The challenge posed by newly discovered cryptic species: disentangling the environmental niches of long-eared bats

Marianne D. Rutishauser1*, Fabio Bontadina1,2,3, Veronika Braunisch1,4, Sohrab Ashrafi1,5 and Raphaël Arlettaz1

ABSTRACT

Aim The discovery of cryptic species poses new challenges for species conservation. Species distributions and conservation status have to be re-evaluated, and the ecological requirements within the species complex have to be re-assessed to recommend adequate conservation guidelines. The recent discovery in Central Europe of the cryptic bat species Plecotus macrobullaris (Kuzjakin 1965) calls for a new appraisal of all three Plecotus species in that area.

Location Switzerland.

Methods Using mostly DNA-identified records, we investigated the environmental niches (ecological niche factor analysis) of the three long-eared bat species at the landscape scale and modelled their potential distributions. Discriminant analysis was used for interspecific niche comparisons.

Results The occurrence of all three species was best explained by proximity to rural settlements and warm summer temperature. Plecotus auritus (Linnaeus, 1758) was positively associated with transition zones from forests to other habitats within heterogeneous landscapes; Plecotus austriacus (J. Fischer, 1829) was more frequently found in orchards and vineyards. Plecotus macrobullaris was linked mostly with deciduous forests. P. auritus had the broadest niche, with occurrence predicted in most forested regions throughout Switzerland. The slightly narrower niche of P. macrobullaris mainly encompassed areas in the Central and Southern Alps. P. austriacus showed a very narrow niche and was predicted mainly in the lowlands, with its habitat requirements overlapping those of P. macrobullaris. Although a range overlap was predicted between P. austriacus and P. macrobullaris, current observations suggest a mostly parapatric distribution in Switzerland.

Main conclusions The projected distributions confirm previous knowledge for P. auritus, but shed new light on the other two species. In contrast to the newly discovered P. macrobullaris, which is actually widespread in the Southern Alps of Switzerland, P. austriacus is restricted to warmer cultivated lowlands and thus may have suffered from recent major land use changes. We suggest reclassifying P. austriacus to a higher conservation status.

Keywords Chiroptera, conservation status, discriminant analysis, ecological niche factor analysis, niche characteristics, Plecotus, potential distribution, Switzerland.
INTRODUCTION

The use of molecular techniques in the study of the genetic variation within taxonomic groups has resulted in the recognition of many cryptic species – that is, species that were originally not distinguished from others owing to high morphological similarity (Bickford et al., 2007), contributing to a better appraisal of biodiversity on planet. In mammals, for instance, the world-wide number of known species increased by 10% within the last 15 years, with 60% of the new findings being cryptic species (Ceballos & Ehrlich, 2009). For both ecologists and conservation biologists, cryptic species represent a challenge, as their discovery often questions the apparent commonness of previously known species that in fact can be rarer than was realized. Consequently, the distribution and conservation status of all species within the cryptic complex must be re-assessed and niche partitioning must be re-evaluated in order to guide conservation action.

Bat species represent 21% of the 5487 so far recognized mammalian species (Schipper et al., 2008). Since 1993, at least 98 new bat species have been described world-wide (Ceballos & Ehrlich, 2009), and even in a well-studied area such as Europe, many new species have been identified or upgraded from the subspecies level since the introduction of protein and DNA screening (e.g. Arlettaz et al., 1997; Mayer et al., 2007). In 2001, a new cryptic species of long-eared bat, Plecotus macrobullaris, was discovered (Kiefer & Veith, 2001; Spizenberger et al., 2002, 2003). This species bears a striking resemblance to two of its sibling species, Plecotus auritus and Plecotus austriacus (Kiefer & Veith, 2001; Pavlinić & Tvrtković, 2004; Ashrafi, 2010; Ashrafi et al., 2010), which both occur across Central Europe. The new species was verified in the mountainous regions from the Pyrenees over the Alps to the Alborz Mountains in Iran (Garin et al., 2003; Juste et al., 2004; Tvrtkovic et al., 2005). In Switzerland, P. auritus was known to occur in woodland up to the timberline (Beck et al., 1995), whereas P. austriacus was mainly restricted to the lowlands (Beck, 1995). The discovery of P. macrobullaris brought into question our understanding of long-eared bats in the region and called for a re-assessment of the geographical distribution, conservation status and ecological niche requirements of all three Plecotus species, especially in the Alps.

