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RESEARCH ARTICLE

The distribution and behaviour studies on a new invasive Buprestid species, *Lamprodila festiva* (Coleoptera: Buprestidae) in Romania

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Abstract
The Cypress Jewel Beetle (*Lamprodila* (*Palmar*) *festiva* subsp. *festiva* (Linnaeus 1767)), is a formerly rare and protected species, present in the red lists of most Western European countries. In Romania, before 2015 it was present only in the South-Western region and less than 10 specimens were collected. The species recently changed its host plants from wild Cupressaceae (*Juniperus sp., Cupressus sp.*) to cultivated Cupressaceae, the most affected one being *Thuja occidentalis*. In 2013 the first big invasion was recorded in Budapest, Hungary. In 2015, the species was found in two horticultural seedling stations in Bucharest. More recently, in 2017, it was recorded in Cluj-Napoca, and in 2018, in Timișoara, Oradea, Suceava. The article provides a short description of the adults, larva and the signs of the attacks on the host plants. The potential distribution of the species is discussed based on climate niche modelling. The daily behaviour of the adults and their preferences, based on observations, is also discussed. Equally, the bioethics related to this attack is analysed.

Keywords
Cypress Jewel Beetle, Cupressaceae, invasive species, Buprestidae, distribution, adult behaviour

Introduction

Lamprodila (*Palmar*) *festiva* is one of the four species of the genus *Lamprodila* in Romania. It belongs to the family Buprestidae (called Metallic Wood Borers or Jewel Beetles).

The species of the genus *Lamprodila* are middle sized beetles (5 to 15mm). The colour is green, green-blue or golden-green with or without copper-red margins and with black or dark-blue spots on the dorsal surface.

The subgenus *Palmar*, which *Lamprodila festiva* is a part of, is characterised by a plumper and more oval body, and by dark spots which exceed the width of the interstriae of the elytra.

There are three closely related taxa in this subgenus: The first is *Lamprodila* (*Palmar*) *festiva* subsp. *festiva* (Linnaeus, 1758), with a European distribution (Volkovitsh and Karpun 2017). It is the single species of the subgenus present in Romania (Nitzu et al. 2016, Ruicănescu 2013). The second is *Lamprodila* (*Palmar*) *festiva* subsp. *holzschuhi* (Hellrigl, 1972), endemic in the Asian part of Turkey (Hellrigl 1972). Another very close taxon is *Lamprodila* (*Palmar*) *cretica* (Zabransky, 1994), endemic in Crete (Volkovitsh and Karpun 2017, Zabransky 1994). All three taxa develop in Cupressaceae, and have a similar habitus and behaviour (Hellrigl 1972, Zabranski 1994, Volkovitsh and Karpun 2017). Their taxonomic status needs revision (Volkovitsh and Karpun 2017).

Lamprodila festiva has a plump oval body, 6–10 mm long, eclectic green, with some blue shimmer (pronotum sometimes a bit with golden shimmer) with dark blue spots on the elytra. The ventral side and the legs are eclectic green. The antennae are dark blue. The male genitalia (aedeagus) is spear-head shaped, dark-brown (Fig. 1, left). The ovipositor is long, soft, membranous, twice-folded, with 2 small appendages in the apical zone (Fig. 1, right). It is entirely hidden in the abdomen when not used.

The larva is typical for the Buprestidae, having the first thoracic segment much larger and flattened, and sclerotized on the both dorsal and ventral surfaces. The abdominal segments are narrow, the 2nd and 3rd thoracic segments make the transition from the 1st larger segment to the narrow abdominal segments. Larval tegument is soft, white-yellowish, a bit darker on the 1st thoracic plate. The head is dark brown, strongly sclerotized. The dorsal thorax surface is covered by microscopic spinules, which make the larva rough and ready for moving forward, because its legs are vestigial. The shape of the spinules is very important in species identification (Volkovitsh 2017). The mature larva measures about 22 mm in length (Fig. 2).

