# Habitat characterization of two *Pinguicula* species (Lentibulariaceae) in the western Alps

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**Background and aims** – The habitat conditions and population characteristics of the Italian members of the genus *Pinguicula* are presently still scarcely known. In this study we carried out a comparative research on two butterwort species occurring in the same mountain area, but very different in their distribution, the endemic of the western Alps *Pinguicula arvetii* and the circumboreal *P. vulgaris*.

**Methods** – We sampled 36 stands of *P. arvetii* and 29 stands of *P. vulgaris* with squared plots of 0.25 m². In each plot we collected data on geology, topography, hydrochemistry and soil, made a complete inventory of plant species, used for calculating the Ellenberg indicator values, and assessed population density and proportion of flowering individuals. Moreover, we calculated indices of size of vegetative and reproductive structures. We compared the environmental characteristics of sites occupied by the two target species and performed ordinations to assess differences in habitat conditions and to analyse the relationships between characteristics of the two butterworts along environmental gradients.

**Key results** – The two butterworts showed significant differences in site elevation and in most of the ecological indicator values. Consistently, the analyses of species composition of stands revealed that the endemic *P. arvetii* occurred more commonly at higher-elevation habitats, characterized by a greater incidence of species typical of snowbeds, screes and sandy and skeletal poor grasslands. Individual and population characteristics differed in their position along the ordination axes, with a negative relationship between population density and proportion of flowering individual in both the *Pinguicula* species.

**Conclusions** – The main environmental variable discriminating sites occupied by the two butterworts was the elevation. Growth and reproductive performances of the two *Pinguicula* species were influenced by site conditions, but environmental gradients differently affected individual and population characteristics of the two target species.

**Key words** – Ecological indicator values, Flower size, Habitat conditions, *Pinguicula arvetii*, *Pinguicula vulgaris*, Rosette size.

## INTRODUCTION

The co-occurrence within the same genus of species having restricted geographic range (endemic species) and others having widespread distributions has long been an intriguing topic for ecologists and evolutionary biologists (Brown et al. 1996, Webb & Gaston 2003). Several authors suggested that endemic plant species may differ from their widespread congeners in terms of habitat and resource-use features (Kessler 2000, Ojeda et al. 2001), but only Lavergne et al. (2004) provided strong evidence for ecological differentiation among the two groups of species through a comparative analysis of ecological and biological traits in twenty congeneric pairs of

endemic and widespread plants. Their study was focused on the French Mediterranean flora, whereas, to our knowledge, nobody has hitherto undertaken an analogous research on the flora of the Alps, as much rich in endemic species (Aeschimann et al. 2004). In this perspective, we tried to compare the habitat conditions of two species with different distribution range belonging to the genus *Pinguicula*. All members of the genus are passive animal trappers (Heslop-Harrison 1978) and their carnivory is currently interpreted as an adaptive strategy to unfavourable conditions occurring in habitats characterized by nutrient poor soils (Aldenius et al. 1983, Adamec 1997). Nevertheless, the root nutrient uptake from

the soil is not so negligible in the butterworts and is restricted by other factors than the presence of available nutrients, such as low temperature, low pH and others (Güsewell & Koerselman 2002). Consequently, variation in environmental conditions can influence trait performances of these carnivorous plants, providing a possible ecological hub for differentiation in habitat use of congeneric species.

*Pinguicula* L. constitutes the second most diverse genus within the Lentibulariaceae family coming after *Utricularia*. It consists of about 100 currently accepted species (Degtjareva et al. 2006, Rodondi et al. 2010), many of them distributed in arctic, alpine, temperate or Mediterranean regions of the Northern Hemisphere (Legendre 2000, Cieslak et al. 2005). The latest monograph of the genus *Pinguicula* was published by Casper (1966). Six years later the same author contributed accounts of the genus for Flora Europaea (Casper 1972). Nevertheless, both contributions deserve to be updated because several new species, which had surprisingly remained undiscovered in many world places including Europe, were meanwhile described. In particular, several of these new entries in the genus were reported from Italy (Tammaro & Pace 1987, Casper & Steiger 2001, Conti & Peruzzi 2006, Ansaldi & Casper 2009, Peruzzi & Gestri 2013, Innangi & Izzo 2014, Bacchetta et al. 2014). Moreover, the list of species of *Pinguicula* occurring on the Italian mountains was recently enriched by *Pinguicula arvetii* Genty, which was not taken into account by Casper (1966, 1972) and which was previously reported only from the French Alps by Aeschimann et al. (2004) and Legendre et al. (2014). This species was also recently documented for the Italian side of the western Alps by several authors as reported in Zaccara Bertolini et al. (2013). In conclusion, the genus *Pinguicula* is presently represented in Italy by fifteen species, eight of them occurring on the Italian Alps. Among the latter, two result endemic of the Alps (*P. arvetii* and *P. poldinii*) (Aeschimann et al. 2004).

Such unexpected results of the recent efforts in the taxonomic studies of the Italian members of *Pinguicula* can be explained considering that the new species are in most cases separated only by small differences in flower morphology from the closest species already known (see Conti & Peruzzi 2006, Peruzzi & Gestri 2013). It is enough for understanding why they remained so long undiscovered and, consequently, their habitat preferences, ecology and population dynamics are practically unknown.

On the other hand, the habitat requirements of the *Pinguicula* species described since a longer time and, therefore, better known and more easily distinguishable in the field, are equally not clearly defined or somewhat controversial. Indeed, the ecological indicator values reported by Ellenberg et al. (1992), Borhidi (1995), Pignatti et al. (2005), and Landolt et al. (2010) for two species of the genus, namely *P. vulgaris* and *P. leptoceras*, are not always concordant.

