adults of *M. asper*. For these reasons, it is recommended to attach plates which explain the monitoring and the importance of the log piles. The surveillance and the plates, described above, are means to reduce these risks. A final aspect to be considered is the interaction with other monitoring activities. Methods employed for the monitoring of *Rosalia alpina* (Linnaeus, 1758) might also be attractive for *M. asper*. It is recommended to allow a distance of a least 1,000 m between monitoring stations for the different species.

Counting, quantification and data sharing

In order to assess the conservation status of populations of *M. asper* for a given season and for a given area, a reference value is calculated as follows:

1. For each session, calculate the total number of individuals (males + females) by adding up the number of individuals found in each wood pile;
2. Calculate the mean value of the total number of individuals counted in each session, excluding the session with the lowest number. Removing the lowest count, as proposed for other insect species (Trizzino et al. 2013), allows the elimination of eventual outlier values due to adverse climatic conditions (e.g. low temperature and/or rainfall) or other factors which do not represent the local population and is meant to reduce the variability of the final value.

Table 3 reports an example of calculation of the mean value of the individuals counted. The mean value obtained is the reference value for the assessment of the conservation status of the species in a given year for a single monitoring station (i.e. 7 log piles). This value allows the comparison of long-term data and the identification of the population trend. However, the statistical analysis of trends in monitoring data is complex (e.g. Schmidt and Meyer 2008, Schmucki et al. 2016). The range of values

Table 3. Example of the calculation of the reference value for the monitoring of *M. asper* in one site. Sessions represent single evenings when the wood piles were checked. The numbers given in the columns Lp. 1 – Lp. 7 represent the total number of *M. asper* observed on one log pile. (Lp. = Log pile).

	Lp. 1	**Lp. 2**	**Lp. 3**	**Lp. 4**	**Lp. 5**	**Lp. 6**	**Lp. 7**	**Total**
Session 1	1	2	4	3	2	3	1	16
Session 2	3	6	5	5	6	3	3	31
Session 3	3	5	7	7	7	4	5	38
Session 4	2	4	3	2	2	0	0	13
Session 5	4	7	8	5	3	5	4	36
Average value for the four counts with the highest average total								30.25

obtained during the MIPP project varied between 2.5 (Parco Naturale Regionale delle Prealpi Giulie, in 2016) and 32.75 (Bosco della Fontana, in 2015).

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References

Anonymous (2015) Operativna Programa "OKOLNA Sreda 2007 – 2013 G." Proekt "Terenni prouchvaniya na razprostranenie na vidove / otsenka na sŭstoyanieto na vidove i khabitati na teritoriyata na tsyalata strana – I faza", 1–14.

Audisio P, Baviera C, Carpaneto GM, Biscaccianti AB, Battistoni A, Teofili C, Rondinini C [Eds] (2014) Lista Rossa IUCN dei Coleotteri saproxilici Italiani. Comitato Italiano IUCN e Ministero dell'Ambiente e della Tutela del Territorio e del Mare, Roma, 1–132.

Bărbuceanu D, Niculescu M, Boruz V, Niculescu L, Stoleriu C, Ursu A (2015) Protected saproxylic Coleoptera in "the Forests in the southern part of the Cândeşti Piedmont", a Romanian Natura 2000 Protected Area. Analele Universităţii din Craiova, seria Agricultură Montanologie Cadastru (Annals of the University of Craiova – Agriculture, Montanology, Cadastre Series) 45: 18–25.

Bologna MA, Rovelli V, Zapparoli M (2016) *Morimus asper funereus* (Sulzer, 1776) (Morimo funereo). In: Stoch F., Genovesi P. (Eds), Manuali per il monitoraggio di specie e habitat di interesse comunitario (Direttiva 92/43/CEE) in Italia: specie animali. ISPRA, Serie Manuali e linee guida, 141/2016, 76–77.

Breuning S (1942) Ḗtudes sur les Lamiares. 11 Tribus: Phrissomini. Novitates Entomologicae 23(85–88): 102–139.

Bringmann HD (1996) Die *Morimus* – und *Acanthoderes*-Arten Bulgariens (Col., Cerambycidae). Entomologische Nachrichten und Berichte 40(4): 237–239.

Campanaro A, Bardiani M, Spada L, Carnevali L, Montalto F, Antonini G, Mason F, Audisio P (2011) Linee guida per il monitoraggio e la conservazione dell'entomofauna saproxilica. Quaderni Conservazione Habitat 6, Cierre Grafica, Verona, 1–8. [with CD-rom].

Carpaneto GM, Campanaro A, Hardersen S, Audisio P, Bologna MA, Roversi PF, Sabbatini Peverieri G, Mason F (2017) The LIFE Project "Monitoring of insects with public participation" (MIPP): aims, methods and conclusions. In: Carpaneto GM, Audisio P, Bologna MA, Roversi PF, Mason F (Eds) Guidelines for the Monitoring of the Saproxylic Beetles protected in Europe. Nature Conservation 20: 1–35. https://doi.org/10.3897/natureconservation.35.12761

Carpaneto GM, Baviera C, Biscaccianti AB, Brandmayr P, Mazzei A, Mason F, Battistoni A, Teofili C, Rondinini C, Fattorini S, Audisio P (2015) A Red List of Italian Saproxylic Beetles: taxonomic overview, ecological features and conservation issues (Coleoptera). Fragmenta Entomologica 47: 53–126. https://doi.org/10.4081/fe.2015.138

Cerretti P (2008) *Morimus* (*asper*) *funereus*. In: Manuale di indirizzo per la gestione delle aree tutelate del Friuli Venezia Giulia, Progetto "SARA Sistema Aree Regionali Ambientali – Costituzione del Sistema regionale delle aree naturali". ALLEGATO B. http://www.regione.fvg.it/rafvg/cms/RAFVG/

Chiari S, Bardiani M, Zauli A, Hardersen S, Mason F, Spada L, Campanaro A (2013) Monitoring of the saproxylic beetle *Morimus asper* (Sulzer, 1776) (Coleoptera: Cerambycidae) with freshly cut wood piles. Journal of Insect Conservation 17: 1255–65. https://doi.org/10.1007/s10841-013-9606-4

Danilevsky ML (2013) A check-list of longicorn beetles (Coleoptera, Cerambycoidea) of Europe, 1–41. http://www.cerambycidae.net/europe.pdf

Danilevsky ML (2015) A new species of the genus *Morimus* Brullé, 1832 (Coleoptera, Cerambycidae) from Central Europe. Humanity Space International Almanac 4: 215–219.

Danilevsky ML, Gradinarov D, Sivilov O (2016) A new subspecies of *Morimus verecundus* (Faldermann, 1836) from Bulgaria and a new subspecies of *Morimus asper* (Sulzer, 1776) from Greece (Coleoptera, Cerambycidae). Humanity Space International Almanac 5: 187–191.

Dojnov B, Vujčic Z, Božić N, Margetić A, Vujčic ZM, Nenadović V, Ivanović J (2012) Adaptations to captive breeding of the longhorn beetle *Morimus funereus* (Coleoptera: Cerambycidae); application on amylase study. Journal of Insect Conservation 16: 239–247. https://doi.org/10.1007/s10841-011-9411-x

Drovenik B, Pirnat A (2003) Strokovna izhodišča za vzpostavljaneùje omrežja NATURA 2000 (Hrošči – Coleoptera). Biološki inštitut Jovana Hadžija ZRC SAZU, Ljubljana, 1–88.

Fusu L, Stan M, Dascălu MM (2015) Coleoptera. In: Iorgu IS (Ed.) Ghid Sintetic pentru Monitorizarea Speciilor de Nevertebrate de Interes Comunitar din România, București, 44–61.

Genovesi P, Angelini P, Bianchi E, Dupré E, Ercole S, Giacanelli V, Ronchi F, Stoch F [Eds] (2014) Specie e habitat di interesse comunitario in Italia: distribuzione, stato di conservazione e trend. ISPRA, Serie Rapporti, 194/2014, Roma, 1–330.

Gnjatović I, Žikić V (2010) Cerambycids of southeast Serbia (Coleoptera, Cerambycidae). Biologica Nyssana, 1(1–2): 111–115.

Hardersen S, Cuccurullo A, Bardiani M, Bologna MA, Mason F, Maura M, Maurizi E, Roversi PF, Sabatini Peverieri G, Chiari S (2017) Monitoring the saproxylic longhorn beetle *Morimus asper* – investigating wood characteristics, season, time of the day and odour traps. Journal of Insect Conservation 21: 231–242. https://doi.org/10.1007/s10841-017-9970-6

Hedin J, Isacsson G, Jonsell M, Komonen A (2008) Forest fuel piles as ecological traps for saproxylic beetles in oak. Scandinavian Journal of Forest Research 23: 348–357. https://doi.org/10.1080/02827580802269991

Juillerat L, Vögeli M (2004) Gestion des vieux arbres et maintien des Coléoptères saproxyliques en zone urbaine et periurbaine. Centre Suisse de Cartographie de la Faune, Neuchatel, 1–20.

Jurc M, Ogris N, Pavlin R, Borkovic D (2008) Forest as a habitat of saproxylic beetles on Natura 2000 sites in Slovenia. Revue d’Écologie (Terre et Vie) 63: 53–66.

Koren T (2010) Longhorn beetles (Coleoptera: Cerambycidae) in Central Istria. Entomologia Croatica 14: 77–84.

Leonarduzzi G (2016) Applicazione di un nuovo metodo di monitoraggio di *Morimus asper/funereus*, specie protetta dalla Direttiva Habitat: caso di studio nel Parco Naturale delle Prealpi Giulie. Tesi di Laurea, Università degli Studi di Udine, Dipartimento di Scienze Agroalimentari, Ambientali e Animali (tutors: Zandigiacomo P, Maurizi E), Udine, 1–56.

Leonarduzzi G, Onofrio N, Bardiani M, Maurizi E, Zandigiacomo P, Bologna MA, Hardersen S (2017) Attraction of different types of wood for adults of *Morimus asper* (Coleoptera, Cerambycidae). In: Campanaro A, Hardersen S, Sabbatini Peverieri G, Carpaneto GM (Eds) Monitoring of saproxylic beetles and other insects protected in the European Union. Nature Conservation 19: 135–148. https://doi.org/10.3897/natureconservation.19.12659.

López-Vaamonde C, Pino-Pérez JJ, Martínez-Fernández A, Fernández Abelleira JC, Devesa-Regueiro S (1993) Contribución al conocimiento de los Cerambycidae Latreille, 1804 de interes forestal (I): *Morimus asper* (Sulz., 1776) en Galicia (Coleóptera, Polyphaga). Congreso Forestal Español, Lourinzán, I.1993, Ponencias y comunicaciones 3: 317–322.

Luce J-M (1996) *Morimus funereus* Mulsant, 1863. In: Helsdingen PJ van, Willemse L, Speight MCD (Eds) Background information on invertebrates of the Habitats Directive and the Bern Convention. Part I – Crustacea, Coleoptera and Lepidoptera. Nature and Environment 79: 59–63.

Merkl O, Hegyessy G (2008) *Morimus funereus*. In: A madárvédelmi (79/409/EK) és azélőhelyvédelmi (92/43/EK) irányelveknek megfelelő monitorozás előkészítése című Átmeneti Támogatás projekt (2006/018–176–02–01), Unpublished Final report, 29–38.

Mulsant E (1863) Histoire naturelle des Coléoptères de France. Longicornes, Annales de la Societe d’Agriculture, Histoire naturelle et Arts utiles de Lyon: 97–320.

Parisi V, Busetto A (1992) Scarabaeidae, Lucanidae, Cerambycidae. Revisione dei coleotteri presenti nella collezione "A. Leosini". Museo di Storia Naturale, Università di Parma, 1–70.

Pavan M (1948a) Sulla durata del ciclo biologico di *Morimus asper* Sulz. (Col. Cerambycidae). Bollettino di Zoologia 15: 33–36. https://doi.org/10.1080/11250004809440056

Pavan M (1948b) Uovo, stadio ovulare e primo stadio larvale, in *Morimus asper* e *Lamia textor*. Bollettino di Zoologia 15: 49–64. https://doi.org/10.1080/11250004809440059

Picard F (1929) Faune de France, 20. Cerambycidae. P. Lechevalier, Paris, 1–167.

Polak S (2012) Phenology and mating behaviour of *Morimus funereus* (Coleoptera, Cerambycidae). In: Maja J. (ed.) Saproxylic beetles in Europe: monitoring, biology and conservation. Studia Forestalia Slovenica 137: 43–52.

Porta A (1932) Fauna Coleopterorum Italica. Vol. V, Rhynchophora-Lamellicornia. Stabilimento Tipografico Piacentino (Piacenza), 1–476.

R Development Core Team (2015) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org

Robertson BA, Hutto RL (2006) A framework for understanding ecological traps and an evaluation of existing evidence. Ecology 87: 1075–1085. https://doi.org/10.1890/0012-9658(2006)87[1075:AFFUET]2.0.CO;2

Romero-Samper J, Bahillo P (1993) Algunas observaciones sobre la distribución y biología de *Morimus asper* (Sulzer, 1776) (Coleoptera: Cerambycidae) en la Península Ibérica. Boletin de la Asociacion Española de Entomologia 17: 103–122.

Rossi de Gasperis S (2015) Distribution patterns and population analysis of threatened longhorn beetles in forest habitats of Central Italy. PhD Thesis, Department of Science, Roma Tre University, 1–119.

Rossi de Gasperis S, Passacantilli C, Redolfi De Zan L, Carpaneto GM (2016) Overwintering ability and habitat preference of *Morimus asper*: a two-year mark-recapture study with implications for conservation and forest management. Journal of Insect Conservation 20: 821–835. https://doi.org/10.1007/s10841-016-9913-7

Sama G (1988) Coleoptera, Cerambycidae. Catalogo Topografico e Sinonimico. Fauna d'Italia, 36. Ed. Calderini, Bologna, 1–216.

Sama G (2002) Atlas of the Cerambycidae of Europe and Mediterranean area. 1: northern, western, central and eastern Europe. British Isles and continental Europe from France (excl. Corsica) to Scandinavia and Urals. V. Kabourek, Zlin, 1–173.

Sama G (2007) Insecta Coleoptera Cerambycidae. In: Ruffo S, Stoch F (Eds) Checklist and distribution of the Italian fauna. Memorie del Museo civico di Storia naturale di Verona , Sezione Scienze della Vita, 17: 217–219. [with CD-ROM]

Sama G, Löbl I (2010) Cerambycidae, Western Palaearctic taxa, eastward to Afghanistan excluding Oman and Yemen and the countries of the former Soviet Union. In: Löbl I, Smetana A (Eds) Catalogue of Palaearctic Coleoptera. 6. Chrysomeloidea. Apollo Books, Stenstrup 84–334.

Sama G, Rapuzzi P (2011) Una nuova Checklist dei Cerambycidae d'Italia (Insecta Coleoptera Cerambycidae). Quaderni di Studi e Notizie di Storia Naturale della Romagna 32: 121–164.

Schmidt BR, Meyer AH (2008) On the analysis of monitoring data: Testing for no trend in population size, Journal for Nature Conservation 16(3): 157–163. https://doi.org/10.1016/j.jnc.2008.05.001

Schmucki R, Pe'er G, Roy DB, Stefanescu C, Van Swaay CAM, Oliver TH, Kuussaari M, Van Strien AJ, Ries L, Settele J, Musche M, Carnicer J, Schweiger O, Brereton TM, Harpke A, Heliölä J, Kühn E, Julliard R (2016) A regionally informed abundance index for supporting integrative analyses across butterfly monitoring schemes. Journal of Applied Ecology 53: 501–510. https://doi.org/10.1111/1365-2664.12561

Solano E, Mancini M, Ciucci P, Mason F, Audisio P, Antonini G (2013) The EU protected taxon *Morimus funereus* Mulsant, 1862 (Coleoptera: Cerambycidae) and its western Palaearctic allies: systematics and conservation outcomes. Conservation Genetics 14: 683–69. https://doi.org/10.1007/s10592-013-0461-3

Stanić V, Ivanović J, Janković-Hladni M, Nenadović V, Marović R (1985) Feeding habits, behavior, oviposition and longevity of the adult cerambycid beetle *Morimus asper funereus* Muls. (Col., Cerambycidae) under laboratory condition. Acta Entomologica Jugoslavica 21: 87–94.

Sturani C (1981) Notizie biologiche e corologiche su alcuni Coleotteri Cerambicidi d'Italia, specialmente delle regioni settentrionali, insulari e limitrofe. Rivista Piemontese di Storia Naturale 2: 17–54.

Trizzino M, Audisio P, Bisi F, Bottacci A, Campanaro A, Carpaneto GM, Chiari S, Hardersen S, Mason F, Nardi G, Preatoni DG, Vigna Taglianti A, Zauli A, Zilli A, Cerretti P (2013) Gli artropodi italiani in Direttiva Habitat: biologia, ecologia, riconoscimento e monitoraggio. Quaderni Conservazione Habitat, 7. CFS-CNBFVR, Centro Nazionale Biodiversità Forestale. Cierre Grafica, Sommacampagna, Verona, 1–256.

Vernik M (2014) Collecting data of distribution for four Natura 2000 species of beetles (Coleoptera) in Slovenia – website www.sporocivrsto.si (Poster), 4. Slovenski entomološki simpozij, 9. in 10. maja 2014, Fakulteti za naravoslovje in matematiko Univerze v Mariboru, Koroška.

Vrezec A (2008) Fenološka ocena pojavljanja imagov štirih vrst varstveno pomembnih saproksilnih hroščev v sloveniji: *Lucanus cervus*, *Cerambyx cerdo*, *Rosalia alpina*, *Morinus* (sic) *funereus* (Coleoptera: Lucanidae, Cerambycidae). Acta Entomologica Slovenica 16: 117–126.

Vrezec A, Ambrožič Š, Polak S, Pirnat A, Kapla A, Denac D (2009) Izvajanje spremljanja stanja populacij izbranih ciljnih vrst hroščev v letu 2008 in 2009 in zasnova spremljanja stanja populacij izbranih ciljnih vrst hroščev. *Carabus variolosus*, *Leptodirus hochenwartii*, *Lucanus cervus*, *Morimus funereus*, *Rosalia alpina*, *Bolbelasmus unicornis*, *Stephanopachys substriatus*, *Cucujus cinnaberinus*, *Rhysodes sulcatus*. Nacionalni inštitut za biologijo, Ljubljana.

Vrezec A, Ambrožič Š, Kapla A (2010) Biology and ecology of flightless cerambycid *Morimus funereus* (Mulsant, 1862) as a background for monitoring application: laboratory and large-scale field study. In: Jurc M, Repe A, Meterc G, Borkovič D (Eds) 6th European Symposium and Workshop on Conservation of Saproxylic beetles, June 15–17, 2010, Ljubljana.

Vrezec A, Ambrožič Š, Kapla A (2012) An overview of sampling method tests for monitoring schemes of saproxylic beetles in the scope of Natura 2000 in Slovenia. In: Jurc M (Ed.) Saproxylic beetles in Europe: monitoring, biology and conservation. – Ljubljana: Slovenian Forestry Institute, Silva Slovenica, Studia Forestalia Slovenica 13: 73–90.

World Conservation Monitoring Centre (1996) *Morimus funereus*. In: The IUCN Red List of Threatened Species 1996: e.T13875A4359886. https://doi.org/10.2305/IUCN.UK.1996.RLTS.T13875A4359886.en [06 April 2016]

Supplementary material 1

Excel sheet, Volume of log piles

Authors: Sönke Hardersen, Marco Bardiani, Stefano Chiari, Michela Maura, Emanuela Maurizi, Pio Federico Roversi, Franco Mason, Marco Alberto Bologna

Data type: specimens data

Link: https://doi.org/10.3897/natureconservation.20.12676.suppl1

Supplementary material 2

Field-sheet *Morimus asper*

Authors: Sönke Hardersen, Marco Bardiani, Stefano Chiari, Michela Maura, Emanuela Maurizi, Pio Federico Roversi, Franco Mason, Marco Alberto Bologna

Data type: Field-sheet

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Training of a dog for the monitoring of *Osmoderma eremita*

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Abstract

One aim of the MIPP Project (http://www.lifemipp.eu) was to develop non-invasive monitoring methods for selected saproxylic beetles. In this paper, a method is proposed for monitoring the larvae of *Osmoderma eremita* in their natural habitat (i.e. hollow trees), using a conservation detection dog (CDD). Wood mould sampling (WMS), the standard method to detect hermit beetles and other saproxylic insects inside tree hollows, is time-consuming and exposes the target species and the whole saproxylic communities to some risks. In contrast, CDDs pose no risk to the species living inside trees while, at the same time, offer a powerful tool for surveying the insects. In this paper, the methods applied to train the dog are presented, together with the results for accuracy (the overall proportion of correct indications), sensitivity (the proportion of correct positive indications) and specificity (the proportion of correct negative indications) obtained once the CDD had been fully trained. Results are presented for nitrocellulose filters with the odour of the target species, for larvae living inside hollow trees, for frass and for the remains of adults. A comparison of the efficiency between CDD and WMS showed that employing the dog was much less time-consuming than WMS.

The literature on training CDDs for nature conservation tasks, with particular reference to cases involving Coleoptera, was also reviewed.