The life cycle is 1–4 years, depending on the temperature and humidity. According to the existing literature, the adults are active in May–July (López-Pérez 2016, Pedersoli 2016). After mating, the female oviposits in the small cracks of the host plant's bark. It does not drill into the bark, because the ovipositor is soft and membranous, with two apical palpators (see the ovipositor, Fig. 1), which are used to check for cracks or crevices in the bark. The larvae mine under the bark and feed

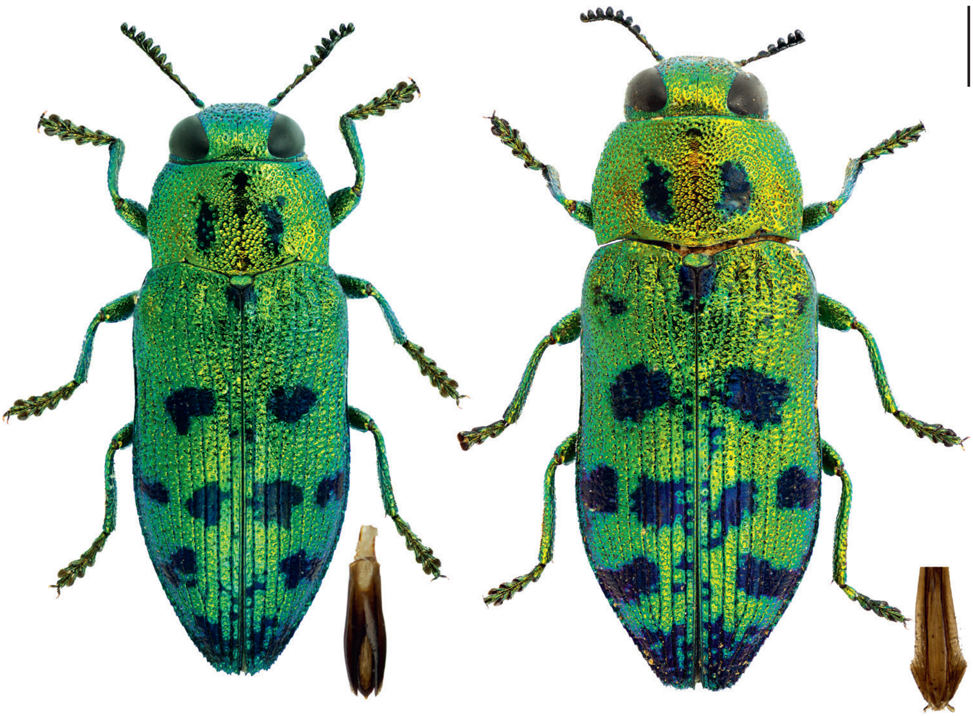

Figure 1. *Lamprodila* (*Palmar*) *festiva festiva* (Linnaeus, 1767) adults: left–male and aedeagus, right–female and ovipositor (Cluj-Napoca, 16.06.2017) (scale bar = 1 mm).

on the cambium, then the sap wood. The tunnels are flat, about 3 times wider than the thickest part of the larva itself, and sinuous. The larvae cut a big amount of xylem vessel, so ten larvae can kill a 7 m tall white cedar. The mature larva digs deeper in the sapwood to make the pupal chamber. The pupa is oriented with the head to the exterior. The emergency holes are "D" or oval shaped and 2–3 millimetres in the long diameter.

Before 2010, the species was known to attack only wild trees from the Cupressaceae family, and had a distribution limited to the surroundings of the Mediterranean sea, and parts of France (Volkovitsh and Karpun 2017).

We were very surprised to see images from Budapest (Hungary) in the summer of 2012, published in social media (Hungarian Natural History Museum 2012), where tens of specimens of *L. festiva* were collected from some white cedars (*Thuja occidentalis*) broken after a summer storm (Németh 2013). This suggested a change in host plants for the species, from wild to cultivated trees, and an expansion towards the north-east.