The accumulating volume of taxonomic and chorological data on the Italian members of *Pinguicula* and the contrasting scarcity of information on their autoecology stimulated us to investigate the habitat conditions of some species belonging to this genus. Consequently, we carried out a comparative ecological research on two target species, whose general distribution partially overlaps in the western Alps, where they

share quite similar habitats, so that the possible differences in their habitat use would not be so obvious. According to Lavergne et al. (2004) we examined whether habitat and vegetative and reproductive traits differ between an endemic species and its widespread congener. As endemic species we chose *Pinguicula arvetii*, a species of possible hybrid origin from a cross between *P. leptoceras* and *P. reichenbachiana* (Legendre et al. 2014), whose distribution is restricted to the western Alps. The selected widespread congener was *Pinguicula vulgaris*, a well-known species having a circumboreal distribution. In our experience, only this couple of *Pinguicula* species could be suitable for this kind of research in the Alps. The two target species are relatively widespread in the suitable habitats of the studied area where they can also cohabit at the same sites.

We addressed our aims by focusing on three key questions:
(1) Are the habitat conditions of the two species of *Pinguicula* under study different?
(2) If yes, which are the abiotic and biotic variables characterizing these different habitats?
(3) Do vegetative and reproductive features of the two butterwort species vary in relation to habitat conditions?

## MATERIALS AND METHODS

### Study area

The comparative analysis of the site ecology of the two target species was performed within a study area corresponding to the distribution range of *Pinguicula arvetii*, which is restricted to the western Alps (44°30′–45°30′N 7°00′–7°30′E, approximately). This area includes, from South to North, a number of different valleys on the Italian side of Cottian and Graian Alps (Varaita, Po, Sangone, Susa, Viù, Soana valleys), all lying in the Piedmont region, to which we added the valley of Guil on the French side of the Cottian Alps, from where *P. arvetii* was primarily reported by Arvet-Touvet (1873) (fig. 1).

The geology of the study area is characterized by the metamorphic rocks of the Pennidic domain. The rocks belonging to the "Calcescisti e Pietre Verdi" complex are largely predominant. "Calcescisti" (calcareous schists) include micaceous calcareous schists and limestones, whereas the "Pietre Verdi" (ophiolites) include metamorphic mafic and ultramafic bedrocks such as prasinites, metagabbros and serpentinites.

The climate in the valleys of the study area is mostly differentiated by the total precipitation varying from about 800 to 1500 mm per year (De Biaggi et al. 1990). The Soana, Viù and Sangone valleys are more humid with an average annual rainfall between 1200 and 1500 mm. The high Po valley is a bit less humid, with an average annual rainfall of about 1100 mm, whereas the middle Susa valley and the high Varaita and Guil valleys receive less than 1000 mm of precipitation yearly. Temperature data of the period 1951–2010 for the altitudinal range from 1000 to 2500 m a.s.l. (where *Pinguicula* populations occurred), derived from the closest long-term weather stations and calculated according to the methodology proposed by Regione Piemonte (1980), indicate
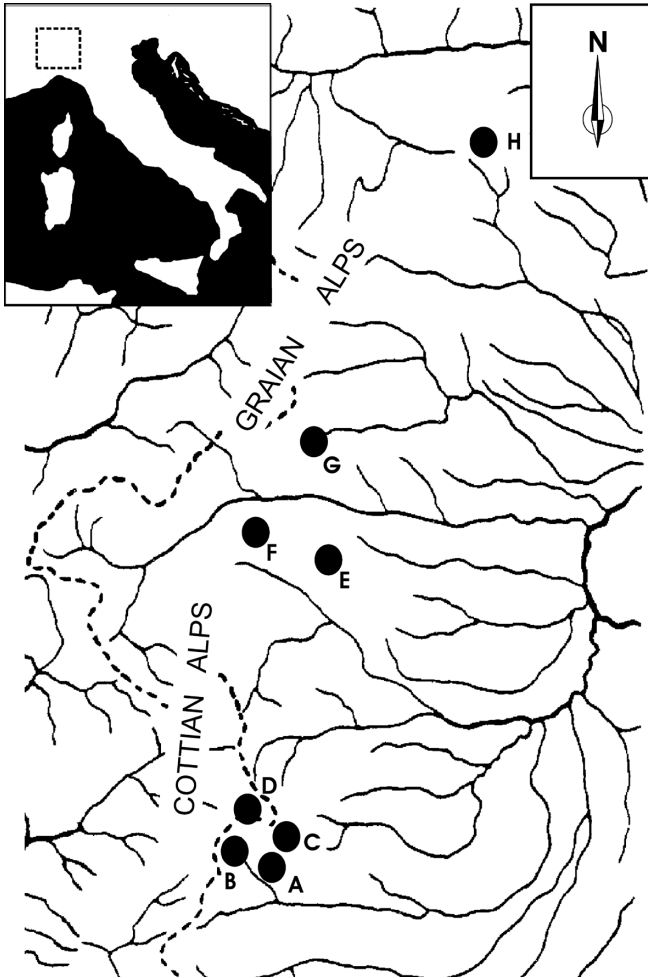
**Figure 1** – Schematic map of the study area. A: Varaita Valley (Vallone di Vallanta – Italy); B: Varaita Valley (close to Colle dell'Agnello – Italy); C: Po Valley (Italy); D: Guil Valley (France); E: Sangone Valley (Italy); F: Susa Valley (Italy); G: Viù Valley (Italy); H: Soana Valley (Italy).

a mean annual temperature from about 1 to 9°C. The coldest month is January (average temperatures comprised between 0 and -5°C) and the warmest one is July (average temperatures comprised between 15 and 25°C).