Keywords
Conservation detection dog, *Osmoderma eremita*, saproxylic beetles, Habitats Directive, monitoring methods, conservation biology

Introduction

In the last few decades, conservation detection dogs (CDDs) (Beebe et al. 2016) have been increasingly used for the protection of wildlife due to their keen sense of smell for locating biological targets. These skills offered by dogs for the collection of wildlife data are well recognised and documented in recent reviews (Helton 2009, Dahlgren et al. 2012, Johnen et al. 2013, Beebe et al. 2016). The first case of dogs employed in nature conservation dates back to the 1890s in New Zealand when they were used to find endangered bird species: the kiwi (*Apteryx* sp.) and the kakapo (*Strigops habroptila* Gray, 1845) (Hurt and Smith 2009, Bebee et al. 2016). At present, the use of CDDs is widespread throughout the world for assisting in an array of activities, including detection of wildlife (plants and animals), carcasses of birds and bats, scats, pathogens and other biological materials (Bebee et al. 2016). In the majority of projects, dogs were used for the detection of scats, while direct searching for wildlife is the second most important use and, only in a minority of cases, carcasses were detected (Bebee et al. 2016). The two main reasons why species are being searched for are: i) they are rare and endangered and ii) they are alien species. In the management of endangered and threatened species, dogs are mostly used to search for mammals (Wasser et al. 2004, Browne et al. 2006, Hurt and Smith 2009, Long et al. 2007, Coppolillo et al. 2015), birds (Browne et al. 2006, Hurt and Smith 2009) and reptiles (Browne 2005, Cablkand Heaton 2006, Cablk et al. 2008, Nussear et al. 2008, Hurt and Smith 2009). Dogs have been used to search for accidentally imported alien species particularly focusing on reptiles (Savidge et al. 2011), rodents (Gsell et al. 2010) and nematodes (Richards et al. 2008). Conservation dogs are also employed to detect the occurrence of birds and bats killed by turbines in order to evaluate the impact of wind farms on wildlife (Arnett 2006, Paula et. al 2011).

Concerning invertebrates, pest, alien and invasive insect species are the main targets and indeed, conservation dogs were used for the first time to detect the gypsy moth *Lymantria dispar* (Linnaeus, 1758) (Wallner and Ellis 1976). Since then, many species belonging to different insect orders have been searched by dogs: Coleoptera (Nakash et al. 2011, Errico 2012, Hoyer-Tomiczek and Sauseng 2013, Kelley 2013, Hoffman 2014, Suma et al. 2014, Coppolillo et al. 2015, Hoyer-Tomiczek et al. 2016), Diptera (Welch 1990), Hemiptera (Pfiester et al. 2008, Rolón et al. 2011), Hymenoptera (Lin et al. 2011, Brocos and González 2015), Isoptera (Brooks et al. 2003, Zahid et al. 2012) and Lepidoptera (Wallner and Ellis 1976). So far, dogs have been used in a limited number of projects to locate protected or endangered insects, such as bumblebees (Waters et al. 2011, O'Connor et al. 2012).

In the LIFE Project MIPP (Monitoring of Insects with Public Participation, www.lifemipp.eu, Action A.4: "Acquisition and training of Osmodog"), a CDD was trained to find the larval stages of the hermit beetle *Osmoderma eremita* (Scopoli, 1763) in its natural habitat (see Mason et al. 2015 and Carpaneto et al. 2017). *O. eremita* is an endangered saproxylic beetle protected in the European Union by the Bern Convention and listed in annexes II and IV of the Habitats Directive 92/43/EEC and also reported as "Near Threatened" in IUCN Red Lists of Threatened Species (Nieto et al. 2009, Nieto and Alexander 2010). This species is associated with old hollow broad-leaved trees in mature forest ecosystems, as well as outside closed forests (e.g. pasture woodlands) (Ranius et al. 2005, Giangregorio et al. 2015, Maurizi et al. 2017).

The CDD of the MIPP project is the first case of a dog searching for an endangered beetle species, *O. eremita*. Indeed, a special feature of conservation dogs is that they can detect cryptic and/or elusive species (Hurt and Smith 2009) and this makes them excellent tools to locate insects. In general, the use of dogs in nature conservation offers many advantages. For example, it can facilitate the location of target species in unreachable habitats (Chambers et al. 2015, Hoyer-Tomiczek et al. 2016), thus decreasing the risk of disturbance and performing non-destructive sampling (Chambers et al. 2015), with particular reference to saproxylic species with larvae living inside hollow trees (Hoyer-Tomiczek et al. 2016). Moreover, the use of dogs minimises the sampling effort, in terms of number of personnel involved in fieldwork and time spent in the field (Browne et al. 2006, Harrison 2006, Duggan et al. 2011, Paula et al. 2011) and reduces the bias in sampling (Wasser et al. 2004, Browne et al. 2006). All of these characteristics fit well with the *O. eremita* study case. Indeed, without the aid of a dog, most of the sampling methods commonly used to detect (and monitor) the hermit beetles involve wood mould sampling (Ranius et al. 2005, Chiari et al. 2014) and passive or active traps (Ranius 2001, Svensson and Larsson 2008, Chiari et al. 2013, Maurizi et al. 2017), which are characterised by the disadvantages listed above. Locating larvae inside trunks using wood mould sampling is the main method which allows the identification of breeding sites of hermit beetles and other saproxylic insects. It consists of digging inside tree cavities to find specimens or their remains. However, it is not often possible with wood mould sampling to investigate all cavities of a tree; in particular, cavities located high up or with narrow openings are very difficult to explore. A further drawback of this method is the physical risk for the target species and for the whole saproxylic community living inside the trunk (Tikkamäki and Komonen 2011, Chiari et al. 2014). Last, but not least, a dog-handler team only requires a few minutes to analyse a single tree, while wood mould sampling is a time-consuming technique (Chiari et al. 2014).

Searching for larvae has some additional advantages as adults have a short period of activity (Maurizi et al. 2017) and population size of adults fluctuates during the activity period and in correlation with environmental factors. In contrast, larvae are always present inside hollow trees during their development which lasts about 3 years. In this paper, a summary of the training techniques for dogs employed in nature conservation, with particular reference to the cases involving Coleoptera is presented. The information obtained by this literature review was used to plan and perfect the training strategy for the dog.

Dog breed and training

The choice of the right dog is critical to the success in the training for finding insects. First of all, a dog must have a suitable *drive* for the specific task required and these motivational characteristics differ between breeds. As indicated by Cablk and Heaton (2006), choosing a breed with a mix of *hunt drive* and *play drive* would be preferable for a CDD. In general, pure-bred dogs are preferred to mixed breeds, as the traits of mixed dogs could be unpredictable (Dahlgren et al. 2012). Recently, it has been shown that breeds that had been originally and specifically selected for scent work (e.g. Beagle, German Pointer, Bracco Italiano), do, in fact, demonstrate a higher olfactory acuity than breeds that had not been selected for such purpose (e.g. English greyhound, Siberian husky) (Polgár et al. 2016). Furthermore, it would be preferable to choose a dog from a specific line that has already shown traits and skills suitable for the specific work. Finally, individual traits are very important in a given breed: dogs can differ in physical and psychological traits (e.g. agility, physical fitness, various aspects of intelligence, predisposition to collaboration and curiosity), all of which can strongly influence the quality of work (Dahlgren et al. 2012). In previous studies, the breed most used in searches for Coleoptera were Golden Retriever (Nakash et al. 2000, Suma et al. 2014), Labrador Retriever (Errico 2012, Coppolillo et al. 2015) and Border Collie (Hoffman 2014, Hoyer-Tomiczek et al. 2016).

It is preferable to use adult dogs for the fieldwork as juvenile dogs may have lower levels of attention and concentration than adults (Hurt and Smith 2009). In addition, adult dogs can perform research routines better than juvenile dogs (Suma et al. 2014). Hurt and Smith (2009) suggest that dogs can be ready to work when they are between 12 and 24 months old. However, the training can start earlier (Dahlgren et al. 2012). In particular, before starting the actual training, the dog needs to undertake base obedience training (Welch 1990, Richards et al. 2008) and needs to be introduced to the search routines through easy search games by encouraging the dog to find toys or other fun targets (Dahlgren et al. 2012).

Living targets, in this case insect larvae, have an odour which is characteristic for each species and specific for the environment in which they live. This is a very important parameter to consider when choosing the scent target to use for the training. Indeed, the saproxylophagous and xylophagous species, living inside wood mould or creating galleries in trunks, have a broad scent bouquet due to different sources of odours present in their habitat (e.g. fungi, sawdust and other organic materials) (Hoyer-Tomiczek et al. 2016). For this reason, as the target for the dog, the training routines include living specimens as well as material from their natural habitat and the training must proceed by presenting odours of increasing complexity (Errico 2012, Suma et al. 2014, Hoyer-Tomiczek et al. 2016). After the first target, a dog can learn to recognise more odours (see below) and, in some cases, more than 20 odours can be recognised (Long et al. 2007, Coppolillo et al. 2015).

The method to train conservation dogs in finding live animals is similar to those used to find unanimated and non-biological targets and it is based on the positive reinforcement of the dog's behaviour (Hurt and Smith 2009, Braun 2013, Johnen et al. 2013). The method is built on rewarding the dog with food or play as the primary reinforcement, immediately after correct signalling. It was used in all studies in which dogs detected Coleoptera (Nakash et al. 2011, Errico 2012, Kelley 2013, Hoffman 2014, Suma et al. 2014, Hoyer-Tomiczek et al. 2016). Clickers, devices that emit a double-click sound (Smith and Davis 2008), can be used as positive secondary reinforcement when the trainer is distant from the dog and cannot give the primary reinforcement immediately after correct signalling (Braun 2013). Although the clicker is mainly a predictor for food (Johnen et al. 2013), it has other complex implications for the dog (Smith and Davis 2008). Training with positive reinforcement should be a progressive process. During the first phase of the training, the dog must be "imprinted" on the target odour (Hoyer-Tomiczek et al. 2016); in practice, the dog must learn that the recognition of a specific odour is linked to a reward. In this context, *imprinting* has the meaning of "teaching odour discrimination skills to a dog" as reported by Fjellanger (2003). The imprinting starts when the dog makes, for the first time, an olfactory contact with the target odour and immediately receives the reward. This scent detection routine should be repeated until the dog has learned clearly and unambiguously how to find and indicate its target (Hoyer-Tomiczek et al. 2016). The target can be presented to the dog *naked* or inside a small perforated box.

During the second phase of training, the scent discrimination phase, the dog learns to discriminate between the target odour and other scents by consolidating, at the same time, the search behaviour and the signalling display; this process is also called "generalisation" of an odour (Hurt and Smith 2009). The generalisation can be stimulated by offering to the dog simultaneously the target odour and other scenting materials and rewarding only after correct signalling. Several kinds of settings can be arranged for the training: the scenting target and non-target material can be presented simultaneously to the dog or one or more targets can be hidden in natural settings or in fenced training areas (Braun 2013). Although the training area must have similar characteristics to those where the real research work will be carried out, the area must be free of the target to avoid confusing the dog (Errico 2012). During the discrimination phase, it is very important to correctly build the communication between the dog and the handler, to develop the dog's search behaviour and to reinforce the focus on the target odour (Hurt and Smith 2009). All search routines and behaviour must be shaped and reinforced by the trainer, by encouraging the dog until it can perform long search sessions without loss of attention (Johnen et al. 2013, Hoyer-Tomiczek et al. 2016). To activate the dog's search behaviour, a specific word from the handler (e.g. *search* or *find it*) must be associated to the specific work that the dog has to perform (Wallner and Ellis 1976, Welch 1990, Rolón et al. 2011, Hoyer-Tomiczek et al. 2016).

Johnen et al. (2013) suggest that the duration of training for sniffer dogs can vary between 7 days and 16 months, but for CDD this period may be longer, as searching for biological targets in nature is likely to be complicated by the presence of numerous olfactory stimuli or by the smell of species related to the target species (Wallner and Ellis 1976, Hurt and Smith 2009). The basic training period (imprint phase) for inexperienced dogs searching for insects is between 1 to 3 months and the subsequent discrimination phase lasts for a further 6 to 7 months (Wallner and Ellis 1976, Suma et al. 2014, Hoyer-Tomiczek et al. 2016). Dogs that have already been trained to search for biological targets and in fieldwork need less time to be ready to work (Brooks et al. 2003, Lin et al. 2011). The total length of training is related to numerous factors, such as the trainer's experience, the trainer's skills, the characteristics of the breed and the traits of individual dogs (Hurt and Smith 2009, Johnen et al. 2013). The weekly rate of training and the daily length of the working sessions can vary in function from the level of training, the familiarity with fieldwork and the skills of the trainer. Different authors indicate a rate from 3 to 5 training sessions per week; each session can last from 2 to 4 hours with breaks related to the level of fatigue in the dog and to temperature (Harrison 2006, Hurt and Smith 2009, Lin et al. 2011, Suma et al. 2014).

A dog can be trained to offer an active or passive response to the target odour (Braun 2013). A passive response consists of pointing with its nose towards the target and/or stopping and sitting close to the scent source (Long et al. 2007). This response is preferable in cases in which the dog might potentially cause disturbance or frighten the target species and also in cases in which the dog might be at risk (by aggressive or dangerous species) (Braun 2013). Active signalling includes various reinforcing behaviour in addition to pointing, such as scratching, barking or sitting and looking at the scent source and handler (Hoyer-Tomiczek et al. 2016). If needed, all these behavioural activities can be enhanced and shaped by delaying the reward after correct signalling (Johnen et al. 2013, Hoyer-Tomiczek et al. 2016). In general, an active response is suitable for conservation dogs searching for beetles.

Creating a cohesive and efficient dog-handler team is critical to the success of the work; the dog must be able to search, locate and signal the target, while the handler has to make this possible by managing the dog in the field and, at the same time, correctly interpreting the behaviour of the dog. It is important to recognise that the searching ability varies between different dog-handler teams (Johnen et al. 2013, Hoyer-Tomiczek et al. 2016). In particular, the handler must: 1) motivate and handle the dog during the work; 2) create a relationship of trust with the dog and maintain a relaxed atmosphere during each search session; 3) understand the dog's searching behaviour and its reactions in the presence of a possible target, as the dog's reactions may depend on several factors such as its psychophysical conditions or weather conditions (Long et al. 2007, Hurt and Smith 2009, Dahlgren et al. 2012); 4) reward the dog adequately after correct signalling, as each activity in the field is simultaneously a training session for it and 5) take care of the dog during the field search, taking into account its needs and other factors that may cause fatigue and loss of concentration, such as thirst, long work sessions on steep terrain or adverse weather conditions that could compromise the quality of work (Dahlgren et al. 2012).

Several authors suggest that the best parameters to describe the ability of conservation dogs in correctly locating their target are the overall percentage of correct indications and the percentage of correctly detected targets for the total number of targets (Wallner and Ellis 1976,Welch 1990, Engeman et al. 2002, Brooks et al. 2003, Cablk and Heaton 2006, Long et al. 2007, Richards et al. 2008, Gsell et al. 2010, Lin et al. 2011, Waters et al. 2011, Suma et al. 2014, Hoyer-Tomiczek et al. 2016). Although these parameters are often characterised by different names, for this work, the definition used by Allouche et al. (2006) was adopted in relation to the assessment of presence-absence predictive models i.e. accuracy (the overall proportion of correct indications), sensitivity (the proportion of correct positive indications) and specificity (the proportion of correct negative indications).

In the majority of the previously listed cases, the average accuracy for conservation dogs was around 90%, although it was variable. Low accuracy may be caused by several factors, such as: age of the dog, insufficient training, problems in the dog-handler team communication, inexperience of the trainer and/or handler (Savidge et al. 2011, Johnen et al. 2013). Exercise and training can increase the accuracy of the dog: dog-handler teams with more experience have a higher accuracy (Savidge et al. 2011). Moreover, dogs employed in new environments have initially low accuracy although this can be increased with training (Wallner and Ellis 1976).

While working in the field, several sources of disturbance can further decrease the accuracy of the search: temperature, wind, fatigue and presence of wild animals, their traces or humans (Hurt and Smith 2009, Dahlgren et al. 2012, Hoyer-Tomiczek et al. 2016). It is important to consider that the accuracy can be artificially increased by the handler; in fact, a handler who knows the hiding places of the targets can unconsciously give this information to the dog (Clever Hans effect) or the dog can follow the odour trail made by the trainer while hiding the target (Lin et al. 2011, Johnen et al. 2013). For this reason, accuracy must to be assessed with a "double blind test", in which the location of the targets is unknown to both the dog and the handler and special precautions must be taken to avoid helping the dog to find its target (Brooks et al. 2003, Cablk and Heaton 2006, Johnen et al. 2013, Hoyer-Tomiczek et al. 2016).

Methods

Breed choice and training methods

The breed of dog selected as a CDD suitable for searching *O. eremita* was the Golden Retriever, a breed widely used to search for biological targets. In fact, the olfactory capabilities of these dogs and their nature make them easy to train and to handle during fieldwork. The dog, which was named Teseo (Figure 1), was chosen from a specific line from which many individuals have been employed as CDDs to find illegally imported animals and animal parts (CITES Service for the Comando Unità Tutela Forestale Ambientale e Agroalimentare Carabinieri – CUTFAA, the former Italian State Forestry Corps). Teseo started working with its trainer/handler at the age of 6 months and the actual fieldwork being carried out once the dog had reached adulthood (24 months), at the time when the trainer considered the dog to be well trained.

Figure 1. Teseo wearing the harness (Photo by Fabio Mosconi).

The training was carried out in successive steps, as a function of the age of the dog and according to the level of skill (Table 1). A collection permit was issued by the Ministero dell'Ambiente e della Tutela del Territorio e del Mare - DG Protezione della Natura e del Mare (U.prot PNM 2012-0010890 del 28/05/2012) to handle larvae of *O. eremita* for dog training.

Age 6–9 months: base training

During the early months of the dog's life, preparatory activities for the next training steps were carried out: i) basic obedience training, in which the dog learned some basic

Table 1. Summary of the training progression of Teseo.

Dog age (months)	Location	Training	Rate
6–9	Fenced training area and public parks	Basic obedience training Search games Agility activities	2 to 3 times/week
9–16	Fenced area	Imprinting phase (target: living larvae)	2 to 5 times/week
16–24	Natural areas *Osmoderma*-free	Discrimination phase (target: nitrocellulose filters with the smell of the target) Preliminary discrimination tests (target: living larvae of *Oryctes nasicornis*, *Gnorimus variabilis*)	5 times/week

commands (e.g. stay, come, sit etc.); ii) search games, i.e. hiding small toys or pieces of food and rewarding the dog with play and iii) agility activities, in order to improve the oral and gestural communication between the dog and handler. During this phase, 2 or 3 training sessions were performed each week in a fenced area. Each session lasted for no more than 2 hours with several breaks to avoid stressing the puppy.

Age 9–16 months: imprinting phase

From this phase onwards, the dog wore a harness when working (Figure 1), both during training and fieldwork. Additionally, the dog was conditioned with a clicker and a special word (i.e. "search") was associated with starting the searches. To imprint the dog to the target odour, some *O. eremita* larvae were used. These larvae were kept in boxes filled with the wood mould collected from their natural habitat. During the initial trials, the larva was washed with water and kept in a perforated box. Simultaneously, empty boxes were presented as control. The washing was necessary to ensure that the dog was imprinted with the pure smell of the target by eliminating other odours related to the specific microhabitat in which the larvae live. The dog was rewarded with the clicker, praise and small pieces of food only when coming into contact with the target odour and when showing a reaction. When the dog had learned to recognise the target odour, the training continued by hiding the washed larva in places easy to find for the dog (i.e., inside basal cavities of trees, under leaves on the ground or under bark), within a fenced training area. Disposable latex gloves were used when handling larvae, to avoid transferring the target odour to the hands of the trainer. This training phase ended when Teseo had successfully learned to locate and signal the target to the trainer. At a later date, the signalling by the dog was shaped and reinforced by delaying the reward as described by Hoyer-Tomiczek et al. (2016) until Teseo had learned proper signalling behaviour.

In the following phases, the larvae were placed inside perforated vials without washing in order to better protect them during the work (Figure 2). This allows positioning the target deep inside cavities without the risk of losing the larvae and the dog can learn the scent bouquet of the larvae (i.e. the scent of the larva plus odours of

Figure 2. Perforated vials in which the larvae were inserted to protect them during training (Photo by Fabio Mosconi).

wood mould). During each training session, the dog searched one tree a time and was rewarded only when it signalled the correct tree.

During every training day, 1 to 5 consecutive sessions were carried out with a single target hidden in a tree and this was repeated for 2 to 5 times a week. The number and the length of the daily working sessions were gradually increased as was the number of trees without the target. After 1 or 2 sessions, the dog was allowed to rest and to play for 5–15 minutes. The imprinting phase ended when the dog had learned to search and unambiguously signal the target.

Age 16–24 months: discrimination phase

During the discrimination phase, the training sessions were carried out as simulations of real fieldwork. The training was conducted in natural and semi-natural areas suitable for *O. eremita* (Table 2). In some areas, a number of independent sub-areas were de-

Table 2. Summary of the areas in the province of Rome (Latium, Italy) where the training and the accuracy tests were carried out. SA: sub-areas for training sessions; TREES: tree species present in the area and in which the target was hidden (Qi: *Quercus ilex*; Qs: *Quercus suber*; Pt: *Populus tremula*); TR: training areas; AC: accuracy test areas; Oasi = private Nature Reserve; PRU = Regional Urban Park; RN = Nature Reserve; Villa = city park with annexed historical buildings.

Area	SA	TREES	TR	AC
RN Monte Mario	1	Qi; Qs	x	x
RN Monte Mario	2	Qi	x	
Villa Doria Pamphilj	1	Qi; Qs	x	x
Villa Doria Pamphilj	2	Qi; Qs	x	x
Villa Doria Pamphilj	3	Qi	x	x
Villa Doria Pamphilj	4	Qi; Qs	x	
PRU del Pineto	1	Qs	x	x
PRU del Pineto	2	Qs	x	x
PRU del Pineto	3	Pt	x	
RN Insugherata	1	Qi; Qs	x	
Oasi LIPU Castel di Guido	1	Qi	x	

fined to increase the number of training sites and these were used in a haphazard order to avoid familiarisation of the dog to individual areas. As far as it is known, *O. eremita* has never been reported from any training site used in the present research.