In Romania, before 2010, less than 10 specimens were, and all of them were from the South-Western part of the country - Baia de Aramă (Mehedinți) (07.1956, 1961, N. Săvulescu leg., stored in Museum Grigore Antipa Bucharest's collection)

Figure 2. *Lamprodila* (*Palmar*) *festiva festiva* (L.) – larva. (Photo Tamás Németh, Hungarian Natural History Museum, Budapest) (scale bar = 1 mm).

and Bistrețu (Mehedinți), (15.07.1966, I. Firu leg., Museum of Oltenia, Craiova's collection) (Ruicănescu 2013, Panin et al. 2015).

After the attack in Hungary, a visual inspection of white cedars in Cluj-Napoca was carried out each summer. For a couple of years, no emergency holes were observed, or anything to prove a Buprestid attack. In 2015 another publication (Nitzu et al. 2016) mentioned an invasion, this time in two ornamental trees breeding nurseries in Bucharest.

In June 2017 one living specimen was finally found in Cluj-Napoca on the ground, after a night rain. Other specimens were observed and collected in the next days. Then emergency holes were also observed. The attacked trees were easy to recognise, symptoms ranging from dried branches or dried canopy to resin leaks and emergency holes on the log and branches (Fig. 3).

Beside the specimens collected in Cluj-Napoca, individuals or other indications of their presence were detected in the following localities: Oradea (9–10.06.2018, B. Mărcuș), Timișoara (3–4.06.2018, F. Prunar), Suceava (28.05.2018) (A. Cazacu & M-L. Duduman), "Ciprian Porumbescu" camping village (Suceava) (11.06.2018) (A. Cazacu), Călimănești (Vâlcea) (3.07.2018, A. Ștefan-Fotin).

The aim of the paper is to investigate the diurnal behaviour of the adult males and females of *Lamprodila festiva*, and the potential distribution area of the species in Romania in the context of the recent expansion of areal and change of host trees for this species.

Material and methods

Local invasion assessment (*Cluj-Napoca*)

The host trees and shrubs within Cluj-Napoca were investigated for specimens of *Lamprodila festiva*. We started the study in June 2017, when the first adult of the *L. festiva* was observed and collected in Cluj-Napoca (12.06.2017). GPS coordinates were collected for each *L. festiva* occurrence, as well as for occurrences of host trees. The spatial information was centralized in GIS software (ArcGIS, ESRI 2011), which was used to create a map with the distribution of the species in the city of Cluj-Napoca (Fig. 4).

Behaviour study

In the period of adult phenophase (May to July) the adults' activity was observed and annotated, between 9^{00}–19^{00}.

Figure 3. Signs of the development of *Lamprodila festiva*: left–weak and partially dry canopy; right–leak of resin (current larval activity) and emergency holes (adults already emerged).

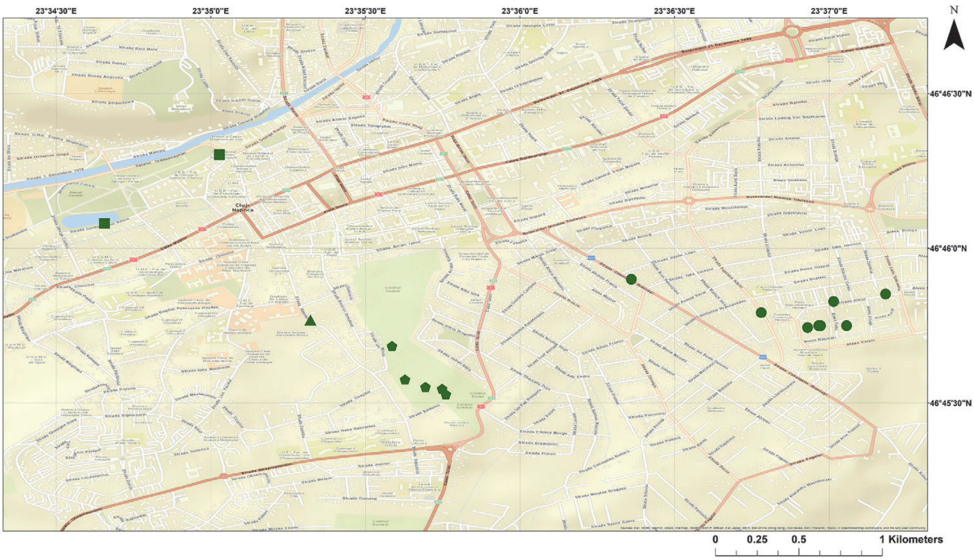

Figure 4. The diagram representation of the presence of the cypress jewel beetles in the studied areas in Cluj-Napoca: green square – Central Park; green pentagon – Central Cemetery; green triangle – Botanical Garden area; green circle – Gheorgheni.