**Data collection**

In summer 2012 and 2013, 36 stands of *Pinguicula arvetii* and 29 stands of *P. vulgaris* were exclusively sampled in the western Alps providing a reasonable cover of the whole study area. The two species could be distinguished from each other in the field evaluating the following morphological features (see electronic appendix 1): *P. vulgaris* has a pink-purple corolla, white at the throat, with lobes of lower lip oblong, divergent, not overlapping or touching, and a cylindric-subulate spur; in *P. arvetii* the corolla is blue with the lobes of lower lips whitish, obovate and overlapping; spur is cylindrical (Casper 1966, 1972, Legendre et al. 2014).

All the data collected in the field were taken within squared plots of 0.25 m² located at sites showing differences

in vegetation composition and/or topographic features, with a minimum distance of about few hundred metres. This plot size was estimated to be optimal in terms of encompassing only the ecologically relevant dimension of the presumed micro-habitat of the target species. For each plot the name of the site was indicated and its location precisely defined also by reporting DMS coordinates (electronic appendix 2), which were measured using a hand-held GPS (GPS map 60 CSx, Garmin, USA).

In order to obtain an environmental characterization of the sites for detecting differences between the two species regarding habitat conditions, we collected data on geology, topography, hydrochemistry and soil in each plot. The litho-type underlying the plots was primarily defined during the field surveys. Subsequently, the lithotypes were grouped into broader categories (limestones, calcareous schists, and mafic and ultramafic rocks). Among the topographic features, elevation, aspect and slope angle were measured with portable altimeter and compass. Aspect angular data were converted into MEXPO unit (Storm 1996), ranging from 0 to 2 for the NE and SW exposure, respectively. In addition, aspect, slope, and latitude data were used for calculating the yearly potential solar radiation received by each plot under a Radiation Index (RI) according to the first equation of McCune & Keon (2002). Water conductivity was measured wherever it was possible (i.e. about 90% of plots) using a portable conductivity meter (HD 9213-R1, Delta Ohm, Italy). Soil samples for chemical analysis were taken from the uppermost horizon from the surface to the depth of 5–10 cm. This is the zone most densely rooted by *Pinguicula*, where the interaction between plants and soil factors is most intensive. Leaves, roots and twigs were removed from the collected soil samples that were then oven-dried at 40°C for a week. Then they were gently crushed in a mortar and passed through a sieve with a mesh size of 1 mm. Soil pH was measured in deionised water 1:2.5 and soil organic matter content was gravimetrically determined by the loss-on-ignition method. Exchangeable calcium (Ca), chromium (Cr), iron (Fe), magnesium (Mg), manganese (Mn), nickel (Ni), potassium (K) and sodium (Na) concentrations were measured by flame atomic absorption spectrometer (Varian AA240FS, Varian, USA). Total phosphorous (P) concentration was determined by the colorimetric method of molybdenum blue using a spectrophotometer (Uvikon 931, Kontron Instruments, UK). Total N concentration was determined by dry combustion with an elemental analyser (EA 1110, Carlo Erba, Italy).

Moreover, to define the floristic assemblage of sites from which to draw inferences on the environmental gradients and habitat characteristics of sites occupied by the two butterworts, a complete inventory of vascular plants and bryophytes was carried out in each plot (electronic appendix 3). First, the percentage cover of each species was visually estimated by its frequency in a grid of 5 cm × 5 cm cells and, subsequently, cover data were transformed according to the following ordinal scale: 1 = 0.1–10%, 2 = 11–20%, 3 = 21–30%, 4 = 31–40%, 5 = 41–50%, 6 = 51–60%, 7 = 61–70%, 8 = 71–80%, 9 = 81–90%, 10 = 91–100%. In addition, for both vascular plants and bryophytes, the total cover was visually estimated. Secondly, a sociological valence corresponding to the phytosociological optimum according to Ae-

schimann et al. (2004) was assigned to each vascular species of the inventory. A sociological valence was also assigned to those bryophytes for which the phytosociological optimum could be derived from literature (Grabherr & Mucina 1993, Gerdol & Tomaselli 1997); to the other bryophytes the ecological valence was assigned based on Dierßen (2001). Finally, the species having coincident phytosociological optima were grouped together and the incidence of the resulting socio-ecological groups (i.e. the total species cover of each group) within each plot was calculated and expressed in percentage.

The nomenclatural and taxonomic source for vascular plants was Aeschimann et al. (2004), except genus *Alchemilla* for which we followed Festi (2000); the names of bryophytes were in accordance with Aleffi et al. (2008).

The difference in the environmental factors between sites occupied by the two target species was also investigated by means of indirect indication based on plant cover data, because in relatively species-poor habitat abundance data may give more reliable results (Diekmann 1995). Ellenberg indicator values (EIVs) of co-occurring species of vascular plants and bryophytes were used for light (L-EIV), temperature (T-EIV), continentality (K-EIV), moisture (F-EIV), soil reaction (R-EIV) and nutrients (N-EIV) (the last not set for bryophytes; Ellenberg et al. 1992). For vascular plants we used the EIVs adapted to the Italian flora by Pignatti et al. (2005), because our study was mostly focused on the Italian Alps. The value of each ecological indicator for each plot was obtained by calculating the weighted average of all values of those species co-occurring in the plot (Diekmann 2003).

In order to evaluate the influence of habitat conditions on growth and reproduction of the two target species, we related the variation of population and individual characteristics of the two *Pinguicula* species to the above-mentioned environmental features. To this aim, the number of individuals (from hereafter called population density) and the proportion of flowering individuals were assessed within each plot. Moreover, on individuals showing reproductive structures we also carried out morphometric measurements of vegetative and reproductive structures. At each plot, at least 25% of total flowering individuals was measured. In the plots with the lowest number of individuals, such percentages corresponded to ten and fifteen individuals for *P. vulgaris* and *P. arvetii*, respectively. On these adult plants, we counted the number of leaves in the rosette and multiplied it by the length of the longest leaf in order to obtain an index of rosette size (see Svensson et al. 1993, Thorén & Karlsson 1998, Méndez & Karlsson 2004). Moreover, we measured the length of the highest flower scape (see Heslop-Harrison 2004) and the front and longitudinal size of the corolla (Rice 2011), whose product was used for calculating an index of flower size.