This study explores the environmental niches (hereafter referred to as ‘niche’ for simplicity) and predicts the potential distributions of all three sibling species of Plecotus in Switzerland, with the objective to re-assess both conservation status and management prescriptions. We applied a presence-only species distribution model using mostly genetically identified specimens, supplemented, in the case of the rarer P. austriacus, by a few properly re-identified museum specimens (skull and body morphometrics). We further interpreted the distribution patterns of the three species and identified differences in their realized niches by determining the most important eco-geographical predictors for occurrence at the landscape scale. Niche breadth and niche overlap were also quantified, while areas of potential occurrence in Switzerland were determined in order to prioritize areas for future research and conservation action.

MATERIAL AND METHODS

Study site

The study was carried out in Switzerland, Central Europe. The country covers a total area of 41,293 km² subdivided into six biogeographical regions (Fig. 1, Gonseth et al., 2001, Fig. S1) Four regions encompass parts of the Alps, the largest mountain range in Europe, where the low valley floors are cultivated and rather densely populated. With increasing elevation, the valley floors are managed more extensively as meadowland or pastureland. At higher elevations, the slopes are covered with predominantly coniferous forests up to 2300 m a.s.l., although grassland, crops and vineyards occur on some foothills and at middle elevation. The southern part of the Alps, Valais (Western Central Alps) and Ticino (Southern Alps) are characterized by a mild climate with deciduous forests prevailing in the Ticino valleys. The Jura, a range of calcareous hills in the north-west, consists of predominantly large coniferous woodlands and pastures. Between these two mountain ranges lies the lower Swiss Plateau, which is densely populated, intensively cultivated and characterized by small and fragmented forests.

Species data

Because knowledge of current distribution patterns is of major interest for conservation, we focused on verified specimens sampled within the last 25 years (since 1984; Fig. 1, Fig. S1). Changes in land use during the last decades preclude the use of old records because the conditions found at that time may be different from the present ones.

As a visual separation of the three species has been shown to be unreliable (Ashrafi et al., 2010), we used ascertained species records, verified through DNA analyses of either individually collected faeces or tissues (Kiefer et al., 2002; Ashrafi et al., 2010) or assessed by applying the sophisticated morphometric identification key developed by Ashrafi et al. (2010) which results from a linear combination of eight external measurements. To obtain a sufficiently large dataset for the rare P. austriacus, we additionally included nine skulls (M. Ruedi, Museum of Natural History of Geneva, unpublished data), which were measured for 15 external characters according to a recently developed method (I. Pavlinić, unpublished data). For this species, we also had to cut back our defined time frame by including seven records from before 1984 (Supplementary material, Table S1). The oldest record from 1948 stemmed from a locality where the species is still present today (confirmed presence within the 5 × 5 km monitoring square, CSCF, 2011), and the other six records could be included because the environmental conditions in the surrounding area have not fundamentally changed. Consequently, our dataset encompasses all currently
Figure 1  (a) Potential distribution of Plecotus auritus in Switzerland, modelled on the basis of 45 genetically ascertained locations. (b) Potential distribution of P. austriaeus in Switzerland, modelled on the basis of 21 either genetically or morphometrically (skull) ascertained locations. (c) Potential distribution of P. macrobullaris in Switzerland modelled on the basis of 49 genetically ascertained locations.
accessible and verified records from museum collections and regional bat workers in Switzerland (Juste et al., 2004; Ashrafi et al., 2010; Mattei-Roesli, 2010, A. Kiefer, unpublished data) supplemented by data specifically collected in the framework of this study.

The large majority of data stem from summer roosts or their vicinity. To obtain independence between samples, we considered only one record per colony site and applied a minimum distance of 1300 m between sample sites, which roughly corresponds to the average foraging distance from the roost observed in Switzerland (Ashrafi, 2010).