Small nitrocellulose filters were impregnated with the target odour by placing the larva in small containers, filled with filters for at least 8 hours. Filters, prepared in this way, can retain the target odour for a long period, if stored in hermetic containers. These filters are small and can be easily hidden in very small cavities and are thus invisible to the dog. These filters also allow the undertaking of long training sessions with multiple targets, simulating areas with a low or a high population density of *O. eremita*. A further important point in favour of the filters is the fact that the use of live larvae of the target species (i.e. a protected insect), can be substantially reduced. Disposable latex gloves and tweezers were used when handling filters to avoid transferring the target odour to the hands of the handler and field assistants.

In this phase, during each training session, 3 to 15 filters were hidden in randomly selected trees. To avoid the smell left behind by the trainer while placing the filters and which could influence the dog, all trees to be searched (those with and without target) had been touched by the trainer prior to the actual session. Only then was Teseo permitted to search one tree at a time, alternating between trees with and without targets (Figure 3). The dog was rewarded with the clicker, praise and small pieces of food every time it correctly signalled. In case of signalling a tree not containing the target, no reward was given and the handler simply led the dog to the next tree. Every training session always finished with finding the target. To this end, a filter was hidden inside a tree in a place which was easy to find. Once the final target had been found, Teseo was rewarded as usual and a few minutes were dedicated to play. The length and the complexity of the single sessions were gradually increased up to a maximum of 50

Figure 3. Teseo searching on a tree according to the indication of the handler (Photo by Emilia Capogna).

minutes of work with about 60 trees searched. During this phase, fieldwork lasted up to a maximum of 5 days per week, with 1 to 3 training sessions interspersed by 15–20 minutes of rest. Some double-blind training sessions were carried out with the help of a field assistant in order to avoid errors in the searches induced by the handler. The double-blind tests were also useful to assess when the dog had reached a level of training appropriate to work in a field study, i.e. when the dog, during a 50 minutes training session, stayed below a maximum error rate of about 15%.

Preliminary discrimination tests

Tests were carried out to verify whether Teseo misidentified larvae of species closely related to *O. eremita* and potentially syntopic in natural habitats. Some preliminary tests were carried out using larvae of *Oryctes* (*Oryctes*) *nasicornis* (Linnaeus, 1758) (Coleoptera: Scarabaeidae: Dynastinae) and *Gnorimus variabilis* (Linnaeus, 1758) (Coleoptera: Scarabaeidae: Cetoniinae). In every test, one larva of *O. eremita* was presented with one of the other species listed above. The larvae were hidden in perforated boxes and randomly mixed with empty boxes. Each day, 2 to 4 tests were carried out with a 10 minutes' break between two successive tests. The dog was rewarded only after signalling correctly.

Measurement of the accuracy

Accuracy, sensitivity and specificity were calculated following Allouche et al. (2006) as reported in Table 3, from data gathered in 8 wooded areas with two different approaches. A first set of tests was carried out in 6 sites without populations of *O. eremita* although they did contain hollow or fractured trees suitable for the larvae of this species (Table 4).

Table 3. Definition and formulae to calculate Accuracy, Sensitivity and Specificity. CPS: correct positive signalling (total n° of targets present in trees and correctly detected and signalled by the dog); CNS: correct negative signalling (total n° of trees without target and not signalled); NT: total n° of trees investigated; TT: total n° of targets present in the trees; TND: target not detected (total n° of targets present in the trees and not detected); NR: no reaction; TWD: targets wrongly detected (total n° of trees without target signalled by the dog as if the target was present).

Accuracy	the overall proportion of correct indications ("the rate of correct classification", Allouche 2006)	CPS+CNS/NT
Sensitivity	the proportion of correct positive indications ("the probability that the model will correctly classify a presence", Allouche 2006)	CPS/TT(CPS+TND)
Specificity	the proportion of correct negative indications ("the probability that the model will correctly classify an absence", Allouche, 2006)	CNS/NR(CNS+TWD)

Table 4. Areas (AREA) and sub-areas (SA) in the province of Rome where the accuracy tests with nitrocellulose filters were carried out. TREES: tree species where filters were hidden (Qi: *Quercus ilex*; Qs: *Quercus suber*); NT: total n°of trees investigated; TT: total number of targets. Indication by Teseo: NR: total n° of "no reaction", recognised as "target not present", PS: total n° of "partial signalling" recognised as "false signalling", CS: total n° of "complete signalling" recognised as "target detected"; Results: CPS: total n° of correct positive signalling (total n° of target correctly detected by the dog), TND: total n° of not-detected targets, CNS: correct negative signalling (NR to a tree without target), TWD: target wrongly detected (wrong CS to a tree without target); ACC: accuracy (CPS+CNS/NT); SEN: sensitivity (CPS/CPS+TND); SPE: specificity (CNS/CNS+TWD).

Area	SA	TREES	NT	TT	NR	PS	CS	CPS	TND	CNS	TWD	ACC	SEN	SPE
RN Monte Mario		Qi; Qs	49	6	44	3	5	4	2	42	1	93.87	66.66	97.67
Villa Doria Pamphilj	1	Qi; Qs	37	5	27	6	10	5	0	27	5	86.49	100	84.37
Villa Doria Pamphilj	2	Qi; Qs	46	6	36	8	10	6	0	36	4	91.30	100	90.00
Villa Doria Pamphilj	3	Qi	60	7	49	8	11	6	1	48	5	90.00	85.71	90.57
PRU del Pineto	1	Qs	20	4	16	2	4	4	0	16	0	100	100	100
PRU del Pineto	2	Qs	51	6	45	1	6	3	3	42	3	94.18	50.00	93.33
MEAN												92.64	83.73	92.66

Table 5. Accuracy measurements in Forcella Buana (FB) and San Vito (SV) considering larvae and frass and remains of adults (F+R) as target. NT: total number of trees investigated; TT: total number of targets present in trees; L13 and L16: total number of trees colonised by larvae of *O. eremita* in 2013 and 2016 respectively; F+R: total number of trees in which frass (F) and/or remains of adults (R) were found in 2013 and 2016; NR: no reaction; PS: partial signalling; CS: complete signalling; CPS: correct positive signalling; TND target non-detected; CNS: correct negative signalling; TWD: target wrongly detected.

		TT				Indications by Teseo			CPS				TND				CNS	TWD
Area	**NT**	**L13**	**L16**	**F+R**	**TOT**	**NR**	**PS**	**CS**	**L13**	**L16**	**F+R**	**TOT**	**L13**	**L16**	**F+R**	**TOT**		
FB	84	4	4	15	23	61	7	23	4	1	4	9	0	3	11	14	47	14
SV	48	2	2	6	10	32	8	16	1	2	4	7	1	0	2	3	29	9
TOT	132	6	6	21	33	93	15	39	5	3	8	16	1	3	13	17	76	23

The targets (filters with the target odour) were hidden by a field assistant unbeknown to dog and handler (double blind test) in randomly chosen trees. Before the search, the field assistant touched all trees to be searched to avoid providing olfactory help for the dog. During the search sessions, the handler reported to the field assistant the interpretation of the dog's behaviour, distinguishing between: no reactions (NR, the dog showed no reactions indicating the target); complete signalling (CS, the dog pointed to the target, sat, barked and looked at the scent source and handler). Sometimes the dog performed partial signalling (PS, e.g. the dog did not sit or barked only weakly). In these cases, the handler recognised the reaction as "false signalling" (FS) and reported that the target was not present (considered as "NR"). All results were recorded in a field sheet (Figure 4). The dog was rewarded only for correct signalling. Again, all sessions ended with finding the target.

<table>
<tr><th colspan="3">Session ID</th><th colspan="3">Date</th><th colspan="2">S/E Time</th></tr>
<tr><th colspan="6">Area</th><th colspan="2">Operators</th></tr>
<tr><td rowspan="2">Weather</td><td colspan="2">T(i)</td><td colspan="2">H(i)</td><td colspan="2">W(i)</td><td rowspan="2">Notes</td></tr>
<tr><td colspan="2">T(f)</td><td colspan="2">H(f)</td><td colspan="2">W(f)</td></tr>
<tr><td></td><td></td><td colspan="3">Dog indications</td><td colspan="2">Results</td><td></td></tr>
<tr><td>Tree ID</td><td>Trg</td><td>NR</td><td>PS</td><td>CS</td><td>TP</td><td>FS</td><td>Notes</td></tr>
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The accuracy of Teseo was also measured in two areas (San Vito and Forcella Buana) where the presence of *O eremita* had been ascertained in previous studies by wood mould sampling (Chiari et al. 2014). In 2013 and in 2016, the presence of the target species was recorded for single trees by the presence of larvae of *O. eremita*, frass or remains of adult specimens. Additionally, the presence of larvae of other flower chafer species was recorded. These data were used to perform a series of double blind tests. The dog-handler team searched individual trees and communicated the outcome to the field assistant (Figure 4). Teseo was only rewarded if the presence of *O. eremita* was indicated correctly.

The overall accuracy, sensitivity and specificity (Table 3) were calculated considering as "presence" of *O. eremita* in a tree: records of larvae in any year (2013 and 2016), records of frass and of remains of adults. Accuracy, sensitivity and specificity were calculated with and without considering records of frass and remains (Tables 5 and 6). Additionally, to test if the dog might also signal the presence of larvae of other species of flower chafers, sensitivity was calculated in a separate analysis, considering as "presence" any records of larvae of Cetoniinae in trees without *O. eremita,* as found previously by the wood mould sampling (Table 7). It was expected that Teseo would not recognise the larvae of the flower chafers as the target and, thus, it was expected to observe low values for sensitivity. All results were recorded in a field sheet (Figure 4). The contribution of the handler to the overall accuracy was calculated as the difference between the accuracy including the "partial signalling" as "no reaction" (after the correction of the handler) and the accuracy including the PS as "target wrongly detected" (Table 8).

The results of the evaluation of accuracy are summarised in Table 8.

Efficiency

The efficiency of the dog-handler team in detecting *O. eremita* larvae inside trees in Forcella Buana and San Vito was compared with the efficiency of the wood mould sampling technique in the same areas. The average time spent in 2016 to investigate a single tree by the two methods was calculated as the proportion between the total amount of time spent in the field by all operators and the total number of trees investigated. Two operators were needed to work with the dog and two operators were also required for wood mould sampling for each tree.

Results

Breed choice and training methods

At the end of the training period, Teseo was ready for the fieldwork; the dog was able to detect larvae of *O. eremita* and to signal the presence of its target inside trees by sitting

Figure 4. The CDD team: the dog, the handler and the field assistant ready for a working session (Photo by Emilia Capogna).

down, barking and looking at the scent source and the handler (Figure 5). Sometimes, Teseo scratched the bark if the source of the odour was located in the upper part of a tree. In a single field session, the dog was able to work for about 50 minutes, after which it needed to rest for 15 to 60 minutes before resuming work. Fatigue and high ambient temperatures caused a general decrease in the dog's working ability.

Table 6. Accuracy measurements in Forcella Buana (FB) and San Vito (SV) considering only larvae as target. NT: total number of trees investigated; TT: total number of target present in trees; L13 and L16: total number of trees colonised by larvae of *O. eremita* in 2013 and 2016 respectively; F+R: total number of trees in which frass (F) and/or remains of adults (R) were found in 2013 and 2016; NR: no reaction; PS: partial signalling; CS: complete signalling; CPS: correct positive signalling; TND targets non-detected; CNS: correct negative signalling; TWD: target wrongly detected.

		TT			Indication by Teseo			CPS			TND			CNS	TWD
Area	**NT**	**L13**	**L16**	**TOT**	**NR**	**PS**	**CS**	**L13**	**L16**	**TOT**	**L13**	**L16**	**TOT**		
FB	84	4	4	8	61	7	23	4	1	5	0	3	3	58	18
SV	48	2	2	4	32	8	16	1	2	3	1	0	1	31	13
TOT	132	6	6	12	93	15	39	5	3	8	1	3	4	89	31

Table 7. Accuracy measurement for larvae of flower chafers species. NT: total number of trees; TT: total number of targets. Indication by Teseo: NR: no reaction, PS: partial signalling, CS: complete signalling; Results: CPS: correct positive signalling, TND targets non-detected, CNS: correct negative signalling, TWD: target wrongly detected.

Area	NT	TT	NR	PS	CS	CPS	TND	CNS	TWD
Forcella Buana	84	32	60	6	24	7	25	35	17
San Vito	48	14	30	5	18	5	9	21	13
TOT	132	46	90	-	42	12	34	56	30

Table 8. Summary of Teseo's accuracy (ACC), sensitivity (SEN) and specificity (SPEC). Filters: the filters with odour of the larvae of *O.eremita*; L13, L16: larvae recorded in 2013 and 2016; F+R: frass and remains of adults; FC: larvae of flower chafers; FB: Forcella Buana; SV: San Vito.

Target	Area	Overall accuracy			Accuracy without handler correction			Handler contribution	
		ACC (%)	SEN (%)	SPEC (%)	ACC (%)	SEN (%)	SPE (%)	ACC (%)	SPE (%)
Filters		92.64	83.73	92.66	80.81	83.73	80,07	11,83	12,59
L13, L16, F+R	FB	66.67	39.13	77.05	58.33	39.13	65.57	-	-
	SV	75.00	70.00	76.31	58.33	70.00	55.26	-	-
Mean		70.83	54.56	76.68	58.33	54.56	60.41	12.50	16.27
L13, L16	FB	75.00	62.50	76.31	66.67	62.50	67.10	-	-
	SV	70.83	75.00	70.45	54.17	75.00	52.27	-	-
Mean		72.91	68.75	73.38	60.42	68.75	59.68	12.49	13.70
FC	FB	-	21.87	-	-	-	-	-	-
	SV	-	35.71	-	-	-	-	-	-
Mean		-	28.79	-	-	-	-	-	-

Preliminary discrimination test

The discrimination tests with larvae of *Oryctes* (*Oryctes*) *nasicornis* and *Gnorimus variabilis* showed similar results. Teseo correctly exclusively signalled the larvae of *O. eremita* and

Figure 5. Teseo signalling the target to the handler: **A** sitting beside a tree containing the target, barking and looking at scent source and handler **B** pointing the target, barking and looking at the handler **C** scratching the trunk and barking (Photos A and B by Emilia Capogna, photo C by Sönke Hardersen).

sometimes showed some faint reactions to the larvae of the other species (e.g. sitting beside the source of the odour or barking weakly). After 2 or 3 repetitions of the tests, Teseo showed no reactions to the larvae of *O. nasicornis* and *G. variabilis*. However, it was noticed that during a few of the training sessions following these discrimination tests, the dog committed a higher rate of errors. Nevertheless, after a few training sessions, Teseo recovered his usual level of accuracy. For this reason it was decided to stop these tests.

Measurement of accuracy

When working with nitrocellulose filters, Teseo showed an accuracy of 93%, a sensitivity of 84% and a specificity of 93% (Table 8). When searching trees with cavities occupied by *O. eremita*, accuracy of 71%, sensitivity of 55% and specificity of 77% were calculated by taking into consideration the presence of *O. eremita* live larvae, frass and remains of adults (Table 8). If the presence of frass and remains of adults were not considered, the accuracy was 73%, the sensitivity was 69% and the specificity was 73% (Table 8). When testing whether Teseo was also able to signal the presence of larvae of other species of flower chafers, the dog showed a sensitivity of 29% (Table 8). The direct contributions of the handler to the accuracy and specificity amount to 12% and 13% respectively for the work with filters and 13% and 14% with larvae (Table 8).

Efficiency

The total time spent by the two operators to investigate 149 trees by wood mould sampling amounted to 198 hours (11,880 minutes) (48 trees in 96 hours in San Vito and 101 trees in 138 hours in Forcella Buana). The total time spent by the two operators to investigate 132 trees with Teseo amounted to 855 minutes (48 trees in 376 minutes in San Vito and 84 trees in 479 minutes in Forcella Buana). The mean overall time spent by two operators per tree for wood mould sampling and with the dog amounted respectively to about 80 minutes and to 6 minutes and 50 seconds.

Discussion

The protocol developed to train the conservation detection dog in the MIPP project was successful in teaching the dog the specific task required, i.e. to find larvae of the saproxylic beetle *O. eremita* living inside hollow trees. Therefore the results showed that this rare and elusive beetle can be monitored with the aid of a trained dog. These results are in line with research on other animal species living in wood or in burrows (Brooks et al. 2003, Hoyer-Tomiczek et al. 2016, Nielsen et al. 2016) that were successfully detected with the aid of dogs. In the case of Teseo, the results obtained for accuracy, sensitivity and specificity in natural conditions are high and close to the ones obtained with nitrocellulose filters with the smell of the larvae of *O. eremita* in controlled conditions (Table 8). This supports the fact that the training plan, which involved the use of filters, was well suited for the project aim. The filters were important because they minimised the number of larvae employed for the training. For the trainer, the use of filters was much easier than using live larvae during the training sessions. A further important point is that the dog showed a high sensitivity to the target odour (i.e. the smell of larvae of *O. eremita*) and distinguished reliably between trees harbouring the larvae and trees without them (Table 8).

The highest values were obtained if only larvae were considered as the target (i.e. excluding frass and remains) (Table 8) and this is a further indication that Teseo only signalled the presence of larvae. Similar levels of accuracy were reported by Hoyer-Tomiczek et al. (2016) for field tests on an alien longhorn beetle. It is important to point out that not all the trees in which frass and remains have been found were also colonised by larvae; only the larvae of *Osmoderma* reliably indicate the presence of the species in a tree.

Confirmation that the dog has been successfully imprinted is also provided by the results of the preliminary discrimination test and by the low sensitivity in signalling larvae of other flower chafers species which often share the same cavity with *O. eremita*. In particular, Teseo showed a much lower sensitivity to larvae of flower chafers (29%) when compared to the larvae of *O. eremita* (69%). This result is similar to Brooks et al. (2003), who found that trained dogs falsely signalled termites in 25% of tests when termite-damaged wood without termites was presented. In contrast, Lin et al. (2011) reported false positives in only 4% of tests when working with ants and Pfiester et al. (2008) found false positive rate of 3% on bed bug faeces.

These results on accuracy and sensitivity of Teseo are consistent with those obtained in other studies involving dogs in the search for saproxylic beetles. For example, a sensitivity of 78% was obtained for dogs trained to find the red palm weevil, *Rhynchophorus ferrugineus* (Suma et al. 2014) and Hoyer-Tomiczek et al. (2016) obtained accuracy values between 81% and 94% for larvae of a xylophagous longhorn beetle. They also showed that dogs had a lower accuracy when searching for wood shaving and frass without larvae of the target species, as was also observed for Teseo.

The difference between the values of accuracy measured with nitrocellulose filters and larvae may depend on several factors. In general, working in a natural setting with live targets is certainly more complicated than searching for filters placed by operators. Other studies have also shown that sensitivity was lower under realistic conditions (Hoyer-Tomiczek et al. 2016). In fact, natural populations of insects can have an non-homogeneous distribution and different trees can contain a variable number of larvae and consequently a different concentration of odour. During the fieldwork, it was obvious that, in presence of strong odour sources, the dog detected the target from several metres distance. This effect can be increased by the wind that can carry the target odour away from the source. Chambers et al. (2015) and Hoyer-Tomicek et al (2016) also reported that the scent of species living in trees was transported by wind. These two variables (high concentration of target odour and wind) can confound the results and lead to signalling of trees which do not contain the target, but which are close to trees with larvae of *O. eremita*. Similarly Chambers et al. (2015) and Hoyer-Tomicek et al. (2016) concluded that wind can lead to signalling of the incorrect tree.

A further important point to consider is which factors increase the level of fatigue in the dog and consequently decrease its reliability (e.g. DeShon et al. 2016). For example, trees can be distant from each other, the vegetation can be impervious and scheduled searches might need to be conducted under sub-optimum weather conditions (e.g. high temperatures or rain).

Precautions should be taken to minimise the effects of these factors, such as: i) carry out some preliminary surveys to allow the dog to become familiar with the working area; in fact, it is well known that accuracy measurements in new areas can initially be low and can increase in later surveys (Wallner and Ellis 1976); ii) choose the most suitable time of the day for the activity of the dog, (e.g. avoid the hours with the highest temperatures, rainy days and strong wind) and iii) pay attention to the level of fatigue in the dog, decreasing the duration of the working sessions and increasing the length of the breaks if necessary or even interrupting the daily work if the dog does not show an appropriate level of concentration.

The lower accuracy measured on trees colonised by larvae of *O. eremita* may also depend on factors related to the detection probability (i.e. the probability to detect the larvae in a tree, if present) of the wood mould sampling method. In Forcella Buana and San Vito, results obtained by wood mould sampling were used to validate the accuracy, sensitivity and specificity of the signalling of Teseo. However, Chiari et al. (2014) showed that the detection probability for wood mould sampling can vary between 34% and 50%. This means that a high percentage of trees harbouring *O. eremita* were not correctly identified by wood mould sampling. In these cases, the complete signalling by the dog was incorrectly counted as errors (false signalling).

Another very important factor to take into account is the relationship between the dog and the handler (cf. Beebe et al. 2016). A handler who correctly understands the dog's behaviour during fieldwork can increase the overall accuracy by more than 10%. Although the improvement of communication between the dog and handler can be obtained through specific double blind training sessions, it is clear that differences exist in the searching ability of different dog and handler teams (Hoyer-Tomicek et al. 2016).

Comparison between the methods: conservation detection dog and wood mould sampling

The results of the tests carried out demonstrated that the use of the CDD was a better method to detect larvae of *O. eremita* when compared to WMS. In fact, the dog showed an overall probability of detecting colonised trees in the area of Forcella Buana and San Vito of 73%, that is higher than the detection probability with WMS (34–50%) in the same areas (Chiari et al. 2014). Furthermore, the use of a dog was much less time-consuming than WMS. The average duration needed to examine a single tree by 2 operators was about 80 minutes by WMS and 6 minutes 50 seconds by CDD, respectively. This means that the dog, during a day with good weather conditions, can complete up to 4 work sessions lasting 50 minutes each (with appropriate breaks), for a total of about 60 trees. Thus, the dog can find larvae of *O. eremita* with high accuracy employing less than 1/10 of the time for WMS. In addition, the searches with the dog completely eliminate the risk of harming larvae, adults or other species living in the hollow trees.