## Data analysis

Statistical analyses were carried out in R (R Core Team 2015) version 3.2.0.

The occurrence of different lithotype groups in sites where populations of the two target species were found was analysed by means of the Pearson's $\chi^2$ test.

The comparison of direct (i.e. topographical, hydrochemical and soil variables) and indirect (i.e. EIVs) site characteristics between plots of the two target species was performed through the non-parametric Mann-Whitney test. The same statistical test was used to compare the percentage of the socio-ecological species groups within plots occupied by the two butterworts.

The relationships between floristic assemblages and environmental variables were assessed by means of redundancy analysis (RDA), performed on species abundance data standardized by means of Hellinger transformation (Legendre & Gallagher 2001). Collinearity among environmental variables was verified through the variance inflation factor, with a cut-off level of 10 (Borcard et al. 2011). To identify the best subset of explanatory variables, forward selection was performed, applying the second stopping criterion of Blanchet et al. (2008). Then, permutation tests were used to test the significance of global RDA models, canonical axes and explanatory variables.

For each *Pinguicula* species, the variation of individual and population characteristics along environmental gradients was investigated through principal component analysis (PCA).

Post-hoc interpretation of ordinations was performed fitting environmental variables onto PCAs with the function "envfit" of the R "vegan" package (Oksanen et al. 2015), which finds vectors of factor averages of environmental variables. Then, results were visualised in the ordination diagrams incorporating environmental factors whose correlations with the first two axes, assed by permutation tests, had a probability level < 0.1.

## RESULTS

### Comparison of site characteristics of the two *Pinguicula* species

*Pinguicula vulgaris* was found on all the categories of rock types occurring in the study area. The overall occurrence of lithotype groups in the sites where the two species were sampled was significantly different ($\chi^2$ = 18.08, Df = 3, $P$ < 0.001). In *P. vulgaris* plots the frequency of mafic substrata (metagabbros) was largely prevailing (table 1), whereas in those of *P. arvetii* there was a codominance of metagabbros and calcareous schists. When the lithotypes were individually considered, the only one showing a significantly

**Table 1 – Occurrence of different lithotype groups in sites of the two *Pinguicula* species.**
Occurrence is expressed in percentage; for each lithotype group, $\chi^2$, degrees of freedom (Df) and $P$ values from the comparison between sites occupied by the two target species are shown. Significant differences are highlighted in bold.

|  | *P. arvetii* | *P. vulgaris* | $\chi^2$ | Df | *P* |
|---|---|---|---|---|---|
| Calcareous schists | 44.44 | 20.69 | 3.056 | 1 | 0.080 |
| Limestones | 0 | 20.69 | 5.922 | 1 | **0.015** |
| Metagabbros | 33.33 | 41.38 | 0.168 | 1 | 0.682 |
| Serpentinites | 22.22 | 17.24 | 0.035 | 1 | 0.852 |

**Table 2** – **Environmental features of sites occupied by the two target species.**
Values are mean ± 1SD; Z and *P* values from Wilcoxon test are shown. Significant differences are highlighted in bold.

| | *P. arvetii* | *P. vulgaris* | Z | *P* |
|---|---|---|---|---|
| Elevation (m a.s.l.) | 1987 ± 284 | 1534 ± 303 | 4.831 | **< 0.001** |
| Slope (°) | 36.9 ± 20.7 | 36.7 ± 26.2 | 0.324 | 0.746 |
| Aspect (MEXPO) | 1.01 ± 0.58 | 0.88 ± 0.74 | 0.787 | 0.431 |
| Potential radiation (MJ $cm^{-2}$ $y^{-1}$) | 0.744 ± 0.246 | 0.624 ± 0.302 | 1.617 | 0.106 |
| Conductivity ($\mu$S $cm^{-1}$) | 116 ± 111 | 164 ± 176 | −0.577 | 0.564 |
| pH | 6.39 ± 1.09 | 6.54 ± 1.18 | −0.324 | 0.746 |
| Soil organic matter (%) | 11.6 ± 8.8 | 14.9 ± 15.2 | −0.683 | 0.494 |
| N (mg $g^{-1}$) | 3.64 ± 2.40 | 4.61 ± 3.11 | −1.130 | 0.259 |
| P (mg $g^{-1}$) | 0.418 ± 0.233 | 0.375 ± 0.228 | 0.767 | 0.443 |
| K (mg $g^{-1}$) | 1.20 ± 1.01 | 1.74 ± 2.00 | −1.007 | 0.314 |
| Ca (mg $g^{-1}$) | 1.69 ± 1.79 | 2.40 ± 2.44 | −0.896 | 0.370 |
| Mg (mg $g^{-1}$) | 0.115 ± 0.154 | 0.150 ± 0.204 | 0.590 | 0.555 |
| Na (mg $g^{-1}$) | 0.081 ± 0.067 | 0.089 ± 0.065 | −1.715 | 0.086 |
| Fe ($\mu$g $g^{-1}$) | 603 ± 667 | 500 ± 509 | −0.115 | 0.908 |
| Mn ($\mu$g $g^{-1}$) | 93.1 ± 113.7 | 83.4 ± 96.3 | 0.670 | 0.501 |
| Ni ($\mu$g $g^{-1}$) | 14.7 ± 27.9 | 12.0 ± 20.8 | 0.074 | 0.941 |
| Cr ($\mu$g $g^{-1}$) | 0.95 ± 1.18 | 0.97 ± 1.19 | −0.309 | 0.757 |
| N:P ratio | 10.1 ± 6.4 | 14.4 ± 7.3 | −2.428 | **0.015** |
| Ca:Mg ratio | 41.0 ± 60.3 | 49.9 ± 56.5 | −0.840 | 0.401 |
| Vascular plant cover (%) | 68.7 ± 13.7 | 56.3 ± 18.7 | 2.940 | **0.003** |
| Moss cover (%) | 21.7 ± 22.0 | 25.9 ± 23.0 | −0.702 | 0.483 |
| T-EIV | 3.10 ± 0.58 | 3.74 ± 0.83 | −3.342 | **< 0.001** |
| F-EIV | 5.50 ± 0.84 | 6.15 ± 1.00 | −2.604 | **0.009** |
| K-EIV | 4.73 ± 0.36 | 4.50 ± 0.31 | 2.926 | **0.003** |
| R-EIV | 5.39 ± 1.37 | 4.81 ± 1.80 | 1.362 | 0.173 |
| L-EIV | 7.54 ± 0.28 | 7.25 ± 0.58 | 2.484 | **0.013** |