For the diurnal behaviour study, two areas were selected: The Central Cemetery (Hajongard) and the Public Garden Mercur-Detunata (Gheorgheni).

Both areas fulfil the two requirements:

1–They are public areas, so they can be accessed without distressing the owners;

2–The density of *Lamprodila festiva* is high enough for a study.

Concurrently, a number of living adults were kept alive with branches of *Thuja occidentalis*, and brought back to the laboratory. These specimens were bred in the laboratory, allowing detailed observations of behaviour (observations which cannot be done in the field, such as eating, resting).

Species distribution model

Given the recent expansion in the territory occupied by the species, we have simulated the current climate niche of *Lamprodila festiva*, in order to assess the degree of suitability of the new localities from a climatic point of view. Localities from 11 European countries were obtained from foreign partners (a total of 105 points with GPS coordinates). In addition to these, 246 localities were introduced from the GBIF database (accessed in September the 12th, 2018).

Models were fitted at 10 arcseconds (about 20 km), using just climate variables. This large-scale approach allowed the use of more points (e.g. points up to 20 km location error), and thus a more complete distribution of the species. It also allowed the removal of species locations which were close to one another (if multiple points were within the same 20/20 km grid, they were considered as one point, reducing the sampling bias induced by oversampling in certain areas). Therefore, of the 351 locations introduced initially, just 177 remain to be used in the calibration of models (presences represented as red dots in Fig. 5).

At low resolution, species distributions are mostly determined by climate variables, with variables such as topography, geology, soil being less important (Guisan and Zimmermann 2000). The climate variables used to build the models are from the *bioclim* dataset (Hijmans et al. 2005).

In order to include only the most relevant variables in the distribution models, the importance of each variable was tested by running univariate generalized linear models, with the variable as predictor, and species presence as the response variable. Goodness of fit for each model was calculated (1-residual variance/null variance) and used to rank variables from most to least important.

After this step, the correlation between variables was calculated using the *corrgram* function (Wright 2013), and a threshold of 0.7 was imposed to avoid collinearity.

Based on importance and non-correlation, three variables were retained: (i) isothermality (bio3), (ii) precipitation seasonality (bio15) and (iii) precipitations of the coldest quarter (bio19).