Protocols, materials and equipment

For the base training and the imprint phase, a 3m training leash was used. Subsequently, when the dog had learned to pay attention to the trainer when he was wearing the harness, no leash was used.

Similarly, the leash was used during the initial conditioning with the clicker: conditioning sessions, lasting 5 minutes, were carried out, giving the dog small pieces of tasty food (different to that used for rewarding the dog after correct signalling) as a reward simultaneously with the click. After the dog had well learned to associate the sound of the click with the food, daily sessions (up to 15 minutes) were carried out without the leash. Metallic clickers were preferable as they were more resistant and produce a louder sound. As a reward for a target detected successfully, small pieces of chicken sausage were used. Disposable latex gloves should be used when handling filters or larvae to avoid transferring the target odour to the hands of the trainer, which might confuse the dog.

Constraints, spatial validity and possible interferences

To avoid interferences in the field during training or monitoring sessions:

- There should be as few people as possible and it would be preferable if the dog was familiar with all the people present.
- The field assistant must stay distant and out of the trajectory of the dog-handler team while working in order to avoid creating disturbance.
- If possible, working should be avoided during rainy and windy days.
- If it is necessary to work during periods with very high temperatures, the hottest hours of the day should be avoided and it is suggested that work should be undertaken early in the morning or late in the afternoon.

When trees are close to each other and in dense woods, it was observed that the dog can become confused as the target odour can apparently move from a source tree. In these cases, the dog can perceive the smell beside another tree and this effect is increased by the wind. When these conditions occur, it might be better to work on groups of trees rather than on individual trees.

It was noticed that Teseo performed better when carrying out a maximum of 5 training sessions per week, alternated with 2 days of rest. When the dog was fully trained (i.e. he reached the expected level of accuracy), the training rate could be reduced to 2 or 3 sessions per week (i.e. maintenance training). However, before working in the field, the rate of training should be increased again to 5 times per week. It would be necessary to start at least 6 weeks earlier and to gradually increase the following parameters: the number of weekly training sessions, the number of daily sessions, the number of trees examined (both with and without target) and the general complexity of the sessions. It is recommended to carry out some training sessions in which only one target is used (in the last tree surveyed during the session) in order to get the dog used to working in areas with low population density of the target species (or where the target species might not be present).

Conclusions

A conservation detection dog is a powerful tool for locating *O. eremita* and these results can be useful for the other related European species of *Osmoderma* (Audisio et al. 2007, 2009, Zauli et al. 2016) and in general for other saproxylic insects. In fact, the use of a trained dog is a fast, accurate and non-invasive method that allows the detection of a target species in an area and to identify the colonised trees; this means that a CDD can locate new populations, can confirm the presence of the target species and can assist in the mapping of the distribution of colonised trees in an area, accurately and efficiently. Furthermore, monitoring by means of dogs can be carried out in the same area repeatedly and this allows the detection probability for this method to be obtained. These values can then be compared with detection probabilities for other methods employed for the same species (e.g. wood mould sampling). Repeating the search with a CDD in the same area but in subsequent years would allow changes in the number and distribution of trees occupied by the beetle to be monitored.

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References

Allouche O, Tsoar A, Kadmon R (2006) Assessing the accuracy of species distribution models: prevalence, kappa and the true skill statistic (TSS). Journal of Applied Ecology 43: 1223–1232. https://doi.org/10.1111/j.1365-2664.2006.01214.x

Arnett EB (2006) A preliminary evaluation on the use of dogs to recover bat fatalities at wind energy facilities. Wildlife Society Bulletin 34(5): 1440–1445. https://doi.org/10.2193/0091-7648(2006)34[1440:APEOTU]2.0.CO;2

Audisio P, Brustel H, Carpaneto GM, Coletti G, Mancini E, Piattella E, Trizzino M, Dutto M, Antonini G, De Biase A (2007) Updating the taxonomy and distribution of the European

Osmoderma, and strategies for their conservation (Coleoptera, Scarabaeidae, Cetoniinae). Fragmenta entomologica 39(1): 273–290. https://doi.org/10.4081/fe.2007.124

Audisio P, Brustel H, Carpaneto GM, Coletti G, Mancini E, Trizzino M, Antonini G, De Biase A (2009) Data on molecular taxonomy and genetic diversification of the European Hermit beetles, a species-complex of endangered insects (Coleoptera: Scarabaeidae, Cetoniinae, *Osmoderma*). Journal of Zoological Systematics and Evolutionary Research 47(1): 88–95. https://doi.org/10.1111/j.1439-0469.2008.00475.x

Beebe SC, Howell TJ, Bennett PC (2016) Using scent detection dogs in conservation settings: a review of scientific literature regarding their selection. Frontiers in Veterinary Science 3: 1–13. https://doi.org/10.3389/fvets.2016.00096

Braun B (2013) Wildlife detector dogs - A guideline on the training of dogs to detect wildlife in trade. WWF Germany, Berlin, 1–16.

Brocos G, González D (2015) Velucan: os cans que detectan niños de Vespa Velutina. http://www.campogalego.com/agroalimentacion/velucan-os-cans-que-detectan-ninos-de-vespa-velutina/

Brooks SE, Oi FM, Koehlera PG (2003) Ability of canine termite detectors to locate live termites and discriminate them from non-termite material. Journal of Economic Entomology 96(4): 1259–1266. https://doi.org/10.1093/jee/96.4.1259

Browne C, Stafford K, Fordham R (2006) The use of scent-detection dogs. Irish Veterinary Journal 59(2): 97–104.

Browne CM (2005) The use of dogs to detect New Zealand reptiles scent. Master Thesis of Science in Zoology. Massey University: Palmerston North, New Zealand.

Cablk ME, Heaton S (2006) Accuracy and reliability of dogs in surveying for desert tortoise (*Gopherus agassizii*). Ecological Applications 16(5): 1926–1935. https://doi.org/10.1890/1051-0761(2006)016[1926:AARODI]2.0.CO;2

Cablk ME, Sagebiel JC, Heaton JS, Valentin C (2008) Olfaction-based detection distance: a quantitative analysis of how far away dogs recognize tortoise odor and follow it to source. Sensors 8: 2208–2222. https://doi.org/10.3390/s8042208

Carpaneto GM, Campanaro A, Hardersen S, Audisio P, Bologna MA, Roversi PF, Sabbatini Peverieri G, Mason F (2017) The LIFE Project "Monitoring of insects with public participation" (MIPP): aims, methods and conclusions. In: Carpaneto GM, Audisio P, Bologna MA, Roversi PF, Mason F (Eds) Guidelines for the Monitoring of the Saproxylic Beetles protected in Europe. Nature Conservation 20: 1–35. https://doi.org/10.3897/natureconservation.35.12761

Chambers CL, Vojta CD, Merign ED, Davenport B (2015) Efficacy of scent-detection dogs for locating bat roosts in trees and snags. Wildlife Society Bulletin 39(4): 780–787. https://doi.org/10.1002/wsb.598

Chiari S, Zauli A, Mazziotta A, Luiselli L, Audisio P, Carpaneto GM (2013) Surveying an endangered saproxylic beetle, *Osmoderma eremita*, in Mediterranean woodlands: a comparison between different capture methods. Journal of Insect Conservation 17(1): 171–181. https://doi.org/10.1007/s10841-012-9495-y

Chiari S, Zauli A, Audisio P, Carpaneto GM (2014) Interactions between larvae of the threatened saproxylic beetle *Osmoderma eremita* and other flower chafers in Mediterranean woodlands: implications for conservation. Insect Conservation and Diversity 7(5): 462–469. https://doi.org/10.1111/icad.12069

Coppolillo P, Parker M, Woollett D, Hurt A, Whitelaw A, Richards N, Homan M, Happel H, Rasker R, Richey M (2015) Working Dog For Conservation. http://wd4c.org/

Dahlgren DK, Elmore RD, Smith DA, Hurt A, Arnett EB, Connelly JW (2012) Use of dogs in wildlife research and management. In: Silvy N (Ed.) Wildlife Techniques Manual vol. 1. The Wildlife Society, Washington DC, 140–153.

DeShon DL, Wong WH, Farmer D, Jensen AJ (2016) The ability of scent detection canines to detect the presence of quagga mussel (*Dreissena rostriformis bugensis*) veligers. Management of Biological Invasions 7: 419–428. https://doi.org/10.3391/mbi.2016.7.4.11

Duggan JM, Heske EJ, Schooley RL, Hurt A, Whitelaw A (2011) Comparing detection dog and livetrapping surveys for a cryptic rodent. The Journal of Wildlife Management 75(5): 1209–1217. https://doi.org/10.1002/jwmg.150

Engeman RM, Vice DS, York D, Gruver KS (2002) Sustained evaluation of the effectiveness of detector dogs for locating brown tree snakes in cargo outbound from Guam International Biodeterioration & Biodegradation 49(2-3): 101–106.

Errico M (2012) Asian longhorned beetle detector dog pilot project. In: McManus F, Gottschalk KW (Eds) Proceedings 23rd U.S. Department of Agriculture Interagency Research Forum on Invasive Species, Annapolis (USA), January 10–13, 2012. U.S. Forest Service, Newtown Square, 1–18.

Fjellanger R (2003) The REST concept. In: McLean IG (Ed.) Mine Detection Dogs: Training, Operations and Odour Detection. Chapter 2 Case studies on training mine detection dogs. Geneva International Centre for Humanitarian Demining, Geneva, 53–104.

Giangregorio P, Audisio P, Carpaneto GM, Marcantonio G, Maurizi E, Mosconi F, Campanaro A (2015) Updated distribution of *Osmoderma eremita* in Abruzzo (Italy) and agro-pastoral practices affecting its conservation (Coleoptera: Scarabaeidae). Fragmenta entomologica 47(2): 139–146. https://doi.org/10.4081/fe.2015.142

Gsell A, Innes J, de Monchy P, Brunton D (2010) The success of using trained dogs to locate sparse rodents in pest-free sanctuaries. Wildlife Research 37: 39–46. https://doi.org/10.1071/WR09117

Harrison RL (2006) A comparison of survey methods for detecting bobcats. Wildlife Society Bulletin 34(2): 548–552. https://doi.org/10.2193/0091-7648(2006)34[548:ACOSMF]2.0.CO;2

Helton WS (2009) Canine ergonomics. The science of working dogs. Taylor & Francis Group, LLC, 1–332.

Hoffman E (2014) Canine scent detection of an invasive wood-boring insect, the Brown Spruce Longhorn Beetle, *Tetropium fuscum*, in laboratory conditions. Environmental Science Undergraduate Honours Thesis. Dalhousie University, Halifax, 1–90.

Hoyer-Tomiczek U, Sauseng G (2013) Sniffer dogs to find *Anoplophora* spp. infested plants. In: Lozzia GC (Ed.) *Anoplophora chinensis* & *A. glabrinpennis*: new tools for predicting, detecting and fighting. How to save our forests and our urban green spaces. Journal of Entomological and Acarological Research 45(1) Special Issue: 10–12.

Hoyer-Tomiczek U, Sauseng G, Hoch G (2016) Scent detection dogs for the Asian longhorn beetle, *Anoplophora glabripennis*. EPPO Bulletin 46(1): 148–155. https://doi.org/10.1111/epp.12282

Hurt A, Smith DA (2009) Conservation dogs. In: Helton WS (Ed.) Canine ergonomics. The science of working dogs. Taylor & Francis Group, LLC, 175–194. https://doi.org/10.1201/9781420079920.ch9

Johnen D, Heuwieser W, Fischer-Tenhagen C (2013) Canine scent detection – Fact or fiction? Applied Animal Behaviour Science 148: 201–208. https://doi.org/10.1016/j.applanim.2013.09.002

Kelley P (2013) Dogs detect pheromone. Fumigants & Pheromones 105: 6.

Larsson MC, Hedin J, Svensson G.P, Tolasch T, Francke W (2003) Characteristic odor of *Osmoderma eremita* identified as a male-released pheromone. Journal of Chemical Ecology 29: 575–587. https://doi.org/10.1023/A:1022850704500

Lin HM, Chi WL, Lin CC, Tseng YC, Chen WT, Kung YL, Lien YY, Chen YY (2011) Fire ant-detecting canines: a complementary method in detecting red imported fire ants. Journal of Economic Entomology 104(1): 225–231. https://doi.org/10.1603/EC10298

Long RA, Donovan TM, Paula M, Zielinski WJ, Buzas JS (2007) Effectiveness of scat detection dogs for detecting forest carnivores. Journal of Wildlife Management 71(6): 2007–2017. https://doi.org/10.2193/2006-230

Mason F, Roversi PF, Audisio P, Bologna MA, Carpaneto GM, Antonini G, Mancini E, Sabbatini Peverieri G, Mosconi F, Solano E, Maurizi E, Maura M, Chiari S, Sabatelli S, Bardiani M, Toni I, Redolfi De Zan L, Rossi de Gasperis S, Tini M, Cini A, Zauli A, Nigro G, Bottacci A, Hardersen S, Campanaro A (2015) Monitoring of insects with public participation (MIPP; EU LIFE project 11 NAT/IT/000252): overview on a citizen science initiative and a monitoring programme (Insecta: Coleoptera; Lepidoptera; Orthoptera). Fragmenta entomologica 47: 51–52. https://doi.org/10.4081/fe.2015.134

Maurizi E, Campanaro A, Chiari S, Maura M, Mosconi F, Sabatelli S, Zauli A, Audisio P, Carpaneto GM (2017) Guidelines for the monitoring of *Osmoderma eremita* and closely related species. In: Carpaneto GM, Audisio P, Bologna MA, Roversi PF, Mason F (Eds) Guidelines for the Monitoring of the Saproxylic Beetles protected in Europe. Nature Conservation 20: 79–128. https://doi.org/10.3897/natureconservation.20.12658

Nakash J, Osem Y, Kehat M (2000) A suggestion to use dogs for detecting red palm weevil (*Rhynchophorus ferrugineus*) infestation in date palms in Israel. Phytoparasitica 28(2): 153–155. https://doi.org/10.1007/BF02981745

Nielsen TP, Jackson G, Bull CM (2016) A nose for lizards; can a detection dog locate the endangered pygmy bluetongue lizard (*Tiliqua adelaidensis*)? Transactions of the Royal Society of South Australia 140: 234–243. https://doi.org/10.1080/03721426.2016.1218698

Nieto A, Alexander KNA (2010) European Red List of Saproxylic Beetles. Publications Office of the European Union, Luxembourg, 1–46.

Nieto A, Mannerkoski I, Putchkov A, Tykarski P, Mason F, Dodelin B, Tezcan S (2010) *Osmoderma eremita*. The IUCN Red List of Threatened Species 2010. http://dx.doi.org/10.2305/IUCN.UK.2010-1.RLTS.T15632A4926651.en

Nussear KE, Esque TC, Heaton JS, Cablk ME, Drake KK, Valentin C, Yee JL, Medica PA (2008) Are wildlife detector dogs or people better at finding desert tortoises (*Gopherus agassizii*)? Herpetological Conservation and Biology 3(1): 103–115.

O'Connor S, Park KJ, Goulson D (2012) Humans versus dogs; a comparison of methods for the detection of bumble bee nests. Journal of Apicultural Research 51(2): 204–211. https://doi.org/10.3896/IBRA.1.51.2.09

Paula J, Leal MC, Silva MJ, Mascarenhas R, Costa H, Mascarenhas M (2011) Dogs as a tool to improve bird-strike mortality estimates at wind farms. Journal for Nature Conservation 19(2): 202–208. https://doi.org/10.1016/j.jnc.2011.01.002

Pfiester M, Koehler PG, Pereira RM (2008) Ability of bed bug-detecting canines to locate live bed bugs and viable bed bug eggs. Journal of Economic Entomology 101(4): 1389–1396. https://doi.org/10.1093/jee/101.4.1389

Polgár Z, Kinnunen M, Újváry D, Miklósi Á, Gácsi M (2016) A test of canine olfactory capacity: comparing various dog breeds and wolves in a natural detection task. PLoS ONE 11(5). https://doi.org/10.1371/journal.pone.0154087

Richards KM, Cotton SJ, Sandeman RM (2008) The use of detector dogs in the diagnosis of nematode infections in sheep feces. Journal of Veterinary Behavior 3: 25–31. https://doi.org/10.1016/j.jveb.2007.10.006

Rolón M, Vega MC, Román F, Gómez A, Rojas De Arias A (2011) First report of colonies of sylvatic *Triatoma infestans* (Hemiptera: Reduviidae) in the Paraguayan Chaco, using a trained dog. Plos Neglected Tropical Disease 5(5): e1026. https://doi.org/10.1371/journal.pntd.0001026

Savidge JA, Stanford JW, Reed RN, Haddock GR, Yackel Adams AA (2011) Canine detection of free-ranging brown treesnakes on Guam. New Zealand Journal of Ecology 35(2): 174–181.

Smith SM, Davis ES (2008) Clicker increases resistance to extinction but does not decrease training time of a simple operant task in domestic dogs (*Canis familiaris*). Applied Animal Behaviour Science 110(3–4): 318–329. https://doi.org/10.1016/j.applanim.2007.04.012

Suma P, La Pergola A, Longo S, Soroker V (2014) The use of sniffing dogs for the detection of *Rhynchophorus ferrugineus*. Phytoparasitica 42(2): 269–274. https://doi.org/10.1007/s12600-013-0330-0

Tikkamäki T, Komonen A (2011) Estimating population characteristics of two saproxylic beetles: a mark-recapture approach. Journal of Insect Conservation 15: 401–408. https://doi.org/10.1007/s10841-010-9313-3

Wallner WE, Ellis TL (1976) Olfactory detection of gypsy moth pheromone and egg masses by domestic canines. Environmental Entomology 5(1): 183–186. https://doi.org/10.1093/ee/5.1.183

Wasser SK, Davenport B, Ramage ER, Hunt KE, Parker M, Clarke C, Stenhouse G (2004) Scat detection dogs in wildlife research and management: application to grizzly and black bears in the Yellowhead Ecosystem, Alberta, Canada. Canadian Journal of Zoology 82: 475–492. https://doi.org/10.1139/z04-020

Waters J, O'Connor S, Park KJ, Goulson D (2011) Testing a detection dog to locate bumblebee colonies and estimate nest density. Apidologie 42: 200–205. https://doi.org/10.1051/apido/2010056

Welch JB (1990) A detector dog for screwworms (Diptera: Calliphoridae). Journal of Economic Entomology 83(5): 1932–1934. https://doi.org/10.1093/jee/83.5.1932

Zahid I, Grgurinovic C, Zaman TK, De Keyzer R, Cayzer L (2012) Assessment of technologies and dogs for detecting insect pests in timber and forest products. Scandinavian Journal of Forest Research 27: 492–502. https://doi.org/10.1080/02827581.2012.657801

Zauli A, Carpaneto GM, Chiari S, Mancini E, Nyabuga FN, Redolfi De Zan L, Romiti F, Sabbani S, Audisio P, Hedenström E, Bologna MA, Svensson GP (2016a) Assessing the taxonomic status of *Osmoderma cristinae* (Coleoptera: Scarabaeidae), endemic to Sicily, by genetic, morphological and pheromonal analyses. Journal of Zoological Systematics and Evolutionary Research 54(3): 206–214. https://doi.org/10.1111/jzs.12127

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Analyses of occurrence data of protected insect species collected by citizens in Italy

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Abstract

Citizen science, the engagement of people in a research project, has grown rapidly in recent years, also for mapping of species of conservation interest. The Life Project "Monitoring Insects with Public Participation" (MIPP) actively promoted collaboration amongst scientists, public administrations and citizens in the collection of occurrence data of nine insect species listed in the Habitats Directive: *Lucanus cervus*, *Osmoderma eremita*, *Cerambyx cerdo*, *Rosalia alpina*, *Morimus asper/funereus*, *Lopinga achine*, *Parnassius apollo*, *Zerynthia cassandra/polyxena* and *Saga pedo*. These species were selected because they share two main characteristics: (i) they are listed in Annexes II and IV of the Habitats Directive and (ii) they are large and relatively easy to identify. From 2014 to 2016, many different strategies were applied to contact and engage the public and approximately 14,000 citizens were reached directly. Additionally, printed and online material informed the public about this project. Citizens could transmit data on the target species, accompanied by a photograph, via the web-site of the project or through a dedicated application (app) for smartphones and tablets. All records were validated by experts based on the photographs sent by citizens. A total number of 2,308 records were transmitted and 1,691 (73.2%) of these were confirmed. Most of the reports were submitted via the website, although the submission via the app increased over time. The species most commonly recorded was *L. cervus*, followed by *M. asper/funereus* and *R. alpina*. Data collected by citizen scientists allowed a detailed analysis to be made on altitudinal distribution and phenology of the species and the results obtained were compared with literature data on altitudinal distribution and phenology. For example, for *L. cervus*, 67% of the records collected were from the altitudinal range 0–400 m a.s.l. Interestingly, the data showed that the phenology of this species changed with altitude.

Keywords

Citizen Science, Habitats Directive, Phenology, Altitudinal distribution

Introduction

Citizen Science (hereafter CS), the engagement of people in order that "scientists and the public work together to investigate and address emergent environmental issues" (Kobori et al. 2016), refers to a wide range of activities that involve lay people at some point in the research process, from research design, to data collection (Bonney et al. 2009), data processing (Raddick et al. 2010) and education (Wiggins and Crowston 2011). CS has grown rapidly in recent years and currently more than 500 English-language CS projects on biodiversity research are known (Kobori et al. 2016) and a number of databases exist (e.g. CitizenScience.org, SciStarter.com). Publication of data from CS projects in peer-reviewed journals is also increasing (Theobald et al. 2015).