different occurrence between the stands of the target species was limestones on which, on the contrary, *P. arvetii* lacked at all, at least in our dataset.

Both target species had a great elevation range, slightly broader for *P. vulgaris*, which was encountered from 960 m to 2220 m a.s.l., whereas *P. arvetii* was found from 1315 to 2420 m. Moreover, stand elevation of the two species was significantly different (table 2), with *P. vulgaris* quite common in the montane vegetation belt between about 1300 and 1700 m, whereas *P. arvetii* was found most commonly in subalpine stations from about 1850 to 2200 m, occasionally ascending up to the low-alpine vegetation belt. The stands of target species did not significantly differ either in slope angle or aspect and, consequently, either in RI. In addition, no significant differences were found for the hydrochemical variables considered.

Among the chemical soil parameters analysed, N:P ratio was the only one that showed a significant difference between sites occupied by the two species under study, with higher values in the plots of *P. vulgaris* (table 2). This depends on the co-occurrence of higher values of total N and lower values of total P in these plots when compared with those occurring in the plots of *P. arvetii*.

The plots occupied by *P. arvetii* showed a significantly higher cover of co-occurring vascular plants in comparison with those occupied by *P. vulgaris* (table 2).

All the indirect explanatory variables (EIVs) showed a significant difference between plots colonized by the two species under study, except R-EIV (table 2) and N-EIV (Z = 0.28, *P* = 0.777; this index was calculated only from vascular plants and not reported in table 2). T- and F-EIV resulted higher in the plots of *P. vulgaris*, whereas K and L-EIV were significantly higher in those of *P. arvetii*.

**Floristic assemblages and habitat characterization**

RDA showed that the plant species composition among plots was significantly explained by the site conditions ($F_{7,57}$ = 2.34, *P* = 0.001; adjusted $R^2$ = 0.13). In particular, the first four canonical axes resulted significant, explaining 10.3% of the variance in floristic composition, as indicated by the accumulated proportion of constrained eigenvalues (0.81). The best subset of explanatory variables consisted in seven environmental factors. Among these variables,

the elevation of sites was the most significant ($F_{1,57} = 3.19$, $P = 0.001$), followed by soil pH ($F_{1,57} = 2.15$, $P = 0.001$), slope ($F_{1,57} = 1.51$, $P = 0.017$), Mg concentration ($F_{1,57} = 1.41$, $P = 0.037$), N:P ratio ($F_{1,57} = 1.41$, $P = 0.036$), Ca:Mg ratio ($F_{1,57} = 1.39$, $P = 0.039$), and Fe concentration ($F_{1,57} = 1.36$, $P = 0.048$). The RDA plot (fig. 2) shows that the floristic assemblages of stands of two butterworts had a widespread distribution along the first two canonical axes. However, the sites occupied by *P. arvetii* mostly occurred at higher elevation in comparison to those of *P. vulgaris*, that seemed to be more frequent at sites characterized by high values of soil N:P ratio.

These results are consistent with the analyses of the incidence of species socio-ecological groups in the stands of the two butterworts (table 3). Indeed, the sites occupied by *P. arvetii*, in comparison with those of *P. vulgaris*, had higher occurrences of species groups characteristic of vegetation types more frequent at high elevation, such as snowbeds, sandy and skeletal poor grasslands, and screes. On the contrary, the incidence of species groups characteristic of poor small-sedge fens and transitional mires, where waterlogged soil limits the decomposition of organic matter, were higher in the stands occupied by *P. vulgaris*. Despite these differences, the prevailing species groups (i.e. primary and secondary acidophytic grasslands and lime-rich small fens) in stands occupied by the two butterworts were the same, indicating a clear overlap in habitat use.