Eco-geographical Variables

The variables tested in the model were selected based on multiple literature reviews on ecological requirements of Plecotus (e.g. Entwistle et al., 1996; Flückiger & Beck, 1995; Fuhrmann & Seitz, 1992; Kiefer & Veith, 1998; Swift & Racey, 1983) and on information obtained from a radiotracking study of the three Plecotus species (Ashrafi, 2010), choosing variables that were expected to be relevant to at least one of the three species (Table 1). In brief, P. auritus has been reported all over Europe, foraging within and along forested areas and orchards (Swift & Racey, 1983; Fuhrmann & Seitz, 1992; Entwistle et al., 1996; Juste et al., 2004; Ashrafi, 2010); Plecotus austriacus has a southerly distribution (Juste et al., 2004) and forages in orchards, gardens, meadows and at forest edges (Flückiger & Beck, 1995; Kiefer & Veith, 1998; Ashrafi, 2010); P. macrobullaris occurs in the mountain ranges from Iran over the Alps to the Pyrenees (Kiefer et al., 2003; Juste et al., 2004), with Ashrafi (2010) reporting foraging habitats in heterogeneous deciduous forests as well as over meadows.

Table 1 The 15 eco-geographical variables (EGVs) used for the analysis and their characteristics. For Boolean variables, the frequency within a radius of 1300 m (fq) or the distance (dist) to the respective feature was used. Variables were discarded from the final models for the following reasons: 1 = contribution to marginality and specialization < 0.1 for all three species, 2 = correlation with a retained EGV > 0.75. Sources of information: Geostat (Geo-data of the Swiss federal statistics office), Swisstopo (Federal office of topography), WSL (Swiss federal institute of forest, snow & landscape research). The original variable resolution was 100 × 100 m

<table>
<thead>
<tr>
<th>Variable category</th>
<th>EGVs</th>
<th>Description</th>
<th>Data source</th>
<th>Survey period</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forest</td>
<td>Coniferous forest (≥ 90%) (fq)</td>
<td>90–100% conifers</td>
<td>Geostat</td>
<td>1990/92</td>
</tr>
<tr>
<td></td>
<td>Mixed forest (fq)</td>
<td>&gt; 10% and &lt; 90% conifers</td>
<td>Geostat</td>
<td>1990/92</td>
</tr>
<tr>
<td></td>
<td>Deciduous forest (≥ 90%) (fq)</td>
<td>90–100% deciduous trees</td>
<td>Geostat</td>
<td>1990/92</td>
</tr>
<tr>
<td>Semi-open habitats</td>
<td>Forest border length (mean)</td>
<td></td>
<td>Swisstopo</td>
<td>2004</td>
</tr>
<tr>
<td></td>
<td>Forest edges (fq)</td>
<td></td>
<td>Swisstopo</td>
<td>2004</td>
</tr>
<tr>
<td></td>
<td>Open forest (fq)</td>
<td></td>
<td>Swisstopo</td>
<td>2004</td>
</tr>
<tr>
<td></td>
<td>Shrubbery (fq)</td>
<td>Hedges and single trees</td>
<td>Swisstopo</td>
<td>2004</td>
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<tr>
<td></td>
<td>Orchards and vineyards (fq)</td>
<td></td>
<td>Swisstopo</td>
<td>2004</td>
</tr>
<tr>
<td>Open habitat</td>
<td>Meadows (fq)</td>
<td></td>
<td>Swisstopo</td>
<td>2004</td>
</tr>
<tr>
<td>Settlements</td>
<td>Towns (dist)</td>
<td></td>
<td>Swisstopo</td>
<td>2004</td>
</tr>
<tr>
<td></td>
<td>Rural settlements (dist)</td>
<td>Villages &amp; single buildings</td>
<td>Swisstopo</td>
<td>2004</td>
</tr>
<tr>
<td>Geology</td>
<td>Limestone (fq)</td>
<td>Limy bedrock</td>
<td>Swisstopo</td>
<td>1964</td>
</tr>
<tr>
<td>Climate</td>
<td>Summer temperature (mean)</td>
<td></td>
<td>WSL</td>
<td>1961–1990</td>
</tr>
<tr>
<td>Landscape and habitats</td>
<td>Patch richness (fq)</td>
<td>Computed (according to McGarigal et al., 2002)</td>
<td>Swisstopo</td>
<td>2004</td>
</tr>
<tr>
<td></td>
<td>Patch diversity (fq)</td>
<td>on forest, shrubbery, meadows and settlements</td>
<td>Swisstopo</td>
<td>2004</td>
</tr>
</tbody>
</table>

All eco-geographical variables were prepared as raster maps with a 100 × 100 m resolution. Continuous variables were calculated from binary land cover data by calculating the minimum distance to the particular feature type or by calculating feature frequency within a radius of 1300 m (modules ‘DistAn’ and ‘CircAn’ in BIOMAPPER 4.0, Hirzel et al., 2007), again reflecting the average foraging range of a colony (Ashrafi, 2010). In FRAGSTATS (McGarigal et al., 2002), we computed two landscape diversity metrics based on four landscape types (settlement, forest, shrubbery and meadows). Patch richness measures the number of different landscape types present within a predefined radius around each grid cell. Patch diversity (Simpson’s diversity index) additionally provides information about the proportional distribution of area among landscape types. These indices were calculated and averaged within a circular moving window (r = 1300 m).