The CS approach has been applied for insect monitoring in a great number of projects with a wide range of geographical scales, for example:

- Urban: butterflies monitoring in the cities of Chicago, New York and Tokyo (Matteson et al. 2012, Washitani et al. 2013); Invasive Alien Bumblebee in Hokkaido (Kadoya et al. 2009).
- National: butterfly monitoring in Germany (http://www.science4you.org), Ireland (Donnelly et al. 2014) and Malaysia (Wilson et al. 2015); anglers monitoring initiative of UK (http://www.riverflies.org); UK Ladybird Survey (UKLS; http://www.ladybird-survey.org); insect monitoring in South Africa (Lovell et al. 2009); Swedish Species Observations System (http://www.artportalen.se).
- Continental: Monarch Larva Monitoring Project (http://monarchlab.org/mlmp); migration and trends of Monarch butterflies (Oberhauser and Prysby 2008, Howard and Davis 2009, Davis 2015); Fireflies (Firefly Watch USA), Lost Ladybug Project (LLP; http://lostladybug.org); Swiss pan-European study of the migratory behaviour of the Red Admiral butterfly (https://insectmigration.wordpress.com/red-admiral-migration/).

Moreover, CS has been used to assess the impact of climate change on butterflies and moths (Parmesan et al. 1999, Warren et al. 2001, Fox et al. 2014) and to study pollinators (http://greatpollinatorproject.org/, review in Kremen et al. 2011, Toomey and Domroese 2013) and the land-use preferences of flower visitors (Deguines et al. 2012, Fox et al. 2014).

The effort required from citizens for the insect monitoring varies from simple observations to the application of a standard monitoring protocol. Examples for CS standardised monitoring are the European Grassland Butterfly Indicator (van Swaay et al. 2008, 2013) and the bumble bee monitoring scheme of Ireland (Donnelly et al. 2014).

Despite the great number of projects aimed at insect monitoring, compared with the total number of species considered in such programmes, invertebrates have been under-sampled by CS and, within invertebrates, butterflies have been over-sampled and beetles have been under-sampled (Theobald et al. 2015).

In this context, the LIFE11 NAT/IT/000252 Project – Monitoring of Insects with Public Participation (MIPP) – promoted active collaboration amongst scientists, public administrations and citizens in the collection of occurrence data for nine target insect species listed in the Habitats Directive (Mason et al. 2015, Zapponi et al. 2017). The aims of the CS programme, developed during the project MIPP, were: (i) education - increasing public knowledge on the habitat, biology and threats of the target species, (ii) awareness - promoting environmental awareness and changes in attitudes and behaviour of the public and (iii) faunistic knowledge - mapping the current distribution of the species.

The CS MIPP programme can be classified as a "cross sectional surveying" (Tulloch et al. 2013), which means that volunteers are free to choose when and where to collect occurrence data. This "undirected approach" to data collection, may result in more rapid and efficient detection of species which is particularly important for a project with a defined duration and which focused on rare species, even if it is less consistent in data collection compared with a standardised protocol (Matteson et al. 2012). Moreover, MIPP is a "verified citizen science" programme (Gardiner et al. 2012), as validation of data is ensured by specialists. Data collected by citizens is an extremely valuable instrument for studies on ecology and distribution of insects (Widenfalk et al. 2014). For example, Zapponi et al. (2017) found that the dataset obtained in two years by citizens resulted in an increase in the distributional ranges of three beetle species, compared with a national inventory provided by experts.

The project used information and communication technology (ICT) to collect geo-referenced faunistic data while adding ecological data for the site observation was optional. Above all, applications for wireless devices (smartphones and tablets) can potentially turn anyone into a citizen scientist, enabling them to act as remote sensors for all sorts of data. These devices can collect data more efficiently and in an automated way while, at the same time, limiting human errors and incorporating many important data-gathering functions - such as capturing images, audio and text - into a single tool that can "stamp" the date, time and geographic coordinates associated with an observation (Teacher et al. 2013). The rise of the internet has seen a 'new wave' of online CS projects, sometimes termed 'citizen cyberscience' and has greatly improved the ability to find participants and interact with them. Additionally, it has facilitated data collection by communities of local people who were traditionally not involved in scientific projects and has offered new ways to potentially influence how science and policy-making are carried out (Graham et al. 2011, Newman et al. 2012, Haklay 2013, Kobori et al. 2016). The project MIPP also used social media which can produce long-term benefits for the project itself and important outputs for conservation (Jue and Daniels 2015), for example, by recruiting volunteers for field activities, by advertising events or by inviting citizens who had posted pictures of the target species on social media and also by sending their records to the MIPP database. Additionally, they allowed symbolic rewards to be provided to participants to strengthen their loyalty to the project and consequently, to provide a larger quantity of data (Hochachka et al. 2012).

The target species

Osmoderma eremita (Scopoli, 1763) (Coleoptera, Scarabaeidae) is listed as a priority species in Annexes II and IV of the Habitats Directive, its typical habitat being the cavities of old broadleaf trees. The species is presented in detail (biology, ecology and monitoring methods) by Maurizi et al. (2017).

Lucanus cervus (Linnaeus, 1758) (Coleoptera, Lucanidae) is listed in Annex II of the Habitats Directive. Its larvae feed on dead wood from different broadleaved tree species, mainly oaks, in contact with the ground. Biology, ecology and monitoring methods of the stag beetle are presented in detail by Bardiani et al. (2017).

Cerambyx cerdo Linnaeus,1758 (Coleoptera, Cerambycidae) is listed in Annexes II and IV of the Habitats Directive. It typically lives in large, old trees (especially oaks) which are, at least partially, exposed to the sun. Redolfi De Zan et al. (2017) provided details on biology, ecology and monitoring methods for this species.

Rosalia alpina (Linnaeus, 1758) (Coleoptera, Cerambycidae) is listed as a priority species in Annexes II and IV of the Habitats Directive and larvae typically develop in wood of large *Fagus sylvatica* but sometimes also in other broadleafed tree species. Biology, ecology and monitoring methods of *R. alpina* are presented in detail by Campanaro et al. (2017).

Morimus funereus Mulsant, 1863 (Coleoptera, Cerambycidae) is listed in Annex II of the Habitats Directive. However, a recent genetic study (Solano et al. 2013) showed that all European and Turkish populations of the genus *Morimus* Brullé, 1832 should be referred to as *M. asper* (Sulzer, 1776) (a genetically and morphologically highly variable taxon) and *funereus* is considered a sub-species (Solano et al. 2013). The larval development takes place in recently cut wood, stumps and trunks of damaged trees. Biology, ecology and monitoring methods for this species are provided by Hardersen et al. (2017).

Lopinga achine (Scopoli, 1763) (Lepidoptera, Nymphalidae) is listed in Annex IV of the Habitats Directive. Its main habitats are forest clearings and forest margins and its larvae feed on *Brachypodium* grasses. In Italy, the species is confined to the Alps.

Parnassius apollo (Linnaeus, 1758) (Lepidoptera, Papilionidae) is listed in Annex IV of the Habitats Directive, mainly occurring in mountain areas on steep, sunny slopes with sparse vegetation and its larvae feed on *Sedum* ssp. and *Sempervivum*.

Zerynthia polyxena (Geyer, 1828) (Lepidoptera, Papilionidae) is listed in Annex IV of the Habitats Directive; however, recent studies have shown that, in central and southern Italy, an endemic sister species, *Z. cassandra* (Dapporto 2010, Zinetti et al. 2013) is present. The larvae of both species feed on the host-plant *Aristolochia* ssp. and are generally found in open habitats such as forest clearings and edges, slopes, open forests or meadows.

Saga pedo Pallas, 1771 (Orthoptera, Tettigonidae) is a parthenogenetic species listed in Annex IV of the Habitats Directive. It is a xerothermophylous species inhabiting dry meadows, pastures and shrubby hillsides. *S. pedo* feeds mainly on other grasshoppers.

Research Objectives

The objectives of this paper are two-fold: (i) to describe the different strategies adopted to engage people in the LIFE project MIPP and to analyse the results of participation by volunteers and (ii) to use the data transmitted to upgrade knowledge of the altitudinal distribution and the phenology of the target species by comparing these results with the most relevant literature.

Materials and methods

Volunteer engagement

Engaging citizen volunteers to monitor and manage natural resources, track species at risk and conserve protected areas is increasing, especially by non-governmental organisations. The contribution of volunteers to natural sciences is not new; in museums, there are hundreds of millions of plants and animals specimens which have been collected by volunteers. However, with CS, the engagement of volunteers is increasing and now makes it possible to carry out monitoring programmes which constitute a new challenge for science (Cathy et al. 2011, Bordogna et al. 2014). In the MIPP project, volunteers were asked to provide records, accompanied by photographs of the target species through a web-site or via the app “MIPP” for smartphones and tablets.

Different strategies were applied to engage the public from 2014 to 2016. The MIPP staff met citizens “face-to-face” during seminars, workshops and dissemination events in cities, in science museums and in nature reserves. Other means used to contact the public were talks and posters at conferences and guided tours. Additionally, a specific education programme for schools was carried out in several Italian regions (see Carpaneto et al. 2017, for details). A total of 403 activities were carried out during 2014–2016, with approximately 14,000 citizens reached (Table 1). These activities involved pupils from primary to high school, university students, professors and technical personnel from nature reserves. Media-related communication activities included the website of the project, social networks (Facebook, YouTube, Twitter), two documentaries transmitted on national TV, 13 interviews in the Italian TV or Radio, monthly press releases and 127 articles in magazines and newspapers (see Table 2 for details). Additionally, printed and online educational material was offered to disseminate the project objectives and to help citizen scientists to recognise the target species. This material included: identification guides (available online), posters (950 copies), leaflets (70,000 copies), booklets (15,000 copies), comic-strips (36 available online) and notice boards (35 installed in 10 nature reserves). Continuous contact with the public was maintained by publishing online technical reports and news as well as a bimonthly newsletter. In addition, an incentive was offered to participants for gathering numerous faunistic data: the project website kept tracks of their records, displayed their records on maps, provided their status and offered prizes in connection with the number of records sent.

Volunteer data collection and verification procedures

Two main systems were used to transmit records of the target species: (i) the project website (http://www.lifemipp.eu) and (ii) the app "MIPP" for smartphones and tablets. The system only focused on presence data. The website was developed using the J2EE, Servlet and JSP languages. The mark-up languages were based on HTML5 with JavaScript, LESS and SASS. Apache Tomcat was used as the web server. The first version of the website was online in September 2013 and overall 97 versions of the website were released. The development of the smartphone application started in September 2013. In March 2014, the application for Android was released, whereas the first version for iOS and Windows Phone were released in May 2015. A total of 13 versions for Android, five versions for iOS and five versions for Windows Phone were released. Both the website and the app contained the guide for volunteers and was named "How to report" with step by step instructions. Identification sheets were available for all species and included information on taxonomy, distribution, biology, ecology and conservation status.

To report a sighting, the citizen scientist had to complete an online form (via website or via app) which included mandatory and optional fields. The mandatory fields were: nickname, e-mail address, geographic coordinates (inserted manually or automatically), date and hour of sighting, photograph of the target species and the name of the species observed (although a field named "Unidentified" for uncertain data was also available). The optional fields were: location information, insect position, habitat and additional notes.

Table 1. Number of dissemination activities and number of citizens reached from 2014 to 2016.

	2014		2015		2016		Total	
	Activities	Citizens	Activities	Citizens	Activities	Citizens	Activities	Citizens
Seminars and workshops	25	642	31	2100	26	652	82	3394
Divulgation events	19	715	20	968	18	1539	57	3222
Conferences	5	130	2	140	3	0	10	270
Guided tours	8	257	54	1496	4	170	66	1923
Educational activities at school	58	1923	70	1862	60	1370	188	5155
Total	115	3667	177	6566	111	3731	403	13964

Table 2. Media-related dissemination performed from 2014 to 2016.

	2014	2015	2016	Total
Documentaries	1	1	0	2
Press releases	9	12	13	34
Magazines and newspaper articles	42	37	48	127
Interviews in TV or Radio	4	5	4	13
Total	56	55	65	176

According to the recommendations provided by Lovell et al. (2009), an attempt was made to minimise subjectivity of the volunteers (mandatory photographs, online instructions etc.) and to ensure data quality (validation by experts). Digital photographs were necessary to ensure the validity of the data which was verified and approved by experts who confirmed or rejected the identification. Records without photographs were not accepted with the exception of “expert” citizens (people who had already sent a number of correct records, thereby leading to the acceptance of this source) or, for certain species, whether the records were provided with an accurate description of the insect. Finally, each report submitted acquired an automatic field, i.e. date of reporting.

Once a submission was correctly completed, the system sent a notification to the e-mail address of the citizen scientist who recorded the species and to the specific expert. Based on the data provided, the expert assigned one of five different statuses to the record: (i) confirmed (the species has been correctly identified by the citizen and all the other information provided were plausible), (ii) rejected (the photo showed none of the target species or the other information was implausible), (iii) not publishable (the specimens was part of an entomological collection, the same specimens had been already reported, wrong geographical coordinates, the picture was not clear), (iv) interesting but not target (the picture refers to a species of conservation interest but none of the target species, e.g. *Lucanus tetraodon*) and (v) pending (evaluation in progress). All confirmed reports were displayed on the map of the project website. However, the exact location was not disclosed and the site of the sighting was indicated within a range of 10 km from the original geographic position provided. The exact position was hidden from the public because the records were considered "sensitive data", due to the species being protected. Volunteers could access the website using their credentials and consult details and exact positions of all the records provided. The reports were stored in the project database. This database is managed through a MySQL relational DBMS (DataBase Management System) and data are exportable as CSV format. At the end of the project, the data will be available and shared with the National Biodiversity Network (http://http://www.naturaitalia.it/banchedati.do) of the Ministry of the Environment.

Statistical analysis of phenology and distribution

Faunistic records were downloaded from the MIPP database on 14.12.2016 and only those records which had been validated by experts were used. The data set contained, amongst other information, the date of the observation and the coordinates. In order to obtain the elevation for each record, two approaches were followed. Firstly, the Google Maps API was used to return elevations for all points, employing the GPS Visualizer (http://http://www.gpsvisualizer.com/geocoder/elevation.html). Secondly, an analysis was performed using the geomorphology tools (DEM) implemented in QGIS (version 2.14.3-Essen). The average of both values was rounded to the nearest metre and these values were used for further analysis. To investigate the altitudinal distribution of the species recorded, the number of records was plotted for six altitudinal ranges (0–400; 401–800; 801–1,200; 1,201–1,600; 1,601–2000 and 2,001–2,400), as this resolution allowed the altitudinal distribution for all target species to be plotted and a statistical analysis comparing different altitudinal ranges for four species to be undertaken. Subsequently, the altitudinal ranges were superimposed on the proportion of the land-surface area of Italy present in the ranges. These were obtained using the processing tools (reclassify) implemented in QGIS (version 2.14.3-Essen).

To investigate the phenology of the target species, two types of analysis were carried out. Firstly, the records for each species were assigned to three 10-day periods in each month and expressed as percentages. The resulting histograms were plotted. In a second step, the change in the phenology with increasing altitude was analysed. To do this, all records of the various species were pooled for the six altitudinal ranges (0–400; 401–800; 801–1,200; 1,201–1,600; 1,601–2000 and 2,001–2,400) and dates were transformed into day of the year (e.g. 1^{st} of January=1). Subsequently for each altitudinal range, a boxplot was created for the pooled days of the year and the median was calculated, defining the day which represented the peak of activity for each altitudinal range. To compare the length of the activity period of the various species at different altitudes, the days were calculated between first and third quartile which define the time when the central 50% of observations were carried out. Phenological data for the different ranges were analysed with the Kruskal-Wallis rank sum test, as implemented in R version 3.1.3 (R Development Core Team 2010). Phenological changes with increasing altitude were only calculated for those species for which the MIPP database held validated data from more than two altitudinal ranges with more than 25 records and only for those ranges with more than 25 records. Thus, these analyses were carried out for *L. cervus*, *M. asper*, *R. alpina* and *P. apollo*.

Results

A total number of 2,308 reports were transmitted to the project database. Most of these reports (n=1,653, 71.6%) were submitted via the website, whereas only 28.4% of the reports (n=655) were submitted via the app (Table 3). The number of annual reports constantly grew from 2014 to 2016 (Table 3). Most of the dates of the sightings fell within the duration of the CS action (2014–2016) and only a small part of records (n=206, 8.9%) were collected prior to 2014 (Table 3). The percentage of records sent via the website decreased between 2014 and 2016 (from 80.6% to 69.2%), while the submission via the app increased (from 19.4% to 37.4%) (Figure 1). To most of the submitted records, the status "confirmed" was assigned by specialists (1,691 out of 2,308 for the whole dataset) (Table 4) and the distribution of these validated records in Italy is presented in Figure 2. The percentages of the five status categories assigned to the reports (confirmed, rejected, not publishable, not target, pending) for records from the years 2014–2016 are given in Figure 3.

The species most commonly recorded was *L. cervus*, followed by *M. asper* and *R. alpina*. The number of discarded and confirmed records was calculated for all nine-target species (Figure 4) and the proportion of confirmed records varied between 87% for *M. asper* and 96% for *L. cervus*, *M. asper*, *R. alpina*, *P. apollo*, *Z. cassandra*/*polyxena* and *L. achine*. On the contrary, for *C. cerdo*, *O. eremita* and *S. pedo*, only between 44% and 64% of records were confirmed by the experts. The number of sightings per year was calculated for the nine-target species (Figure 5). In general, the number of sightings increased, but some exceptions exist. For example, for *L. cervus,* the number of records from 2014 was higher than from 2015. Similarly, for *C. cerdo* and *P. apollo*, the number of records from 2015 was higher than from 2016. The proportion of records submitted via the app and via the website was calculated for all nine-target species (Figure 6). The app was used to transmit 21% to 31% of records for the five beetle species. In contrast, for butterflies, only 7%–11% of records were transmitted via the app.

A total of 695 citizens submitted at least one record during the three years analysed and the number of participants increased each year (2014: n=182; 2015: n=295; 2016: n=335). Most of the citizens (n=603) transmitted data in only one year, whereas a few provided records during more than one year (n=92). Most of the citizens (n=600) transmitted one to three records, a smaller part of citizens (n=68) submitted

Table 3. Number of records submitted via web, via app and both, for each year and for date of transmission.

Date of transmission	Recorded before 2014			Recorded 2014–2016			All records		
	Web-site	App	Total	Web-site	App	Total	Web-site	App	Total
2014	117	1	118	350	84	434	467	85	552
2015	58	6	64	513	210	723	571	216	787
2016	23	1	24	592	353	945	615	354	969
All reporting date	198	8	206	1455	647	2102	1653	655	2308

Table 4. Number of records submitted via web, via app and both, for the 5 status categories and for date of sightings.

Status category of records	Recorded before 2014			Recorded 2014–2016			All records		
	Web-site	App	Total	Web-site	App	Total	Web-site	App	Total
Confirmed	164	4	168	1169	354	1523	1333	358	1691
Not publishable	6	0	6	7	5	12	13	5	18
Not Target	9	0	9	46	61	107	55	61	116
Rejected	19	4	23	200	207	407	219	211	430
Pending	0	0	0	33	20	53	33	20	53
Tot.	198	8	206	1455	647	2102	1653	655	2308

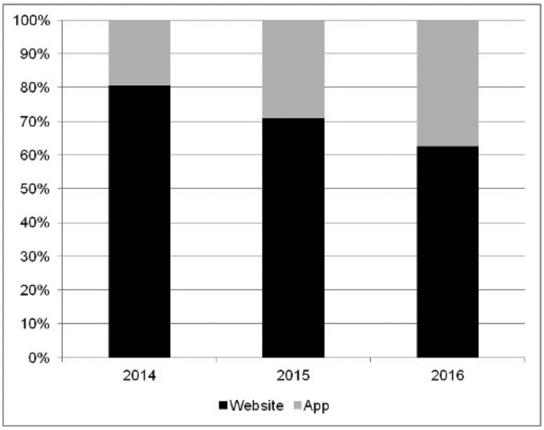

Figure 1. Percentage ratio of reports submitted via web and via app for year (considering the sightings from 2014 to 2016).

Figure 2. Distribution map of confirmed records of the target species collected by citizen scientists during the LIFE MIPP Project.

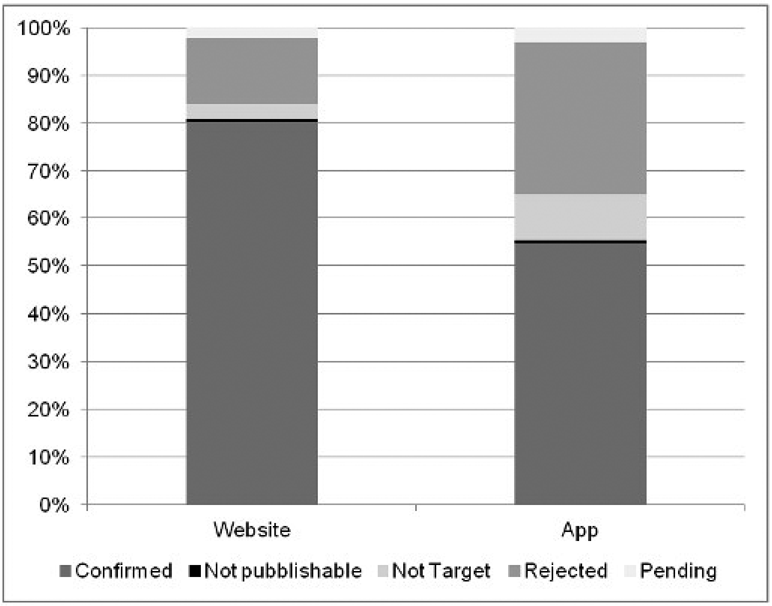

Figure 3. Percentage ratio between the five status categories of the reports (considering sighting date between 2014 and 2016) submitted via web and via app.