## Variation of population and individual characteristics of the two butterwort species

PCA of individual and population variables of the two target species (fig. 3) indicated that the first two ordination axes accounted for 76.8% and 72.5% of the variance in *P. arvetii* and *P. vulgaris*, respectively. In *P. arvetii* (fig. 3A) the stand-level density of population was negatively related with the proportion of flowering individuals, while the size-related characteristics of individuals seemed to be independent from the population ones. In addition, the size of rosette, flower and flower scape were negatively related to the elevation, which conversely seemed to promote the proportion of flowering individuals. Differently, the population density increased with growing nutrient content in the soil, such as Ca and N. Also in *P. vulgaris* (fig. 3B) an opposite trend between the two population characteristics was found, whereas two out of three individual variables, namely rosette size and scape height, seemed to be negatively related with the population density. In this species, the three individual-size characteristics were positively related with the soil reaction and, consistently, showed higher values in stands occurring on limestones.
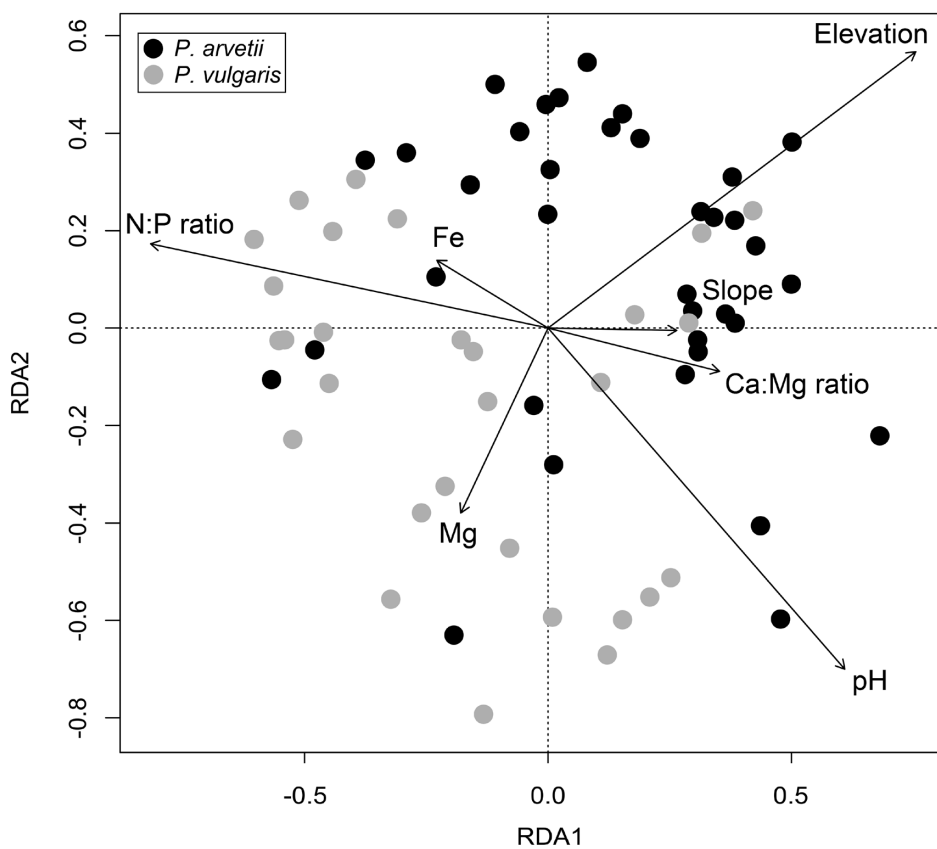


**Figure 2** – RDA plot of the floristic assemblages of plant species in stands (points) of the two butterworts, constrained by environmental variables (arrows).

**Table 3 – Incidence (in percentage ± 1 SD) of socio-ecological groups of species between plots of *Pinguicula arvetii* and *P. vulgaris*.**
For each socio-ecological species group, *P* value (from Wilcoxon test) of the comparison between sites occupied by the two butterworts is shown. Significant differences in incidence of socio-ecological species group between *P. arvetii* and *P. vulgaris* sites are highlighted in bold.

| Socio-ecological species groups | P. arvetii | P. vulgaris | P |
|---|---|---|---|
| Poor small-sedge fens and transitional mires (*Caricetalia nigrae*) | 3.9±8.5 | 15.2±18.6 | **0.007** |
| Primary and secondary acidophytic grasslands (*Nardetea strictae* and *Caricetea curvulae*) | 16.5±14.9 | 17.7±18.3 | 0.947 |
| Lime-rich small sedge fens (*Caricetalia davallianae*) | 15.9±14.9 | 19.9±15.3 | 0.272 |
| Spring and rill swamps (*Montio-Cardaminetea*) | 7.9±11.4 | 6.2±8.8 | 0.553 |
| Snowbeds (*Salicetea herbaceae*) | 2.2±3.6 | 0.6±2.7 | **0.016** |
| Basiphytic primary grasslands (*Elyno-Seslerietea*) | 10.5±11.9 | 5.3±6.8 | 0.060 |
| Nutrient-rich wet grasslands *(Molinio-Arrhenatheretea)* | 10.4±9.8 | 10.3±9.2 | 0.915 |
| Tall herb communities and green alder scrubs (*Mulgedio-Aconitetea*) | 1.5±2.7 | 1.2±2.4 | 0.681 |
| Arid and semiarid grasslands (*Festuco-Brometea*) | 4.1±7.7 | 2.4±3.9 | 0.799 |
| Sandy and skeletal poor grasslands (*Koelerio-Corynephoretea*) | 2.0±3.0 | 0.4±1.5 | **0.009** |
| Scree scope (*Thlaspietea rotundifolii*) | 3.1±4.5 | 1.6±5.2 | **0.027** |
| Coniferous woodlands and dwarf-shrub heaths (*Vaccinio-Piceetea* and *Loiseleurio-Vaccinietea*) | 4.7±6.8 | 2.4±6.1 | 0.089 |
| Deciduous woodlands (*Querco-Fagetea)* | 0.8±2.6 | 2.9±5.4 | 0.054 |
| Acidophytic bryophytes | 3.0±7.0 | 2.4±5.5 | 0.733 |
| Basiphytic bryophytes | 2.2±4.4 | 2.4±8.2 | 0.269 |
| Other species | 11.2±7.6 | 9.0±11.1 | - |
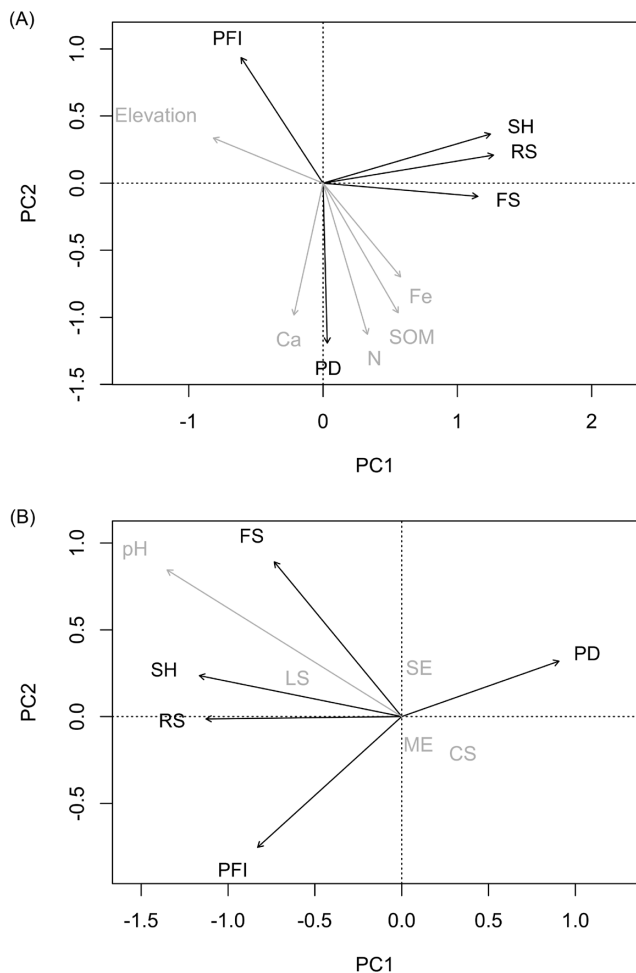