Before applying an ecological niche factor analysis (Hirzel et al., 2002), all eco-geographical variables were normalized by Box–Cox transformation (Sokal & Rohlf, 1981).

Data analysis

Modelling approach

As absence data were not available and the generation of pseudo-absences can be problematic when dealing with cryptic species, we performed an ecological niche factor analysis (ENFA) to investigate the environmental niches and to predict potentially suitable areas for the three species in Switzerland. ENFA is a presence-only distribution model, an advantage when species absence is difficult to assess as in the case of elusive bat species or when absences are considered unreliable as in cryptic species complexes (Hirzel et al., 2010).
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Potential distribution of cryptic long-eared bats

Potential distribution

Based on the resulting predictor set, maps of potential distributions were calculated to identify potentially suitable and non-suitable areas for the three species. Using MacArthur’s broken stick heuristics (MacArthur, 1960; Hirzel et al., 2002), the numbers of those factors were determined (Table S1), which contained a significant amount of information to compute habitat suitability (HS) maps. The median algorithm (Hirzel et al., 2002) was employed which assumes the median of the species’ frequency distribution along each factor to be the optimal approximation of the species’ environmental optimum. Based on this assumption, a partial HS value is assigned to each cell in the study area, which is proportional to the distance of the cell to this optimum in the environmental factor space. HS maps are then produced from a weighted combination of the partial HS values obtained from the selected factors, with weights corresponding to the factors’ contribution to explained information (for details, see Hirzel et al., 2002).

Model validation

Models were evaluated by means of a fivefold cross-validation (integrated in BioMAPPER 4.0, Hirzel et al., 2007), splitting the dataset into five partitions, using each partition in turn to evaluate a model calibrated based on the four remaining partitions. For model evaluation, we used the continuous Boyce index (Hirzel, et al. 2006), an index especially developed for situations where missing absence data rule out the calculation of a confusion matrix. The index is obtained by plotting the ratio between the observed number of evaluation points (P) and the number of evaluation points when assuming a random species distribution (E) against the HS values. The index, defined as the Spearman’s R between P/E and HS, ranges from −1 (wrong model) to 1 (perfect model), with zero indicating a model that performs not better than random (Hirzel et al. 2006, Braunisch & Suchant, 2010).

Niche differentiation

The niches of the three species were compared by discriminant analyses (Legendre & Legendre, 1998) based on the same set of eco-geographical variables measured at the occurrence localities. Discriminant factors were computed so as to maximize the interspecific variance between the three species while minimizing the intraspecific variance at the same time. The correlations of the variables with the discriminant factors indicate in which variables the species’ distributions differ most. An analysis of the frequency distributions of the species locations along the discriminant factors permits comparison of niche breadths and niche overlaps between species. The standardized Levin’s index (B’) (Colwell & Futuyma, 1971) with values ranging from 0 (narrow niche) to 1 (broad niche) was employed to measure the niche breadth (Arlettaz et al., 1997b; Arlettaz, 1999). To analyse niche overlaps, we applied Lloyd’s asymmetric niche overlap index (Hurlbert, 1978). This directional measure accounts for the fact that the niche overlap between two species is mostly non-reciprocal. All calculations were integrated into BioMAPPER 4.0 (Hirzel et al., 2007).

RESULTS

Presence data

We collected 115 Plecotus records from Switzerland (Fig. S1, Table S1). The samples of P. auritus (N = 45) were distributed over all six bioregions in Switzerland (Fig. 1a). Plecotus
*P. austriacus* (*N* = 21) was found in northern (lower Midlands and Jura) and western Switzerland, with only one record at low altitude in the Alps (Western Central Alps; Fig. 1b). *P. macrobullaris* locations (*N* = 49) mainly stemmed from the Central and Southern Alps plus a few samples from the foothills of the north-eastern Alps (Fig. 1c).