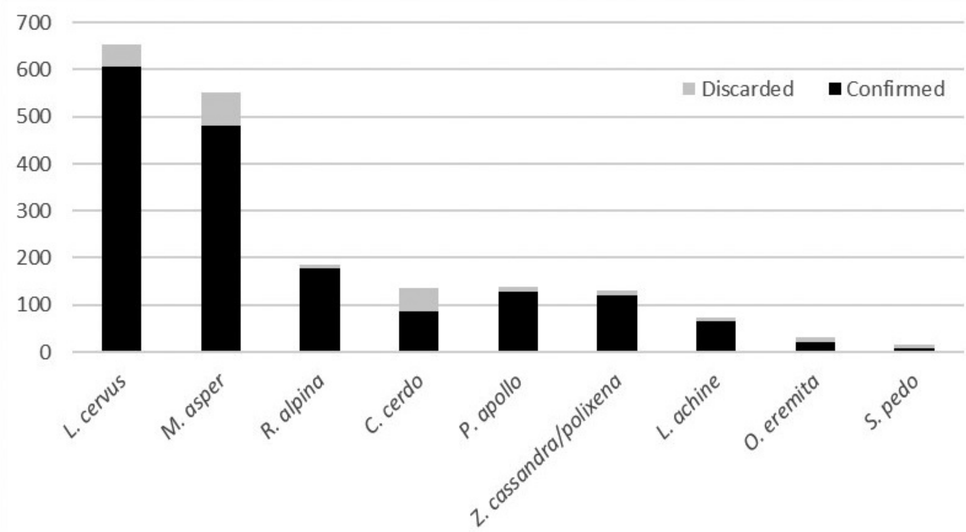

Figure 4. Number of discarded and confirmed records for the nine-target species reported by citizens.

4–10 records each, another group of citizens (n=34) submitted 11–62 records and 1 citizen transmitted 132 records. Figure 7 identifies the number of records in correlation to the number of citizens. Some records without photographs sent by "expert"

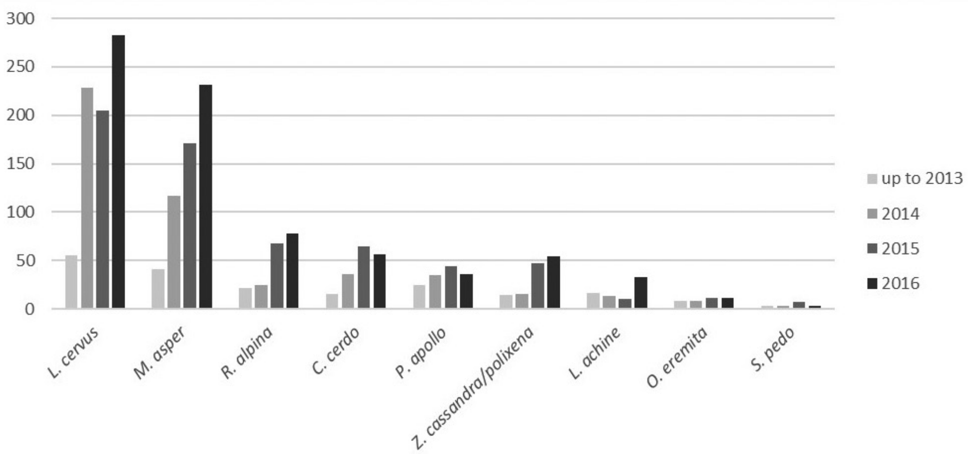

Figure 5. Number of records per year of the nine-target species reported by citizens.

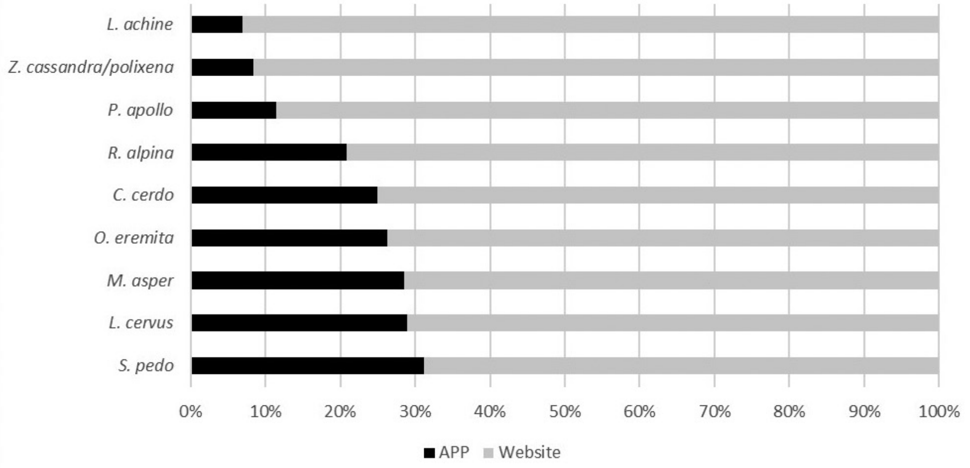

Figure 6. Percentage of records submitted via website and via app for the nine-target species.

citizens (naturalists with a considerable experience in entomology) were accepted, a total of 4.6% of the complete data-set. These mainly concerned *L. cervus* (70 records of 605 records confirmed).

Altitudinal variation

The average difference between the two methods for obtaining altitudes for all points was 8.1 m ±8.8 m standard deviation (SD). The altitudinal distribution of the various species, as revealed by CS data, is presented in Figure 8. These distributions showed

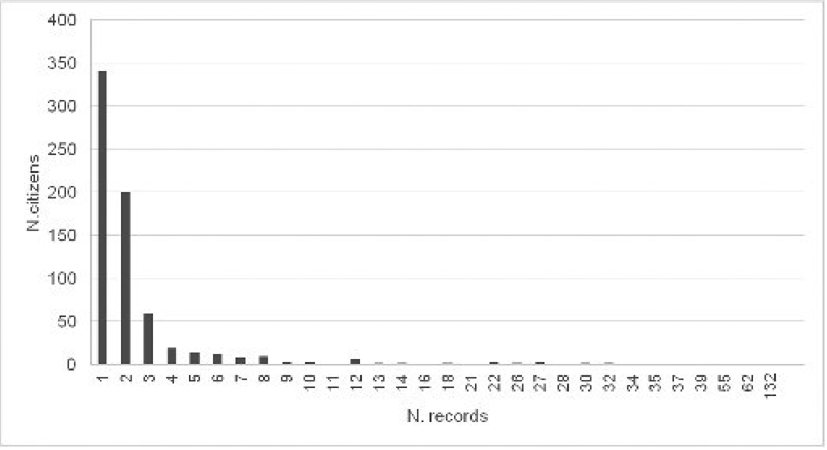

Figure 7. Relationship between the number of provided records and the number of citizens.

distinct patterns for the various species and, for each species, the altitudinal distribution revealed by CS differed substantially from the altitudinal distribution of the Italian territory (Figure 8). For example for *L. cervus*, 67% of observations were carried out in the range 0–400 m a.s.l. but, in Italy, only 18% of the land-surface area are in this altitudinal range. Thus, *L. cervus* was observed more commonly at lower altitudes than would be expected if the species were randomly distributed in the national territory.

The species most commonly recorded at low altitudes was *C. cerdo*, with 79.5% of all observations between 0 and 400 m a.s.l. The lowest record was set at 2 m a.s.l. and the highest at 1,147 m a.s.l. The species with the highest number of observations at high elevations above sea level was *P. apollo*, with 47.6% of all records from the altitudinal range 1,601–2,000 m a.s.l. For this species, the lowest record was from 722 m a.s.l., whereas the highest was from 2,252 m a.s.l. The lowest and highest altitudes for all target species, as revealed by the CS data, are presented in Table 5.

Phenological variation

Phenology for all species is presented in Figure 9. The analysis of phenology in relation to altitude were carried out for *L. cervus*, *M. asper*, *R. alpina*, and *P. apollo* and a total of nine comparisons for different altitudinal ranges were carried out (see below). In all cases, peak activity occurred later with increasing elevation and was on average delayed by 10 days when moving upwards by 400 m a.s.l.

For *O. eremita*, the first observation was from 12 June (2012), while the last observation was from 11 September (2016). Because the histogram (Figure 9) did not

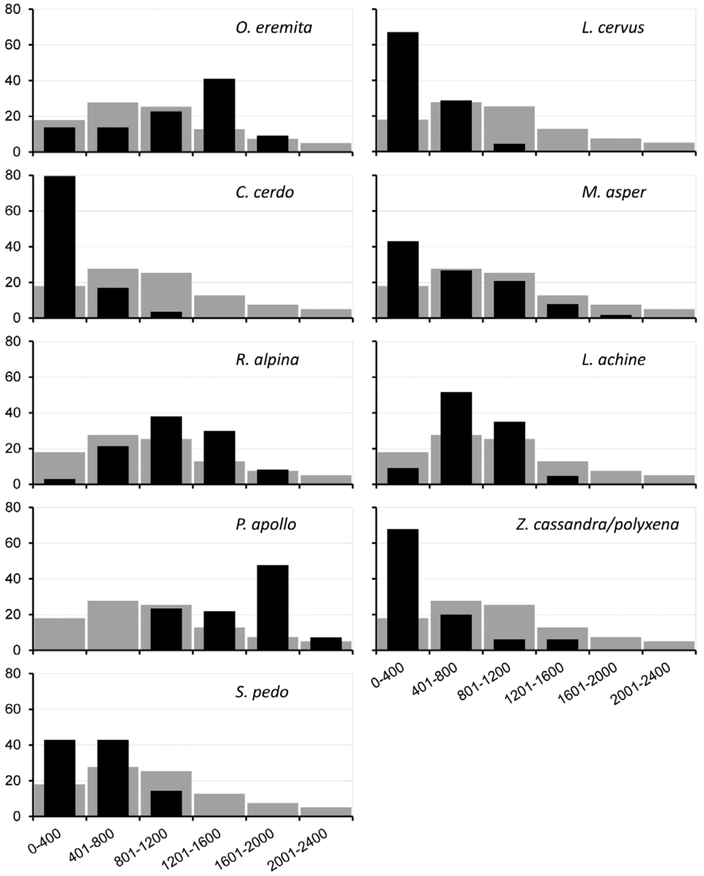

Figure 8. Altitudinal distribution of the target species, as revealed by the citizen science data. The distributions are expressed as percentages of the total number of records and are presented with the altitudinal distribution of the Italian territory as background.

Table 5. The lowest and highest altitude in meters where the target species were recorded according to the citizen science data.

Species	Lowest altitude (m)	Highest altitude (m)
O. eremita	3	1,836
L. cervus	6	1,065
C. cerdo	2	1,147
M. asper	2	1,870
R. alpina	3	1,997
L. achine	179	1,526
P. apollo	722	2,252
Z. cassandra/polyxena	1	1,482
S. pedo	90	860

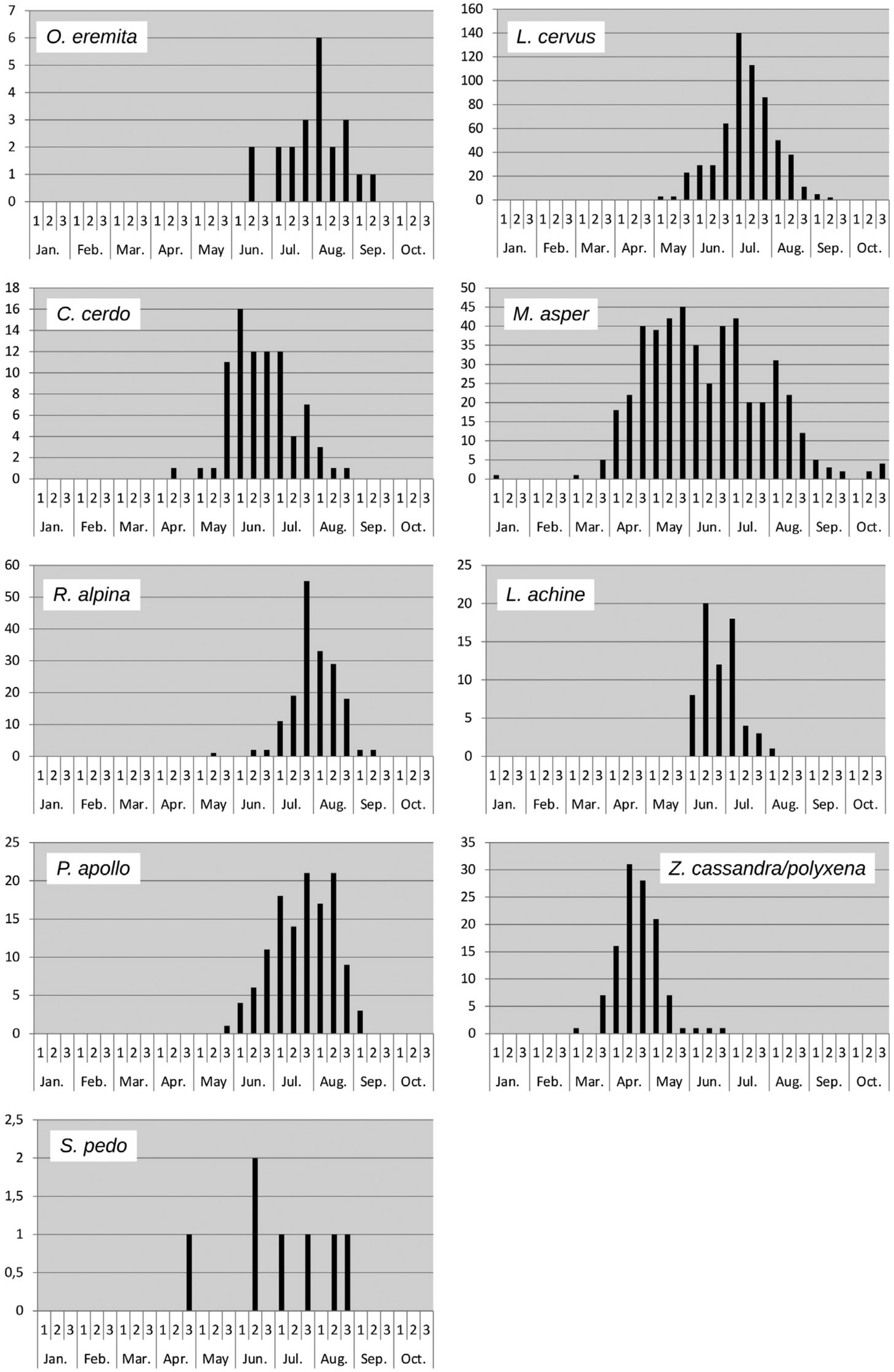

Figure 9. Phenology of the target species, as revealed by the citizen science data.

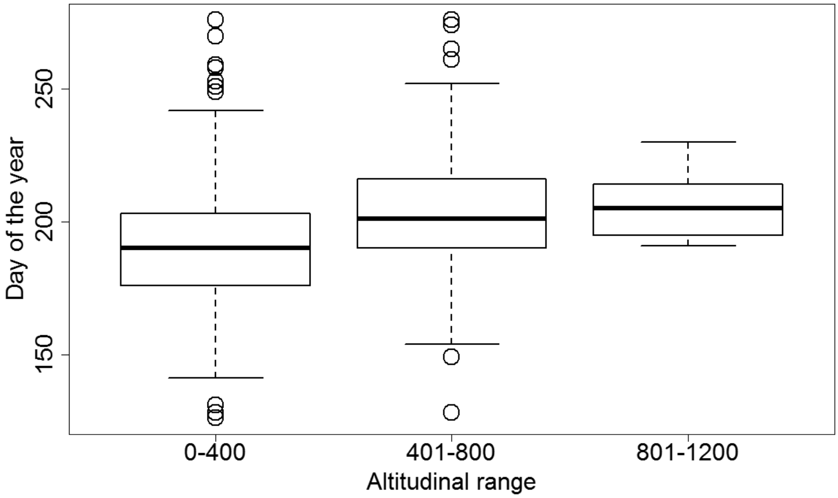

Figure 10. Phenology of *L. cervus* in different altitudinal ranges, based on citizen science data. The boxplots represent the distribution of dates, expressed as "days of the year". The medians for the altitudinal ranges are: 190 (09 July) (0–400 m a.s.l.); 201 (20 July) (401–800 m a.s.l.); 205 (24 July) (801–1,200 m a.s.l.).

show a clear pattern, the central 50% of observations were calculated for *O. eremita*. This analysis showed that the main activity period was between 19 July and 18 August.

The earliest record of *L. cervus* was from 06 May (2015) and the latest from 17 September (2016) while high numbers were observed between the end of June and early August. For this analysis, remains recorded in September and October were not considered, as old remains can be found weeks after the end of the flight period (Campanaro et al. 2011). The phenology of *L. cervus* changed with increasing altitude (Figure 10) and this was highly significant (χ^2 = 167.3, df = 108, p < 0.001). Peak of activity was observed on 09 July for the altitudinal range 0–400 m a.s.l., but 20 July at 401–800 m a.s.l. At higher elevations (801–1,200 m a.s.l.) the peak of activity was observed on 24 July. In addition, the length of the activity period varied considerably for the different altitudinal ranges. Whereas the central 50% of observations were made in 27 days at 0–400 m a.s.l. and in 26 days at 401–800 m a.s.l., the same percentage was observed at the highest altitude investigated (801–1,200 m a.s.l.) in only 19 days.

The earliest record of *C. cerdo* was on18 April (2013) and the latest on 30 August (2016), while high numbers of this beetle were observed between late May and early July. For *M. asper*, the earliest record was on 08 January (2015) and the last one on 29 October (2016). Large numbers of this longhorn beetle were observed for an extended period, which lasted from mid-April to mid-August. As in *L. cervus*, phenological changes between the different altitudinal ranges were marked. The peak of activity for *M. asper* was observed even later in the year as altitude increased (Figure 11) and this trend was highly significant (χ^2 = 209.2, DF = 157, p = 0.003). Whereas the peak of activity was observed at low altitudes (0–400 m a.s.l.) on 23 May, this date was even

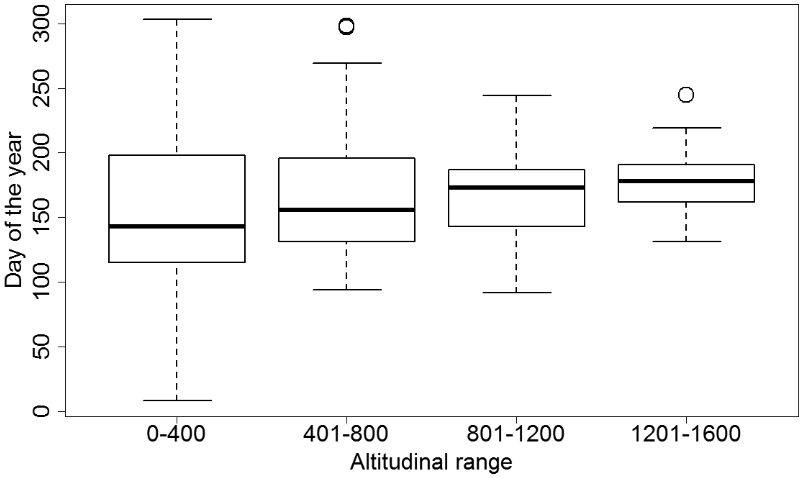

Figure 11. Phenology of *M. asper* at different altitudinal ranges, based on citizen science data. The boxplots represent the distribution of dates, expressed as "days of the year". The medians for the altitudinal ranges are: 143 (23 May) (0–400 m a.s.l.); 156 (05 June) (401–800 m a.s.l.); 173 (22 June) (801–1,200 m a.s.l.); 178 (27 June) (1,201–1,600 m a.s.l.).

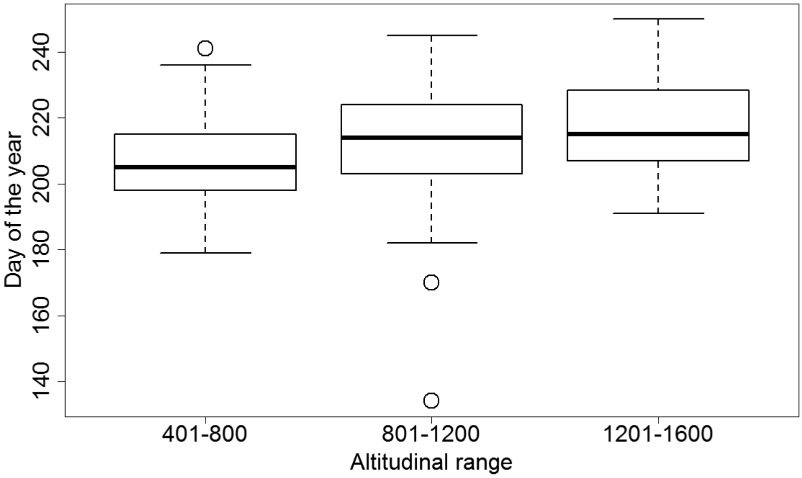

Figure 12. Phenology of *R. alpina* in different altitudinal ranges, based on citizen science data. The boxplots represent the distribution of dates, expressed as "days of the year". The medians for the altitudinal ranges are: 205 (24 July) (401–800 m a.s.l.); 214 (02 August) (801–1,200 m a.s.l.); 215 (03 August) (1,201–1,600 m a.s.l.).

later in the year with increasing elevations. At 401–800 m a.s.l., the peak of activity was observed on 05 June and at 801–1,200 m a.s.l. on 22 June. At 1,201–1,600 m a.s.l., the median fell on 27 June. Again, the length of the activity period decreased with in-

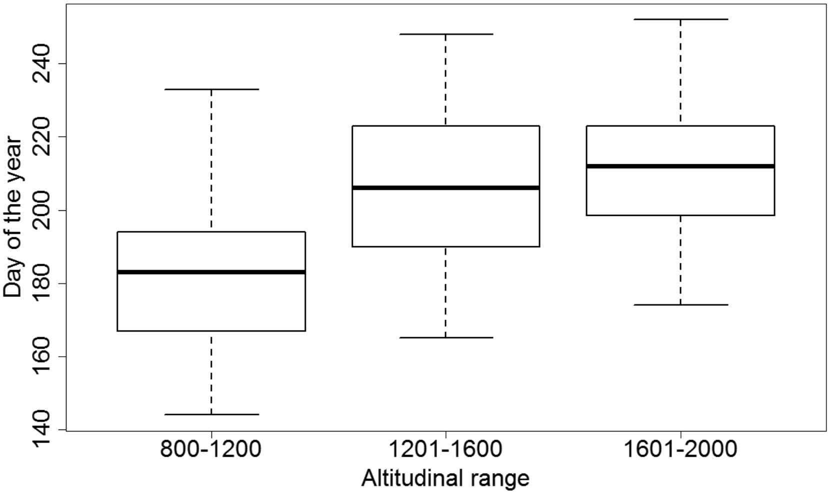

Figure 13. Phenology of *P. apollo* in different altitudinal ranges, based on citizen science data. The box-plots represent the distribution of dates, expressed as "days of the year". The medians for the altitudinal ranges are: 183 (02 July) (800–1,200 m a.s.l.); 206 (25 July) (1,201–1,600 m a.s.l.); 212 (31 July) (1,601–2,000 m a.s.l.)

creasing elevation. Whereas the central 50% of observations of *M. asper* were made in 83 days at 0–400 m a.s.l., the same percentage of data was collected in only 65 days at 401–800 m a.s.l. and in 44 days at 801–1,200 m a.s.l. While at 1,201–1,600 m a.s.l., 50% of observations were collected in only 29 days.