## DISCUSSION

The two species of *Pinguicula* examined in this study showed significant differences in their habitat conditions, ascribing to lithological (table 1), topographical and climatic (table 2) features. Elevation is the main measured environmental variable which significantly differentiated the plots where the two species occurred.

Among the environmental indicators, the EIVs concerning climatic conditions (T-, L-, and K-EIV) resulted significantly different between the sites of the two *Pinguicula* species. A difference concerning T values of the two butterwort species was already reported by Landolt et al. (2010). A significant elevation pattern was found for all these three indicator climatic variables. Because the elevation gradient is characterized by a decrease in mean annual temperature, the two variables showed an opposite trend of variation (electronic appendix 4). L- and K-EIV also differed significantly between the two target species, but, differently from T-EIV, both increased with elevation (electronic appendix 4b & c). This expresses an increasing proportion of light-demanding species and a higher amount of alpine plants with higher K-EIV towards higher elevation. Also, the F-EIV was significantly different between the two species, with higher values at the *Pinguicula vulgaris* plots. Nevertheless, it did not

◄ **Figure 3** – PCA of the individual and population characteristics of *P. arvetii* (A) and *P. vulgaris* (B). Environmental factors are shown in grey; quantitative variables are represented by arrows with length proportional to the correlation with the ordination. Abbreviations: PD, population density; PFI, proportion of flowering individuals; RS, rosette size index; FS, flower size index; SH, scape height; SOM, soil organic matter; CS, calcareous schists; LS, limestones; ME, metagabbros; SE, serpentinites.

show a significant elevation pattern, because the occurrence of wet habitats is, in general, more conditioned by other variables (mainly lithology and topography). The other two EIVs concerning soil conditions (R- and N-EIV) were not significantly different in the plots where two species occurred. The not-significantly different values of R-EIV could be dependent on the occurrence of both *Pinguicula* species on a variety of substrates. Nevertheless, Landolt et al. (2010) reported different R values for the two *Pinguicula* species, with *P. arvetii* occurring on from weakly acid to weakly neutral soils and *P. vulgaris* on from neutral to alkaline. A possible explanation of the divergence is that we sampled *P. vulgaris* only on a limited part of its distribution area, where the more alkaline soils are probably underrepresented with respect to the Eastern Alps. On the other hand, Heslop-Harrison (2004) asserted that the occurrence of *P. vulgaris* in the British Isles seems to be governed mainly by the availability of water rather than by the type of substratum. The N-EIVs were consistent with the general trend regarding soil nutrient availability which was not significantly different between the two species, except N:P ratio. The ratio significantly higher in the plots of *P. vulgaris* could be related to their higher F-EIVs, in accordance with the fact that wetness favours N more than P retention, with a resultant increase in total N:P from drier to wetter sites (Craft & Chiang 2002). It was not surprising that most of the significant habitat differences between the two target species could be derived from the EIVs rather than from direct measures of environmental variables. There are reasons why bioindication could perform better than instrumental measurements of ecological factors. Plants used for bioindication integrate all relevant factors in time and space, whereas actual measurements provide snapshots (Zonneveld 1983, Zelený & Schaffers 2012). Moreover, measurements depend on arbitrary choices of the researcher (e.g. sampling procedures, analytical methods, choice of measured factors) (Szymura et al. 2014).

The moisture indicator values resulting from our study for both target species were lower than those reported for these and other butterwort species from literature; moreover they differed between two species, differently from that reported by Landolt et al. (2010). This was in accordance with our field observation that these species occurred both at sites permanently waterlogged and at ones that could be waterlogged only during a part of the growth season or on slopes close to spring and rills only periodically irrigated, with a higher occurrence of *P. arvetii* for sites only periodically waterlogged. This result could suggest the needs of a general revision of the moisture indicator values for all the butterwort species, because their habitats can range from permanently to only periodically waterlogged sites. This could be accomplished by adding a value for moisture variability as proposed by Landolt et al. (2010) for all wetland species. Another possibility is to fulfil new calibrations of EIVs based on empirically measured environmental variables have been already proposed by several authors (Hill et al. 2000, Lawesson et al. 2003, Tomaselli et al. 2005).