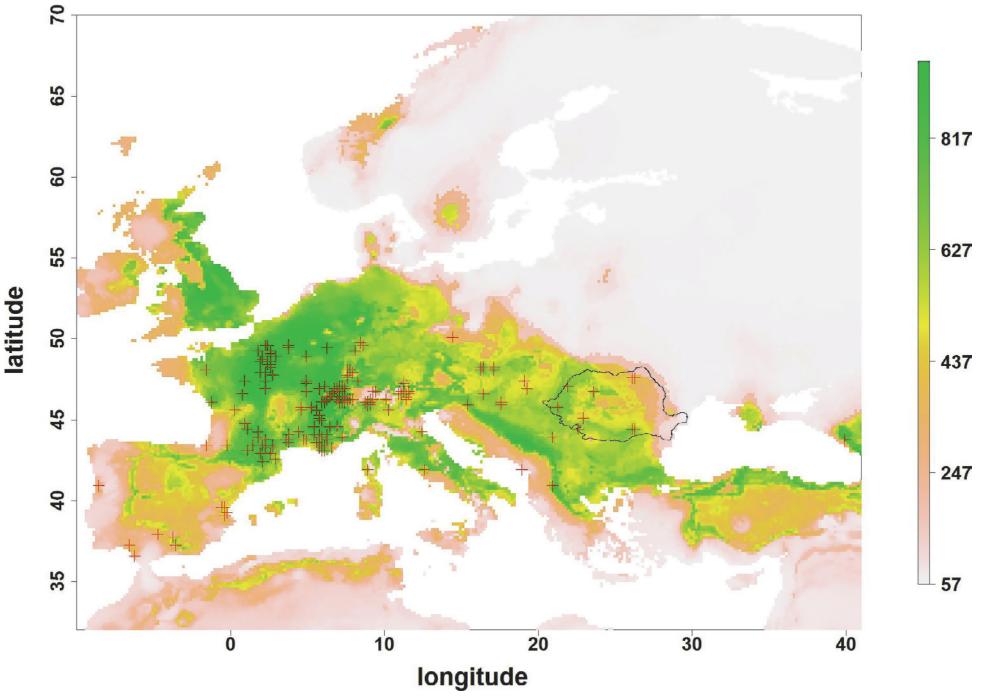

Figure 5. Climate niche suitability (values from 0 – not suitable to 1000 optimal) and known occurrences of *Lamprodila festiva* in Europe (red dots).

Models were fitted at 10 arcseconds (cca. 20 km) using the *biomod2* package (Thuiller et al. 2017). Aside from the species presence dataset, a high number of pseudo-absences was generated (2000 localities, 10 times, using a random strategy). Prevalence has been set to 0.5 (equal weight to absences and presences) (Lomba et al. 2010). 10 evaluation runs were executed, each with an 80/20 split in training and testing. 9 modelling techniques were used (GLM – Generalized Linear Models, GAM – Generalized Additive Models, SRE – Surface Range Envelop, CTA – Classification Tree Analysis, RF – Random Forests, MARS – Multiple Adaptive Regression Splines, FDA – Flexible Discriminant Analysis, ANN – Artificial Neural Networks, GBM – Generalized Boosting Model). As a result, for each raster cell, a number of 900 models were applied (9 modeling techniques x 10 runs (80–20 splits) x 10 Pseudo-Absences generated). An AUC of 0.7 was set as cutoff value (models below the threshold are not used to build the ensemble models).

Results

Local invasion assessment (*Cluj-Napoca*)

The attack concentrated on four main areas in the city: Gheorgheni neighbourhood, "Alexandru Borza" Botanical Garden and surrondings, "Simion Bărnuțiu" Central Park, and the central cemetery "Hajongard".

During our study, we collected 52 adult specimens (11 in 2017 and 41 in 2018). We investigated 215 white cedar trees, 178 of them showed tracks of attack and 7 trees were removed by the local authorities because they were had dried out. The period of adult emergence in Cluj-Napoca was 24.05–21.06.2018.

In Cluj-Napoca, the peak occurrence was observed in the neighbourhood of Gheorgheni, especially between the buildings, where about 8 white cedars of 10 were attacked, but also in the walking sides, where only 6 of 10 trees presented symptoms of attack. In the Central Cemetery, in the lower part (old cemetery) we observed no presence of *L. festiva*, but going to the upper (newer) cemetery the presences became more and more significant.

Inside the perimeter of the Botanical Garden, we observed no presence of the *L. festiva*. But we have observed more or less severe attacks outside the Botanical Garden (Republicii Street, Institute of Psychology and the Oncologic Institute "Iuliu Hațieganu").

In the Central Park area, symptoms were visible only on the trees planted close to the asphalted alleys or concrete paved roads (Fig. 4).

Behaviour study

In the Public Garden Mercur-Detunata and Central Cemetery the daily behaviour of the adults of CJB could be observed (35 observations).

The adults start their activity at about 8^{00}–9^{00} in the canopies of the trees, where they stay on the top side of the branches. They eat the *Thuja* leaves, or the tip of the newly grown branches (personal observations).

In the period 10^{00}–13^{00}, they are in the maximum activity, so they do sunbath and mating, obviously in the top of the canopies, so they are hard to be observed, being acrodendric. In this period, the stems are more or less exposed to the sun (personal observations).