For *R. alpina*, the earliest record was on 13 May (2016) and the last one on 17 September (2015). A large number of this longhorn beetle was observed from early July to late August. In this species, phenological changes between the different altitudinal ranges were also marked (Figure 12), but these were not statistically significant ($\chi^2 = 51.7$, DF = 58, $p = 0.706$). Whereas the peak of activity was observed at the altitudinal range 401–800 m a.s.l. on 24 July, this date was observed at 801–1,200 m a.s.l. on 02 August. At 1,201–1,600 m a.s.l., the peak of activity fell on 03 August. The length of the activity period did not show a clear pattern in correlation with elevation. The central 50% of observations of *R. alpina* were made in 17 days at 401–800 m a.s.l., the same percentage of data being collected in 21 days at 801–1,200 m a.s.l. as well as at 1,201–1,600 m a.s.l.

L. achine was observed between 02 June (2011) and 01 August (2013), with most observations from mid-June to early July.

The earliest record of *P. apollo* was on 24 May (2015) and the latest on 09 September (2014), while high numbers were observed between early July and mid-August. The phenology of *P. apollo* changed with increasing altitude (Figure 13) but this trend was not significant ($\chi^2 = 68.5$, DF = 58, $p < 0.162$). The peak of activity was observed on 02 July for the altitudinal range 800–1,200 m a.s.l., but at 1,201–1,600 m a.s.l. this occurred later, on 25 July. At even higher elevations (1,601–2,000 m a.s.l.), peak activity was observed on 31 July. The length of the activity varied between the different altitudinal ranges, but not in a consistent manner. Whereas, the central 50% of observations were made in 27 days at 800–1,200 m a.s.l. and in 33 days at 1,201–1,600 m a.s.l., the same percentage was observed at the highest altitude investigated (1,601–2,000 m a.s.l.) in 25 days.

For *S.pedo,* the number of records was too small (n=7) to allow for any meaningful analysis of the phenology. For *Z. cassandra* and *Z. polyxena*, the earliest record was on 09 March (2016) and the latest on 30 June (2014), while high numbers were observed exclusively between early April and early May.

Discussion

The present study represents the first application in Italy of the CS approach to the study of insect species listed in the Habitats Directive; it is also the first time that the CS approach has been applied to rare and/or elusive insect species (i.e. *R. alpina*, *O. eremita*, *S. pedo*, *L. achine*).

The CS programme presented in this paper can be considered successful in terms of citizen response, records transmitted, scientific outputs and social outcomes. The number of records provided is high considering the ecology and biology of the target species which are rare, have a localised distribution and have a restricted activity period. The response by citizens followed a positive trend, both considering the number of records provided and the number of citizens involved, thus demonstrating that the interest in the project was maintained over more than one year. The continuous contacts with volunteers (guaranteed by e-mails), the high number of public events and the different dissemination tools, certainly contributed to this result, because "identity and motivation are crucial to maintaining committed volunteers" (Kobori et al. 2016).

The preferred method for transmitting records was the website, even if the percentage of data sent via the app was increasing. One possible reason for this prevalence for using the website could be that photographs of reasonable quality of the target species were easier to take with cameras and a macro lens. The inbuilt cameras of smartphones were less suitable for this purpose. However, the increasing use of the app MIPP over the years might be due to several factors, amongst them being: advances in the usability of the app, better internet connections, better promotion for the app, or more simply, citizens who send records are middle-aged people (average age 44) and increasingly use smartphones and apps.

The high rate of correct validations (73%) confirmed that the majority of the data collected by volunteers were correct. Similarly, Gardiner et al. (2012) found that citizen scientists correctly identified lady beetles in 81–100% of cases. Ratnieks et al. (2016) reported that volunteers correctly identified between 79% and 94% of insects and they showed that the type of training method had a significant effect on identification accuracy. Thus, the ability of citizens in recognising the target species suggested that the information provided to facilitate species identification during the MIPP project was useful or that they had personal expertise in entomology. However, these data, which contained 27% of erroneous records, also showed that validation was a crucial step in a CS project to ensure the scientific quality of the data collected by citizens (cfr. Delaney et al. 2008, Zapponi et al. 2017).

L. cervus was the most frequently recorded species, probably due to the fact that it is a relatively large and common species in northern and central Italy. The high number of records for *R. alpina* was surprising as this species has a fragmented distribution in Italy and is restricted to a very specific habitat (open beech forests). However this species is stunning and recognizable due to its typical colour pattern: the body is velvet blue-gray with black spots. It might also be that this species is becoming more common, as has been observed in Switzerland (Lachat et al. 2013).

The analysis of the participation of citizens in terms of the number of records provided, showed that a small share of participants contributed with many data and many contributed with few data. In other words, a large group of citizens occasionally contributed to the project (providing one to three records) and a small group of citizens contributed constantly (providing dozens of records). This pattern was also reported by Boakes et al. (2016), who examined the composition of three CS datasets and found that most volunteers contributed few records and were active for just one day. Furthermore Boakes et al. (2016) emphasised that the main objective is to encourage citizens to further their skills in the research of the target species and to provide support for new volunteers. An example of the importance of the CS initiative was reported by Zapponi et al. (2017). Using a subset of the dataset analysed here (2 years, 3 species), the authors demonstrated the high value of this CS initiative, as the quality of occurrence data gathered by volunteers was similar to the data collected exclusively by experts. In addition, Widenfalk et al. (2014) emphasised that data collected by citizens represent an extremely valuable instrument for studies on ecology and distribution. It is believed that CS can not only provide collections of valid data, but also represents a positive way to connect people with nature and this can increase the collective knowledge on conservation values and threats for the environment (Devictor et al. 2010).

Phenological and altitudinal variation

The records collected with the CS approach, allowed detailed analysis of altitudinal distribution and phenology of the target species, particularly for those with the highest numbers of records. Additionally, Polgar et al. (2013) stated that, for insect phenology studies, reliable CS data can be a powerful tool for scientific analysis.

The comparison between traditional biological recording schemes and CS approaches to gather data on species distributions was examined by van der Wall et al. (2015). The two recording approaches revealed similar abundances of bumblebee species but different geographical distributions. CS records displayed more extensive geographic coverage, reflecting human population density, thus offering better opportunities to account for recording effort (van der Wall et al. 2015).

The analysis performed on records collected by citizens showed that the patterns of altitudinal distributions for all species differed from that of the Italian land-surface. This means that the species were not observed randomly, but records followed species-specific altitudinal distributions. These patterns might be influenced by different frequencies of visits of citizens to the various altitudes, but these data do not permit this investigation. The abundant data for *L. cervus*, *M. asper*, *R. alpina* and *P. apollo* also permitted the species phenology to be investigated for specific altitudinal ranges and led to the statement that their peak of activity was delayed by 10 days on average for populations recorded with an increase in altitude of 400 m. To the best of the authors' knowledge, such detailed phenological information has not been available prior to this study. In the following paragraphs, the results obtained from the CS data are compared with the information available in literature for all species investigated.

The citizen scientists reported *O. eremita* at altitudes between 3 m and 1,836 m a.s.l. This upper limit is higher than that reported for Italy by scientific literature (Ranius et al. 2005), who stated that the species has been recorded up to 1,500 m a.s.l., similar to the altitudinal ranges reported for Bosnia-Herzegovina (50–1,350 m a.s.l.) and Greece (100–1,200 m a.s.l.) (Tauzin 1994, Ranius et al. 2005). However, one population of the *O. eremita* complex was recently recorded in Calabria (southern Italy) at 2,000 m a.s.l. (Mazzei A. and Brandmayr P. pers. com.). For *O. eremita*, the volunteers recorded it between 12 June and 11 September. These data are in accordance with those of Bologna et al. (2016a) reported for Italy. Whereas, Schaffrath (2003) reported for Germany that adults are active from June to early August and Ranius et al. (2005) stated for Europe in general, that the adults of *O. eremita* are normally found from July to September. However, in some European regions (Germany, Slovenia and Italy), there have been several observations in June and even single findings in April and May (Ranius et al. 2005).

The altitudinal data derived by the records for *L. cervus*, ranged from 6 m to 1,065 m a.s.l., with more than 65% of the observations from sites below 400 m a.s.l. This altitudinal range is very similar to that given by Campanaro et al. (2011) i.e. from sea level up to 1,000 m a.s.l. Across Europe, the altitude at which the beetle lives varies from 5 m to 1,700 m a.s.l. in Bulgaria (Harvey et al. 2011). However, the species is generally reported as abundant below 200 m a.s.l. and, in northern Spain, does not occur above 800 m a.s.l. (Alvarez Laó and Alvarez Laó 1995). *L. cervus* was observed by citizen scientists between 06 May and 17 September. This period is more extended than most other studies; the only exception being Vrezec (2008), who also analysed non-systematically collected data and reported observations for this species from 19 March to 19 September. However, phenological data are generally based on monitoring and report shorter periods of time for the activity of the species. For example, Campanaro et al. (2016) reported sightings from the end of May to the end of August and similarly Harvey et al. (2011) gave the following dates: 24 May to 05 August, Sprecher Übersax and Dürrer (1998) in Switzerland observed *L. cervus* between 25 May and 04 July. The volunteers reported high numbers of records between the end of June and early August, with a clear peak in early July. Most authors agree that high numbers of adults of this species can be observed between mid June and the end of July (e.g., Vrezec 2008, Chiari et al. 2014, Campanaro et al. 2016, Scaccini and Anaclerio 2016, Bardiani et al. 2017, Tini et al. 2017) and a peak of activity is in late June or early July (Campanaro et al. 2016). The CS results confirmed the phenology obtained from the ecological studies in Italy (Chiari et al. 2014, Bardiani et al. 2017, Tini et al. 2017) and showed a close correlation between phenology and altitude. In fact, at increasing altitudes, the length of the activity period decreased, whereas the peak of activity shifted forward.

Although *C. cerdo* was recorded between 2 m and 1,147 m a.s.l. by the CS approach, approximately 80% of all observations were made between 0 and 400 m a.s.l. This altitudinal range is similar to that reported for France, where the species was found below the altitude of 900 m a.s.l. (Horák et al. 2010). The data collected by volunteers for the MIPP project showed that *C. cerdo* was active from 18 April to 30 August, while high numbers of this beetle were observed between late May and early July. In the Iberian Peninsula adults are active from early May to late August, but in southeastern Spain, where average temperatures are higher, adult activity spans from February to June (Peris-Felipo et al. 2011). In France, adults are active from June to September (Bensetti and Gaudillat 2002).

Based on data recorded by citizens, the longhorn species *M. asper* was recorded between 2 m and 1,870 m a.s.l. This altitudinal range is in agreement with data reported by Anonymous (2015) and Bologna et al. (2016b), whereas other authors indicated the upper distributional elevation to be lower (Romero-Samper and Bahillo 1993, Bringmann 1996, Vrezec et al. 2009). The data collected by volunteers for the MIPP project showed that *M. asper* was active for most of the year, from 08 January to 29 October. This long period, which covers more than 10 months, is much longer than the activity periods reported generally in literature (López-Vaamonde et al. 1993, Romero-Samper and Bahillo 1993, Bringmann 1996, Drovenik and Pirnat 2003, Polak 2012, Bărbuceanu et al. 2015). Vrezec (2008), who also analysed data collected non-systematically, found that *M. asper* was active for a similar length of time: from 02 February to 30 September. The long activity period observed with the CS approach is in line with the observation that adults of *M. asper* can overwinter (Polak 2012, Rossi de Gasperis et al. 2016). The finding that the peak of activity was observed even later during the year with increasing altitude, while the length of the activity period decreased with increasing altitude, is in keeping with ecological theory but, to the authors' knowledge, no comparable data have been published for *M. asper*. These dates give important indications when planning monitoring fieldwork. However, it is important to note that, at Bosco Fontana (25 m a.s.l.), in the Po river plain of Italy, the peak of activity was observed even earlier during the monitoring activities carried out during the MIPP project, with a peak observed from mid to late April (Hardersen et al. 2017, unpubl. data). In contrast, the CS data indicated the peak of activity on 23 May at an altitude of 0–400 m a.s.l.

The observations of *R. alpina* were reported by volunteers from 3 m to 1,997 m a.s.l. These elevations are very similar to those stated by Lachat et al. (2013) for Europe, where the species is spread from the sea coast to about 2,000 m a.s.l. Other authors reported the upper limit for *R. alpina* to be lower. For example, Bologna et al. (2016c) reported that this species is present from sea level to 1,500 m a.s.l. Similarly, for Switzerland, Duelli and Wermelinger (2005) reported the species for an altitude up to 1,500 m a.s.l. The CS records are from 13 May to 17 September, with large numbers of observations from early July to late August. These dates are very similar to those provided by Vrezec (2008) for Slovenia, which are also based on data non-systematically collected. Here, *R. alpina* was observed between 04 May and 22 September and 50% of observations were concentrated between 14 July and 08 August. This analysis also showed that, for *R. alpina*, the peak of activity was observed later at higher altitudes.

The butterfly *L. achine* was observed between 02 June and 01 August, with most observations from mid-June to early July. This phenology is similar to that reported for the region Veneto (Italy), where the species was most frequently observed between mid-May and early July (Bonato et al. 2014). The volunteers reported observations at altitudes ranging from 179 m to 1,526 m a.s.l., a range similar to that reported for the Veneto region (300–1,430 m a.s.l.) (Bonato et al. 2014) and for the rest of the Italian Alps, where the species has been recorded up to approximately 1,600 m a.s.l. (Villa et al. 2009). Tolman and Lewington 2008 also reported a similar altitudinal range (200–1,500 m a.s.l.).

Volunteers reported sightings of *P. apollo* from 722 m to 2,252 m a.s.l. and this altitudinal range is similar to that reported for Italy (600–2,300 m a.s.l.) (Villa et al. 2009) and in general (500–2,400 m a.s.l.) (Tolman and Lewington 2008). In contrast, the observations for the Veneto region are at slighter lower elevations, from 400 m to 2,000 m a.s.l. (Bonato et al. 2014). The butterfly *P. apollo* was observed from 24 May to 09 September, but high number of observations were madefrom early July to mid-August. This phenology is very similar to that reported for the Veneto region, where adults have been observed from 13 May to 10 September, with a peak in the second half of July (Bonato et al. 2014). The phenology of *P. apollo* changed with increasing altitude and the peak of activity was delayed as altitude increased. The authors are not aware that this trend has been reported before.

The two butterflies *Z. cassandra* and *Z. polyxena* were observed by volunteers between 1 m and 1,482 m a.s.l., with 68% of observations below 400 m a.s.l. These data are in line with observations from the Veneto region, where the species has been recorded from the plains to 1,200 m a.s.l. (Bonato et al. 2014). On the contrary, Tolman and Lewington (2008) reported the occurrence of this species from 0–1,700 m a.s.l., but generally below 900 m a.s.l. In contrast, Villa et al (2009) reported for Italy that the species is present up to approximately 1,000 m a.s.l. For both butterflies, the earliest record was from 09 March (2016) and the latest from 30 June (2014), while high numbers were observed exclusively between early April and early May.

S. pedo was observed by volunteers between 90 m and 860 m a.s.l., but the small number of records (n = 7) did not permit any meaningful analysis.

In all investigated cases, the peak of activity was observed later with increasing altitude and it was delayed by 10 days on average when moving upwards by 400 m. Additionally, for the two cases where the CS MIPP project provided a large dataset (*L. cervus*, *M. asper*), with 604 and 476 records respectively, the length of the activity period decreased with increasing altitude. These indications are important for the monitoring protocols, as they give some indications for the timing and duration of the monitoring periods at different altitudes (Bardiani et al. 2017, Hardersen et al. 2017).

Generally, the data collected non-systematically by volunteers allowed altitudinal distribution and phenology to be analysed, at least for those species for which a minimum number of records had been collected. Similarly, Zapponi et al. (2017) showed that CS projects can provide reliable distributional data for poorly known species of high conservation priority and previously, Vrezec (2008) had successfully used non-systematically collected data to infer the phenology of saproxylic beetles. Additionally, Schmeller et al. (2009) reported that volunteer-based schemes can yield unbiased results for the status of species. However, creating and maintaining a large-scale CS network is a multi-year, tiered process, requiring a great deal of investment in order to enable it to flourish, expand and remain sustainable (Delaney et al. 2008).

Altogether, our results confirm that the CS approach, if based on an adequate effort of dissemination, is a reliable tool for gathering or implementing information on distribution and phenology of rare and protected species for which an extensive knowledge referred to a wide territory (e.g. national scale) can be lacking or incomplete. The main advantage of our CS approach is that data-collection does not start from preconceived assumptions and thus provides data from sites and/or dates from which entomologists would not have expected the occurrence of the species.

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References

Álvarez Laó CM, Álvarez Laó DJ (1995) Análisis de la mortalidad de ciervos volantes *Lucanus cervus* en carreteras asturianas. Boletín de Ciencias de la Naturaleza – Real Instituto De Estudios Asturianos 43: 15–25.

Anonymous (2015) Operativna Programa "OKOLNA Sreda 2007- 2013 G." Proekt "Terenni prouchvaniya na razprostranenie na vidove / otsenka na sŭstoyanieto na vidove i khabitati na teritoriyata na tsyalata strana – I faza": 1–14.

Bărbuceanu D, Niculescu M, Boruz V, Niculescu L, Stoleriu C, Ursu A (2015) Protected saproxylic Coleoptera in "the Forests in the southern part of the Cândeşti Piedmont", a Romanian Natura 2000 Protected Area. Analele Universităţii din Craiova, seria Agricultură Montanologie Cadastru (Annals of the University of Craiova - Agriculture, Montanology, Cadastre Series) 45: 18–25.

Bardiani M, Chiari S, Maurizi E, Tini M, Toni I, Zauli A, Campanaro A, Carpaneto GM, Audisio P (2017) Guidelines for the monitoring of *Lucanus cervus*. In: Carpaneto GM, Audisio P, Bologna MA, Roversi PF, Mason F (Eds) Guidelines for the Monitoring of the Saproxylic Beetles protected in Europe. Nature Conservation 20: 37–78. https://doi.org/10.3897/natureconservation.20.12687

Bensetti F, Gaudillat V (2002) Connaissance et gestion des habitats et des espèces d'intérêt communautaire – Tome 7 : Espèces animales. La documentation Française, Muséum national d'histoire naturelle, Paris, 1–353.

Boakes EH, Gliozzo G, Seymour V, Harvey M, Smith C, Roy DB, Haklay M (2016) Patterns of contribution to citizen science biodiversity projects increase understanding of volunteers' recording behaviour. Scientific reports. 6. https://doi.org/10.1038/srep33051

Bologna MA, Rovelli V, Zapparoli M (2016a) *Osmoderma eremita* (Scopoli, 1763) (Scarabeo eremita), *O. italicum*, *O. cristinae*. In: Stoch F, Genovesi P (Eds) Manuali per il monitoraggio di specie e habitat di interesse comunitario (Direttiva 92/43/CEE) in Italia: specie animali. ISPRA, Serie Manuali e linee guida: 141/2016, Roma, 64–65.

Bologna MA, Rovelli V, Zapparoli M (2016b) *Morimus asper funereus* (Sulzer, 1776) (Morimo funereo). In: Stoch F, Genovesi P (Eds) Manuali per il monitoraggio di specie e habitat di interesse comunitario (Direttiva 92/43/CEE) in Italia: specie animali. ISPRA, Serie Manuali e linee guida: 141/2016, Roma, 76–77.

Bologna MA, Rovelli V, Zapparoli M (2016c) *Rosalia alpina* (Linaeus, 1758) (Rosalia alpina). In: Stoch F, Genovesi P (Eds) Manuali per il monitoraggio di specie e habitat di interesse comunitario (Direttiva 92/43/CEE) in Italia: specie animali. ISPRA, Serie Manuali e linee guida: 141/2016, Roma, 74–75.

Bordogna G, Carrara P, Criscuolo L, Pepe M, Rampini A (2014) A linguistic decision making approach to assess the quality of volunteer geographic information for citizen science. Information Sciences 258: 312–327. https://doi.org/10.1016/j.ins.2013.07.013

Bonato L, Uliana M, Beretta S (2014) Farfalle del Veneto – Atlante distributiv. Regione Veneto, Fondazione Musei Civici di Venezia, Marsilio Editori, Venezia, 1–391.