The cover of vascular plants (table 2) showed higher values in plots occupied by *P. arvetii* compared to those of *P. vulgaris*, while the moss cover did not differ between the sites of the two target species. These results may be, at least

partially, due to the higher soil moisture of *P. vulgaris* sites, which can limit the growth of vascular plants, and/or to the more developed vegetation of *P. arvetii* plots, more frequently occurring within alpine grassland communities.

RDA (fig. 2) of the relationships between plant species assemblages and environmental features of sites also showed, within a considerable general overlap between the stands where the two butterworts occurred, some remarkable differences in the habitat conditions. The most important of these is the confirmation of the preferential occurrence of *P. arvetii* for higher elevation sites compared to those including *P. vulgaris*, already resulted from direct comparisons of topographic characteristics of the sites of occurrence. Also, for the species assemblages accompanying *P. vulgaris*, RDA confirmed that this butterwort occurred more frequently with respect to *P. arvetii* at stands with higher N:P ratio. Among the other environmental variables influencing the floristic assemblages, soil pH seemed to have a noticeable role; however, the two butterworts studied did not show difference in their occurrence in respect to this soil characteristic.

The analysis of socio-ecological groups of species (table 3) strengthened that *P. arvetii* occurred more frequently at high elevation sites in comparison with *P. vulgaris* and that the two target species seemed to share the core of their habitat use. Nevertheless, despite their relatively similar ecology, when the two species coexist within the same altitudinal range (mostly between 1400 and 2000 m of elevation) and on the same lithotype (ultramafic), they occurred separately within different habitats with a preference of *P. vulgaris* for sites permanently waterlogged, such as fens and transitional mires, whereas *P. arvetii* was preferentially encountered at sites only periodically waterlogged such as springs and rills.

PCA of individual and population variables of the two butterwort species (fig. 3) revealed that in both the two *Pinguicula* species the performances of different characteristics did not follow the same trajectory in the multidimensional space. In particular, the opposite position of population density and proportion of flowering individuals (along the second and the first ordination axes respectively for *P. arvetii* and *P. vulgaris*) could indicate that the optimal conditions for effective establishment of seedlings or hibernacula differ from those promoting flowering. Including a posteriori projections of environmental factors into ordinations showed that the main environmental gradient influencing butterwort performances were related to topography and/or soil properties. In particular, *P. arvetii* (fig. 3A) seemed to be influenced by the stand elevation, promoting the proportion of flowering individuals but limiting the size of vegetative and reproductive structures. These results could reflect that, along an elevation gradient characterized by stressful condition such as low temperatures and short duration of the growing season, this butterwort species displays an increasing effort in sexual reproduction coupled with a decreasing size of individuals. In addition, the proportion of flowering individual seemed to increase also under nutritional constraints. Indeed, this effort in sexual reproduction was negatively related with several soil properties, such as Ca and N content and the percentage of organic matter, which conversely seemed to promote the population density of *P. arvetii*, depending from both sexual and vegetative reproduction and individual survival.

In *P. vulgaris* (fig. 3B) the main environmental gradient influencing the individual and population variables analysed seemed to be related to soil reaction and lithotype underlying stands. This result may indicate that growth performances of this butterwort species are promoted by the more favourable trophic conditions of sites characterized by high pH, mainly occurring on limestones. In addition, lithotypes-related site conditions seemed to affect *P. vulgaris* performances also at the population level. Indeed, the lowest values of population density and proportion of flowering individuals were respectively found on limestones and ultramafic (i.e. serpentinites) substrata.

At several sites, the populations of the two target species were encountered close to each other. Along the elevation gradient this area of overlapping was comprised between about 1400 and 2000 m a.s.l. Nevertheless, this area is actually much more restricted, because above 1800 m *P. vulgaris* occurred on limestones and, less frequently, on calcareous schists, whereas *P. arvetii* was not recorded on limestones, and below 1400 m it occurred very rarely and only on mafic bedrock (metagabbros). At the sites where two species co-occurred, they could be easily distinguished during the flowering period, while under non-optimal phenological conditions the different length of the longest leaf could be a reliable proxy for identifying the occurring species (the average value was $3.81 \pm 0.79$ cm in the populations of *P. vulgaris* and $2.58 \pm 0.65$ cm in those of *P. arvetii*). Consequently, the reciprocal distribution of two species can be predicted from our data, considering that below 1440 m the occurrence of *P. vulgaris* is highly more probable than that of *P. arvetii*, while above 1800 m the latter species becomes the most common butterwort, except on limestones.

In conclusion, our study demonstrated that *P. arvetii* is a butterwort species typically confined to the metamorphic rocks of the Pennidic domain where the "Calcescisti e Pietre Verdi" complex is largely predominant. From the phytogeographical viewpoint, it belongs to a floristic contingent centred in the south-western Alps (Aeschimann et al. 2004, Legendre et al. 2014). According to Lavergne et al. (2004) the endemic status of *P. arvetii* could explain why it differed significantly from the widespread congener *P. vulgaris* for a number of attributes concerning the habitat use and the effects of environmental variables on individual and population traits. This result came to light despite we examined a lower number of traits with respect to Lavergne et al. (2004) and a pair of congener species less markedly different in their habitat with respect to those examined by these authors that focused their attention on endemic species occurring on steep slopes, with high rock cover and low and open vegetation.

## SUPPLEMENTARY DATA

Supplementary data are available at *Plant Ecology and Evolution*, Supplementary Data Site (http://www.ingentaconnect.com/content/botbel/plecevo/supp-data), and consist of: (1) flowers of the two butterwort species (pdf); (2) list of plot locations with corresponding abbreviations (pdf); (3) plot-level cover (in class form 0 to 10) of species and their subdivision in socio-ecological groups (Excel spreadsheet); and (4) relationships between elevation and Ellenberg indicator values (EIVs) of the plots studied (pdf).

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