In the period 13^{00}–15^{00}, the sun starts to lower, the males are still in the maximum activity, flying over their territories and looking for females. But the fertilised females are flying in the lower part of the canopy, to prepare to oviposit (personal observations).

In the period 15^{00}–18^{00}, the females descend on the stems and oviposit. The males guard the canopy, flying up and down. After 18^{30}–19^{00}, the activity stops, the insects going in the upper part of the canopy and preparing for rest (personal observations).

Species distribution modelling

Model performance measured by ROC was above 0.75 for all modelling techniques used (Table 1).

Of the 3 variables included, the most important for most models (7 of 9 techniques) was bio 3 (*isothermality*). For 2 of the 9 modeling techniques (RF and FDA), the most important variable was bio 19 (*precipitation of coldest quarter*) – Table 2.

The ensemble climate model for the species considers most of the continental part of Europe as suitable from a climatic point of view (Fig. 5). The models indicate most of Portugal and Spain as having lower probability of occurrence. This is also true for the Balkan coast of the Adriatic and higher altitudes of the Alps, as well as for the larger part of Russia. In Romania, the region of Moldova appears to represent the eastern climatic limit for the species distribution.

Table 1. Model performance measured by ROC (Area under the curve)

Model	ROC
GLM	0.85463
GAM	0.87172
SRE	0.77392
CTA	0.82715
RF	0.89347
MARS	0.8745
FDA	0.86142
ANN	0.85573
GBM	0.89332

Table 2. Variable importance by modelling technique (scale from 0 – not important, to 1 – very important)

variable	GLM	GAM	SRE	CTA	RF	MARS	FDA	ANN	GBM
bio_3	0.81419	0.76844	0.81798	0.70254	0.44743	0.74059	0.0917	0.67013	0.63605
bio_15	0.18984	0.20438	0.17389	0.33263	0.49089	0.18158	0.71102	0.35549	0.23639
bio_19	0.02142	0.17164	0.22809	0.32804	0.41742	0.1465	0.06321	0.44761	0.26426

Discussions

After observing a number of about 60 females, all have landed on pruned stems, with no moss, lichens or other climbing plants (*Hedera helix, Parthenocissus quinquefolia*). Also, solitary trees were observed to be preferred instead of grouped trees. The females are looking often for the limit between the sun exposed part and the shadowed part. Interesting, but the limewashed stems are preferred (Fig. 6), because the limestone is hydrofuge and the females are looking for dry surfaces. The reason for this is the vital, but very unstable relationship, between the buprestid's larvae and the fungi. The larva consumes cambium, but it cannot decompose the cellulose and lignin by itself. So, it needs some fungi for doing this, but where there are useful

Figure 6. Aspects of the behaviour of *Lamprodila festiva*: left–male lurking for mate on the branches of the white cedar; right–female ovipositing on the stem. After our observation, they even prefer the lime painted stems.

fungi, there are dangerous ones too. In our field observations, we have seen adults trying to emerge from their pupal chambers, being killed by fungi (Fig. 7).

Also, in captivity, a number of specimens were killed by *Aspergillus* fungi (Fig. 8).

Because the trees are public or private property, we could not peel the bark in search for larvae, but in Jendek et al. (2018), there is a photo of a dead larva in its tunnel (Fig. 2A).

We assume the fungus is the reason why the larvae make the sinuous and horizontal tunnels under the bark, which cause the damage of the vascular system of the trees. The individuals of the species look for the optimal temperature and humidity, in order to avoid the fungus attack. These conditions vary due to the direction of the sunrays on the bark.

In regards to its overall distribution, *Lamprodila festiva*, is a Circum-Mediterranean element, being formerly recorded in Southern and South-Central Europe and Northern Africa. The main variable considered responsible for the species climate niche according to the model is *isothermality* (a measure of temperature variability). *Isothermality* has lower values in the continental part of Europe (Russia) and in the Scandinavian Peninsula, as in cool and dry climates temperature variation is higher. *Isothermality* is higher in the immediate vicinity of large water bodies in warm climate, where temperature variation is reduced. The second variable, *precipitation variability*, has higher values in the extreme south of the European continent

Figure 7. Adult of *Lamprodila festiva* killed by fungi while tried to emerge the pupal chamber.