Bonney R, Cooper CB, Dickinson J, Kelling S, Phillips T, Rosenberg KV, Shirk J (2009) Citizen Science: A Developing Tool for Expanding Science Knowledge and Scientific Literacy. BioScience 59: 977–984. https://doi.org/10.1525/bio.2009.59.11.9

Bringmann HD (1996) Die Morimus- und Acanthoderes-Arten Bulgariens (Col., Cerambycidae). Entomologische Nachrichten und Berichte 40: 237–239.

Campanaro A, Bardiani M, Spada L, Carnevali L, Montalto F, Antonini G, Mason F, Audisio P (2011) Linee guida per il monitoraggio e la conservazione dell'entomofauna saproxilica. Quaderni Conservazione Habitat 6, Cierre Grafica, Verona, 1–8. [+CD-ROM]

Campanaro A, Redolfi De Zan L, Hardersen S, Antonini G, Chiari S, Cini A, Mancini E, Mosconi F, Rossi de Gasperis S, Solano E, Bologna MA, Sabbatini Peverieri G (2017) Guidelines for the monitoring of *Rosalia alpina*. In: Carpaneto GM, Audisio P, Bologna MA, Roversi PF, Mason F (Eds) Guidelines for the Monitoring of the Saproxylic Beetles protected in Europe. Nature Conservation 20: 165–203. https://doi.org/10.3897/natureconservation.20.12728

Campanaro A, Zapponi L, Hardersen S, Méndez M, Al Fulaij N, Audisio P, Bardiani M, Carpaneto GM, Corezzola S, Della Rocca F, Harvey D, Hawes C, Kadej M, Karg J, Rink M, Smolis A, Sprecher E, Thomaes A, Toni I, Vrezec A, Zauli A, Zilioli M, Chiari S (2016) European monitoring protocol for the stag beetle, a saproxylic flagship species. Insect Conservation and Diversity 9: 574–584. https://doi.org/10.1111/icad.12194

Carpaneto GM, Campanaro A, Hardersen S, Audisio P, Bologna MA, Roversi PF, Sabbatini Peverieri G, Mason F (2017) The LIFE Project "Monitoring of insects with public participation" (MIPP): aims, methods and conclusions. In: Carpaneto GM, Audisio P, Bologna MA, Roversi PF, Mason F (Eds) Guidelines for the Monitoring of the Saproxylic Beetles protected in Europe. Nature Conservation 20: 1–35. https://doi.org/10.3897/natureconservation.35.12761

Chiari S, Zauli A, Campanaro A, Donzelli PF, Romiti F, Svensson GP, Tini M, Audisio P, Carpaneto GM (2014) Monitoring presence, abundance and survival probability of the stag beetle, Lucanus cervus, using visual and odour-based capture methods: implications for conservation. Journal of Insect Conservation 18: 99–109. https://doi.org/10.1007/s10841-014-9618-8

Dapporto L (2010) Speciation in Mediterranean refugia and post-glacial expansion of *Zerynthia polyxena* (Lepidotera, Papilionidae). Journal Of Zoological Systematics And Evolutionary Research 48: 229–237.

Davis AK, Dyer LA (2015) Long-term trends in eastern North American monarch butterflies: a collection of studies focusing on spring, summer, and fall dynamics. Annals of the Entomological Society of America 108: 661–663. https://doi.org/10.1093/aesa/sav070

Deguines N, Julliard R, de Flores M, Fontaine C (2012) Te whereabouts of flower visitors: contrasting land-use preferences revealed by a country-wide survey based on citizen science. PLoS ONE 7: 1–9.https://doi.org/10.1371/journal.pone.0045822

Delaney DG, Sperling CD, Adams CS, Leung B (2008) Marine invasive species: validation of citizen science and implications for national monitoring networks. Biological Invasions 10: 117–128. https://doi.org/10.1007/s10530-007-9114-0

Devictor V, Whittaker RJ, Beltrame C (2010) Beyond scarcity: citizen science programmes as useful tools for conservation biogeography. Diversity and Distributions 16: 354–362. https://doi.org/10.1111/j.1472-4642.2009.00615.x

Donnelly A, Crowe O, Regan E, Begley S, Caffarra A (2014) The role of citizen science in monitoring biodiversity in Ireland. International Journal of Biometeorology 58: 1237–1249. https://doi.org/10.1007/s00484-013-0717-0

Drovenik B, Pirnat A (2003) Strokovna izhodišča za vzpostavljaneùje omrežja NATURA 2000 (Hrošči – Coleoptera). Biološki inštitut Jovana Hadžija ZRC SAZU, Ljubljana, 1–88.

Duelli P, Wermelinger B (2005) *Rosalia alpina* L.: Un Cerambicide raro ed emblematico. Sherwood 114: 19–23.

Fox R, Oliver TH, Harrower C, Parsons MS, Thomas CD, Roy DB (2014) Long-term changes to the frequency of occurrence of British moths are consistent with opposing and synergistic effects of climate and land-use changes. Journal of Applied Ecology 51: 949–957.https://doi.org/10.1111/1365-2664.12256

Gardiner MM, Allee LL, Brown PMJ, Losey JE, Roy HE, Smyth RR (2012) Lessons from lady beetles: accuracy of monitoring data from US and UK citizenscience programs. Frontiers in Ecology and the Environment 10: 471–476. https://doi.org/10.1890/110185

Graham EA, Henderson S, Schloss A (2011) Using Mobile Phones to Engage Citizen Scientists in Research. Eos 92: 313–315. https://doi.org/10.1029/2011EO380002

Haklay M (2013) Citizen Science and Volunteered Geographic Information – overview and typology of participation. In: Sui DZ, Elwood S, Goodchild MF (Eds) Crowdsourcing geographic knowledge: Volunteered Geographic Information (VGI) in theory and practice. Springer, Berlin, 105–122. https://doi.org/10.1007/978-94-007-4587-2_7

Hardersen S, Bardiani M, Chiari S, Maura M, Maurizi E, Roversi PF, Mason F, Bologna MA (2017) Guidelines for the monitoring of *Morimus asper funereus* and *Morimus asper asper*. In: Carpaneto GM, Audisio P, Bologna MA, Roversi PF, Mason F (Eds) Guidelines for the Monitoring of the Saproxylic Beetles protected in Europe. Nature Conservation 20: 205–236. https://doi.org/10.3897/natureconservation.20.12676

Harvey DJ, Gange AC, Hawes CJ, Rink M (2011) Bionomics and distribution of the stag beetle, *Lucanus cervus* (L) across Europe. Insect Conservation and Diversity 4: 23–38. https://doi.org/10.1111/j.1752-4598.2010.00107.x

Hochachka WM, Fink D, Hutchinson R, Sheldon D, Wong W-K, Kelling S (2012) Data-intensive science applied to broad-scale citizen science. Trends in Ecology & Evolution 27: 130–137. https://doi.org/10.1016/j.tree.2011.11.006

Howard E, Davis AK (2009) The fall migration flyways of monarch butterflies in eastern North America revealed by citizen scientists. Journal of Insect Conservation 13: 279–286. https://doi.org/10.1007/s10841-008-9169-y

Horák J, Büche B, Dodelin B, Alexander K, Schlaghamersky J, Mason F, Istrate P, Méndez M (2010) *Cerambyx cerdo*. The IUCN Red List of Threatened Species 2010. http://http://www.iucnredlist.org/details/4166/1 [downloaded on 28.02.2016]

Jue DK, Daniels JC (2015) A successful model for citizen scientist involvement in building a statewide at-risk butterfly database. Journal of Insect Conservation 19: 421–431. https://doi.org/10.1007/s10841-014-9733-6

Kadoya T, Ishii HS, Kikuchi R, Suda SI, Washitani I (2009) Using monitoring data gathered by volunteers to predict the potential distribution of the invasive alien bumblebee *Bombus terrestris*. Biological Conservation 142: 1011–1017. https://doi.org/10.1016/j.biocon.2009.01.012

Kobori H, Dickinson JL, Washitani I, Sakurai R, Amano T, Komatsu N, Kitamura W, Takagawa S, Koyama K, Ogawara T, Miller-Rushing AJ (2016) Citizen science: a new approach to advance ecology, education, and conservation. Ecological Research 31: 1–19. https://doi.org/10.1007/s11284-015-1314-y

Kremen C, Ullman KS, Thorp RW (2011) Evaluating the quality of citizen-scientist data on pollinator communities. Conservation Biology 25: 607–617. https://doi.org/10.1111/j.1523-1739.2011.01657.x

Lachat T, Ecker K, Duelli P, Wermelinger B (2013). Population trends of *Rosalia alpina* (L.) in Switzerland: a lasting turnaround?. Journal of Insect Conservation 17: 653–662. https://doi.org/10.1007/s10841-013-9549-9

López-Vaamonde C, Pino-Pérez JJ, Martínez-Fernández A, Fernández Abelleira JC, Devesa-Regueiro S (1993) Contribución al conocimiento de los Cerambycidae Latreille, 1804 de interes forestal (I): *Morimus asper* (Sulz., 1776) en Galicia (Coleóptera, Polyphaga). Congreso Forestal Español, Lourinzán, I. Ponencias y comunicaciones 3: 317–322.

Lovell S, Hamer M, Slotow R, Herbert D (2009) An assessment of the use of volunteers for terrestrial invertebrate biodiversity surveys. Biodiversity and Conservation 18: 3295–3307. https://doi.org/10.1007/s10531-009-9642-2

Mason F, Roversi PF, Audisio P, Bologna MA, Carpaneto GM, Antonini G, Mancini E, Sabbatini Peverieri G, Mosconi F, Solano E, Maurizi E, Maura M, Chiari S, Sabbatelli S, Bardiani M, Toni I, Redolfi De Zan L, Rossi de Gasperis S, Tini M, Cini A, Zauli A, Nigro G, Bottacci A, Hardersen S, Campanaro A (2015) Monitoring of insects with public participation (MIPP; EU LIFE project 11 NAT/IT/000252): overview on a citizen science initiative and a monitoring programme (Insecta: Coleoptera; Lepidoptera; Orthoptera). Fragmenta Entomologica 47: 51–52. https://doi.org/10.4081/fe.2015.134

Matteson KC, Taron DJ, Minor ES (2012) Assessing citizen contributions to butterfly monitoring in two large cities. Conservation Biology 26: 557–564. https://doi.org/10.1111/j.1523-1739.2012.01825.x

Maurizi E, Campanaro A, Chiari S, Maura M, Mosconi F, Sabatelli S, Zauli A, Audisio P, Carpaneto GM (2017) Guidelines for the monitoring of *Osmoderma eremita* and closely related species. In: Carpaneto GM, Audisio P, Bologna MA, Roversi PF, Mason F (Eds) Guidelines for the Monitoring of the Saproxylic Beetles protected in Europe. Nature Conservation 20: 79–128. https://doi.org/10.3897/natureconservation.20.12658

Newman G, Wiggins A, Crall A, Graham E, Newman S, Crowston K (2012) The future of citizen science: emerging technologies and shifting paradigms. Frontiers in Ecology and the Environment 10: 298–304. https://doi.org/10.1890/110294

Oberhauser KS, Prysby MD (2008) Citizen science: creating a research army for conservation. American Entomologist 54: 97–99. https://doi.org/10.1093/ae/54.2.103

Parmesan C, Ryrholm N, Stefanescu C, Hill JK, Thomas CD, Descimon H, Huntley B, Kaila L, Kullberg J, Tammaru T, Tennent WJ, Thomas JA, Warren M (1999) Poleward shifts in geographical ranges of butterfly species associated with regional warming. Nature 399: 579–583. https://doi.org/10.1038/21181

Peris-Felipo FJ, Falcó-Garí JV, Jiménez-Peydró R (2011) The diversity of Cerambycidae in the protected Mediterranean landscape of the Natural Park of Carrascal de La Font Roja, Spain. Bulletin of Insectology 64: 87–92.

Polak S (2012) Phenology and mating behaviour of *Morimus funereus* (Coleoptera, Cerambycidae). In: Maja J (Ed.) Saproxylic beetles in Europe: monitoring, biology and conservation. Studia Forestalia Slovenica 137: 43–52.

Polgar CA, Primack RB, Williams EH, Stichter S, Hitchcock C (2013) Climate effects on the flight period of lycaenid butterflies in Massachusetts. Biological Conservation 160: 25–31. https://doi.org/10.1016/j.biocon.2012.12.024

Raddick JM, Bracey G, Gay PL, Lintott CJ, Murray P, Schawinsky K, Szalay AS, Vandenberg J (2010) Galaxy Zoo: exploring the motivations of citizen science volunteers. Astronomy Education Review 9:1 https://doi.org/10.3847/AER2009036

Ranius T, Aguado LO, Antonsson K, Audisio P, Ballerio A, Carpaneto GM, Chobot K, Gjurašin B, Hanssen O, Huijbregts H, Lakatos F, Martin O, Neculiseanu Z, Nikitsky NB, Paill W, Pirnat A, Rizun V, Ruicănescu A, Stegner J, Süda I, Szwałko P, Tamutis V, Telnov D, Tsinkevich V, Versteirt V, Vignon V, Vögeli M, Zach P (2005) *Osmoderma eremita* (Coleoptera, Scarabaeidae, Cetoniinae) in Europe. Animal Biodiversity and Conservation 28: 1–44.

Ratnieks FLW, Schrell F, Sheppard RC, Brown E, Bristow OE, Garbuzov M (2016) Data reliability in citizen science: learning curve and the effects of training method, volunteer background and experience on identification accuracy of insects visiting ivy flowers. Methods in Ecology and Evolution 10: 1226–1235. https://doi.org/10.1111/2041-210X.12581

Redolfi De Zan L, Bardiani M, Antonini G, Campanaro A, Chiari S, Mancini E, Maura M, Sabatelli S, Solano E, Zauli A, Sabbatini Peverieri G, Roversi PF (2017) Guidelines for the monitoring of *Cerambyx cerdo*. In: Carpaneto GM, Audisio P, Bologna MA, Roversi PF, Mason F (Eds) Guidelines for the Monitoring of the Saproxylic Beetles protected in Europe. Nature Conservation 20: 129–164. https://doi.org/10.3897/natureconservation.20.12703

Romero-Samper J, Bahillo P (1993) Algunas observaciones sobre la distribución y biología de *Morimus asper* (Sulzer, 1776) (Coleoptera: Cerambycidae) en la Península Ibérica. Boletin de la Asociacion Española de Entomologia 17: 103–122.

Rossi de Gasperis S, Passacantilli C, Redolfi De Zan L, Carpaneto GM (2016) Overwintering ability and habitat preference of *Morimus asper*: a two-year mark-recapture study with implications for conservation and forest management. Journal of Insect Conservation 20: 821–835. https://doi.org/10.1007/s10841-016-9913-7

Scaccini D, Anaclerio M (2016) *Lucanus cervus* (Coleoptera: Lucanidae) sampling during its flight period in Northern Italy: data from an ongoing survey in an oak forest. Bulletin van de Koninklijke Belgische Vereniging voor Entomologie 152: 163–167.

Schaffrath U (2003) Zur Lebensweise, Verbreitung und Gefährdung von *Osmoderma eremita* (Scopoli, 1763) (Coleoptera; Scarabaeoidea, Cetonidae, Trichiinae). Philippia 10: 157–248.

Schmeller DS, Henry PY, Julliard R, Gruber B, Clobert J, Dziock F, Lengyel S, Nowicki P, Deri E, Budrys E, Kull T, Tali K, Bauch B, Settele J, Van Swaay C, Kobler A, Babij V, Papastergiadou E, Henle K (2009) Advantages of volunteer-based biodiversity monitoring in Europe. Conservation Biology 23: 307–316. https://doi.org/10.1111/j.1523-1739.2008.01125.x

Solano E, Mancini M, Ciucci P, Mason F, Audisio P, Antonini G (2013) The EU protected taxon *Morimus funereus* Mulsant, 1862 (Coleoptera: Cerambycidae) and its western Palaearctic allies: systematics and conservation outcomes. Conservation Genetics 14: 683–69. https://doi.org/10.1007/s10592-013-0461-3

Sprecher-Übersax E, Dürrer H (1998) Über das Vorkommen des Hirschkäfers (Lucanus cervus L) in der Region Basel. Mitteilungen der Entomologischen Gesellschaft Basel 48: 142–166.

Tauzin P (1994) Le genre Osmoderma Le Peletier et Audinet-Serville 1828 (Coleopt., Cetoniidae, Trichiinae, Osmodermatini): systématique, biologie et distribution. Première partie. L'Entomologiste 50: 195–214.

Teacher AGF, Griffiths DJ, Hodgson DJ, Inger R (2013) Smartphones in ecology and evolution: a guide for the app-rehensive. Ecology and Evolution 3: 5268–5278. https://doi.org/10.1002/ece3.888

Theobald EJ, Ettinger AK, Burgess HK, DeBey LB, Schmidt NR, Froehlich HE, Wagner C, HilleRisLambers J, Tewksbury J, Harsch M a, Parrish JK (2015) Global change and local solutions: tapping the unrealized potential of citizen science for biodiversity research. Biological Conservation 181: 236–244. https://doi.org/10.1016/j.biocon.2014.10.021

Tini M, Bardiani M, Chiari S, Campanaro A, Maurizi E, Toni I, Mason F, Audisio P, Carpaneto GM (in press) Use of space and dispersal ability of a flagship saproxylic insect: a telemetric study of the stag beetle (*Lucanus cervus*) in a relict lowland forest. Insect Conservation and Diversity.

Tolman T, Lewington R (2008) Collins Butterfly Guide, Harper Collins Publishers, London, 1–384.

Toomey AH, Domroese MC (2013) Can citizen science lead to positive conservation attitudes and behaviors? Human Ecology Review 20: 50–62.

Tulloch AIT, Possingham HP, Joseph LN, Szabo J, Martin TG (2013) Realising the full potential of citizen science monitoring programs. Biological Conservation 165: 128–138. https://doi.org/10.1016/j.biocon.2013.05.025

Van Swaay C, Van Strien A, Harpke A (2013) The European grassland butterfly indicator: 1990–2011. European Environment Agency, Luxemburg, 1-–4. https://doi.org/10.2800/89760

Van Swaay CAM, Nowicki P, Settele J, Van Strien AJ (2008) Butterfly monitoring in Europe: methods, applications and perspectives. Biodiversity and Conservation 17: 3455–3469. https://doi.org/10.1007/s10531-008-9491-4

Villa R, Pellecchia M, Pesce GB (2009) Farfalle d'Italia. Istituto per i Beni Artistici Culturali e Naturali della Regione Emilia-Romangna, Editirice Compositori, Bologna, 1–375.

Vrezec A (2008) Fenološka ocena pojavljanja imagov štirih vrst varstveno pomembnih saproksilnih hroščev v sloveniji: *Lucanus cervus*, *Cerambyx cerdo*, *Rosalia alpina*, *Morinus funereus* (Coleoptera: Lucanidae, Cerambycidae). Acta Entomologica Slovenica 16: 117–126.

Vrezec A, Ambrožič Š, Polak S, Pirnat A, Kapla A, Denac D (2009) Izvajanje spremljanja stanja populacij izbranih ciljnih vrst hroščev v letu 2008 in 2009 in zasnova spremljanja stanja populacij izbranih ciljnih vrst hroščev. *Carabus variolosus*, *Leptodirus hochenwartii*, *Lucanus cervus*, *Morimus funereus*, *Rosalia alpina*, *Bolbelasmus unicornis*, *Stephanopachys substriatus*, *Cucujus cinnaberinus*, *Rhysodes sulcatus*. Nacionalni inštitut za biologijo, Ljubljana, 1–174.

Warren MS, Hill JK, Thomas JA, Asher J, Fox R, Huntley B, Roy DB, Telfer MG, Jeffcoate S, Harding P, Jeffcoate G, Willis SG, Greatorex-Davies JNN, Moss D, Thomas CD (2001) Rapid responses of British butterflies to opposing forces of climate and habitat change. Nature 414: 65–69. https://doi.org/10.1038/35102054

Washitani I, Yoshioka A, Suda S, Yasukawa M, Kituregawa U (2013) Status of Pseudozizeeria maha revealed by participatory monitoring on butterfly in Tokyo. Iwanami Kagaku 83: 961–996. https://doi.org/10.1890/ES14-00212.1

Widenfalk LA, Ahrne K, Berggren Å (2014) Using citizen-reported data to predict distributions of two non-native insect species in Sweden. Ecosphere 5: 1–16.

Wiggins A, Crowston K (2011) From conservation to crowdsourcing: a typology of citizen science. Proceedings of the Annual Hawaii International Conference on System Sciences: 1–10. https://doi.org/10.1109/HICSS.2011.207

Wilson JJ, Jisming-See SW, Brandon-Mong GJ, Lim AH, Lim VC, Lee PS, Sing KW (2015) Citizen science: the first peninsular Malaysia butterfly count. Biodiversity Data Journal 3: e7159. https://doi.org/10.3897/BDJ.3.e7159

Zapponi L, Cini A, Bardiani M, Hardersen S, Maura M, Maurizi E, Redolfi De Zan L, Audisio P, Bologna MA, Carpaneto GM, Roversi PF, Sabbatini Peverieri G, Mason F, Campanaro A (2017) Citizen science data as an efficient tool for mapping protected saproxylic beetles. Biological Conservation 208: 139–145. https://doi.org/10.1016/j.biocon.2016.04.035

Zinetti F, Dapporto L, Vovlas A, Chelazzi G, Bonelli S, Balletto E, Ciofi C (2013) When the rule becomes the exception. No evidence of gene flow between two *Zerynthia* cryptic butterflies suggests the emergence of a new model group. PLoS ONE 8(6): e65746. https://doi.org/10.1371/journal.pone.0065746

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Books: Goix N, Klimaszewski J (2007) Catalogue of Aleocharine Rove Beetles of Canada and Alaska. Pensoft Publishers, Sofia-Moscow, 166 pp.

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PhD thesis: Dalebout ML (2002) Species identity, genetic diversity and molecular systematic relationships among the Ziphiidae (beaked whales). PhD thesis, Auckland, New Zealand: University of Auckland.

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