**Environmental niche characteristics and potential distributions**

The models revealed the lowest overall marginality of 1.03 and a high tolerance (=1/specialization) of 0.67 for *P. auritus*, indicating that this species is the most generalist among the three. The highest overall marginality in *P. austriacus* (1.40) and *P. macrobullaris* (1.56) indicates that the occurrence of these species depends on environmental conditions that largely deviate from the average conditions in the study area. The tolerance of *P. macrobullaris* (0.45) was intermediate. Finally, the very low tolerance of *P. austriacus* (0.17) illustrates a high sensitivity to deviations from the optimum compared to the other species.

In the following, we focus on variables contributing most to marginality (*M*) and explained specialization (*S*), with coefficients *c(M)* and *c(S) > |0.2|* for at least one species. Additional results can be inferred from Table 2. Positive (or negative) *c(M)* indicates that a habitat feature was more (or less) frequently used compared to its availability, which in the following is referred to as ‘preference’ or ‘avoidance’. Among the variables contributing most to marginality in all three species were proximity (i.e. negative distance) to rural settlements and open forest, as well as coniferous forest and a few samples from the foothills of the north-eastern Alps (Fig. 1c).

<table>
<thead>
<tr>
<th>Eco-geographical variables contribution to:</th>
<th><em>Plecotus auritus</em></th>
<th><em>Plecotus austriacus</em></th>
<th><em>Plecotus macrobullaris</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Marginality (100%)</td>
<td>Expl. S. (7 F., 82%)</td>
<td>Expl. I. (7 F., 91%)</td>
<td>Expl. S. (4 F., 95%)</td>
</tr>
<tr>
<td>Coniferous forest (fq)</td>
<td>0.21</td>
<td>0.17</td>
<td>0.19</td>
</tr>
<tr>
<td>Mixed forest (fq)</td>
<td>0.12</td>
<td>0.19</td>
<td>0.15</td>
</tr>
<tr>
<td>Deciduous forest (fq)</td>
<td>0.14</td>
<td>0.14</td>
<td>0.14</td>
</tr>
<tr>
<td>Forest border length (fq)</td>
<td>0.29</td>
<td>0.33</td>
<td>0.31</td>
</tr>
<tr>
<td>Forest edges (fq)</td>
<td>0.27</td>
<td>0.29</td>
<td>0.28</td>
</tr>
<tr>
<td>Open forest (fq)</td>
<td>0.20</td>
<td>0.16</td>
<td>0.18</td>
</tr>
<tr>
<td>Shrubbery (fq)</td>
<td>-0.18</td>
<td>0.14</td>
<td>0.16</td>
</tr>
<tr>
<td>Orchards and vineyards (fq)</td>
<td>0.09</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>Meadows (fq)</td>
<td>-0.07</td>
<td>0.19</td>
<td>0.13</td>
</tr>
<tr>
<td>Towns (dist)</td>
<td>-0.10</td>
<td>0.05</td>
<td>0.07</td>
</tr>
<tr>
<td>Rural settlements (dist)</td>
<td>-0.67</td>
<td>0.16</td>
<td>0.42</td>
</tr>
<tr>
<td>Limestone (fq)</td>
<td>-0.16</td>
<td>0.16</td>
<td>0.16</td>
</tr>
<tr>
<td>Summer temperature (mean)</td>
<td>0.25</td>
<td>0.15</td>
<td>0.20</td>
</tr>
<tr>
<td>Patch richness (fq)</td>
<td>0.29</td>
<td>0.15</td>
<td>0.22</td>
</tr>
<tr>
<td>Patch diversity (fq)</td>
<td>0.22</td>
<td>0.21</td>
<td>0.22</td>
</tr>
</tbody>
</table>

In *P. auritus*, other important eco-geographical variables contributing to marginality were long forest border lengths...
and the frequency occurrence of forest edges \(c(M)\) of 0.29 and 0.27, both also showing high contributions to specialization \(c(S)\) of 0.33 and 0.29, respectively and coniferous forests \(c(M) = 0.21\). The potential distribution for \(P. auritus\) was computed using the first seven factors (Table S1), which accounted for 91% of the explained information. Potentially suitable areas for this species are present throughout Switzerland (Fig. 1a).