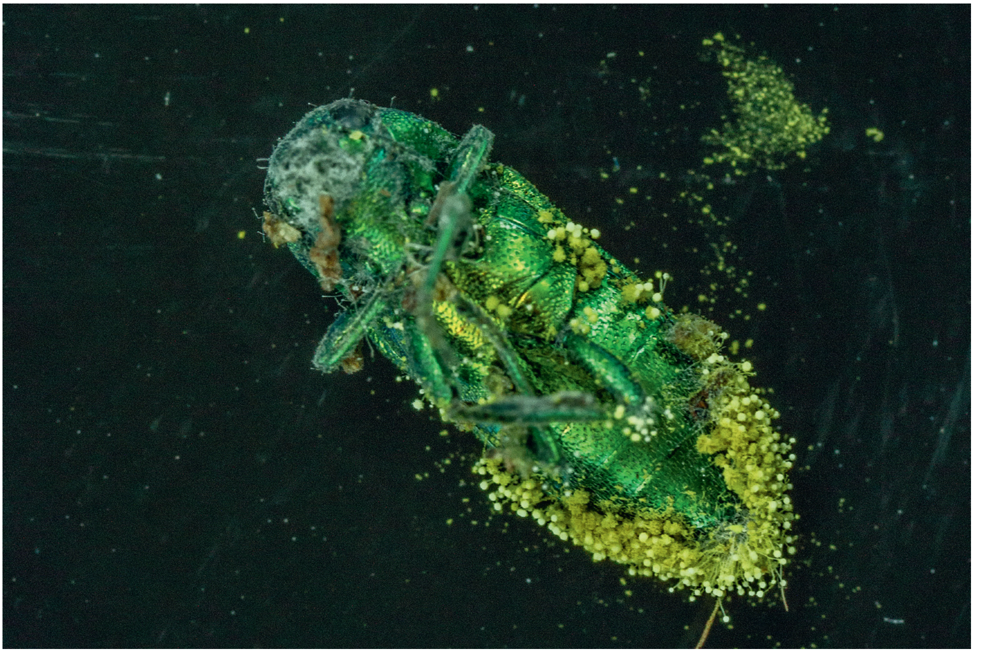

Figure 8. Adult of *Lamprodila festiva* invaded by *Aspergillus* fungi.

(southern parts of Spain, Italy, Greece), on the northern shores of the African continent and in the Middle East (Israel, Lebanon, Syria), corresponding to areas where precipitation falls in an irregular manner during the month (high standard deviation compared to monthly mean of precipitation).

Lamprodila festiva occurs in areas with intermediate values for *isothermality*. It appears to dislike a too continental climate, with high variation of temperature (low *isothermality*), but also climates with little variation of temperatures (high *isothermality*). The areas with high *isothermality* are also very often areas where precipitation of the coldest quarter is higher (areas near the coasts), and where precipitation variability is higher (dry periods alternating with storms).

Some of the newly discovered localities from Romania (Timișoara, Oradea, Călimănești) fitted well in the potential climate niche predicted by the models. Suceava however, appears to be a locality towards the limit of the climatic niche of the species. *Lamprodila festiva* was probably distributed in Suceava together with *Thuja* seedlings which were already populated with eggs or larvae. We doubt about the possibility of installing a permanent population of CJB in Suceava, because of the continental climate, with harsh winters and high temperature variability.

The climate niche models are only partially relevant, as the species distribution is also limited by the distribution of host plant species from the Cupressaceae family, and by cellulose and lignin decomposing fungi, as well as by other factors (i.e. competition, facilitation, etc). The full understanding of the species niche is not attempted here, and the maps should be regarded as potential climate niche based on the distribution of the species so far.

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