\textit{Plecotus austriacus} preferred short distances from towns \(c(M) = -0.30\), as well as a high frequency of orchards and vineyards \(c(M) = 0.36\), and avoided coniferous and open forests \(c(M) = -0.34\) and -0.28, while at the same time showing a low tolerance towards deviations from optimal conditions in these variables \(c(S)\) of 0.25, 0.30, 0.33 and 0.26, respectively (Table 2). The potential distribution of \(P. austriacus\) was computed based on the first four factors (Table S2) explaining 97% of the information. The model indicated potentially suitable areas in the Midlands as well as in some lower alpine valleys including those in Ticino (Southern Alps). Areas of high suitability were often scattered and sparsely connected (Fig. 1b).

In \(P. macrobullaris\), an important variable contributing to both marginality and specialization (Table 2) was a high frequency of deciduous forest \(c(M) = 0.32\); \(c(S) = 0.24\). Meadows and shrubbery \(c(M) = -0.24\) and -0.23, respectively also contributed to marginality, but with negative coefficients indicating an avoidance of these habitats. For \(P. macrobullaris\), we computed the potential distribution based on three factors (Table S1) explaining 88% of the information. The most suitable areas for this species were situated in the Western Central Alps and Ticino (Southern Alps), lower Alpine valleys and some small scattered areas in the Jura and the Midlands (Fig. 1c).

Altitude per se was not included in our models, because it was highly correlated with temperature (Pearson’s \(r = 0.98\)), which is the decisive factor determining species distributions. However, to facilitate a comparison with related studies referring to altitude as a proxy for temperature (e.g. Kiefer et al., 2002; Pavlič & Terković, 2004; Mattei-Roesli, 2010), we also provide the observed and predicted distributions of each species (Fig. 1a-c) with regard to the altitudinal gradient. The distribution maps predicted \(P. auritus\) to occur up to the timberline (c. 2000 m a.s.l.), \(P. macrobullaris\) up to moderate elevation (c. 1500 m), whereas \(P. austriacus\) was restricted to low elevation (below 600 m).

**Model evaluation**

The distribution model of \(P. auritus\) revealed the highest predictive power. The continuous Boyce index \(B_{\text{cont}}\) was comparatively closer to one and had a small standard deviation \(B_{\text{cont}} = 0.77 \pm 0.10\), mean ± standard deviation). The \(B_{\text{cont}}\) values of the remaining models were 0.69 ± 0.34 and 0.56 ± 0.27 for \(P. macrobullaris\) and \(P. austriacus\), respectively. The larger standard deviations indicate a lower robustness of the latter two models, but still an acceptable predictive power.

**Niche differentiation**

The first two discriminant factors accounted for 58% of the total variance (first factor: 37%, second factor: 21%), indicating a rather low level of discrimination between habitat requirements of the three species and an extensive overlap of their niches (Fig. 2). The first discriminant factor maximized the partitioning between \(P. auritus\) and \(P. macrobullaris\), with \(P. austriacus\) taking an intermediate position closer to \(P. macrobullaris\) (Fig. 2). The maxima of the frequency distributions of \(P. austriacus\) and \(P. macrobullaris\) clearly deviated from average global conditions. Along the first factor, species were most discriminated by deciduous forest, summer temperature and patch richness (Fig. 3, Table 3). The species frequency distributions along the second discriminant axis showed a separation of \(P. austriacus\) from the other two species that largely overlapped (Fig. 2). Along this axis, the frequency distribution of \(P. austriacus\) was situated closer towards the pole of orchards and vineyards, whereas those of the other two species were located more towards forests (open and coniferous), forest border length and forest edge frequency (Fig. 3, Table 3).

The standardized Levin’s niche breadth index \(B';\) Table 4) computed over both discriminant factors indicated that the niche of \(P. austriacus\) was distinctly narrower \((B' = 0.17)\) than the niches of the other two species, with the latter two having similar breadths \((P. macrobullaris: B' = 0.30; P. auritus: B' = 0.31)\). Lloyd’s asymmetric niche overlap indices (Table 5) showed a similar pattern over both discriminant factors. The ecological niche of \(P. austriacus\) was more encompassed within the niches of the other two species than the opposite, and the niches of \(P. auritus\) and \(P. macrobullaris\) were more overlapping with each other than with the niche of \(P. austriacus\) (see also Fig. 2).

**DISCUSSION**

Spatial distribution models are useful tools for setting conservation priorities for both species and habitats (Chefaoui & Lobo, 2008) and for discriminating distributions and niche requirements of sympatric sibling species (Sattler et al., 2007). Given the time pressure and limited financial resources in applied conservation, presence-only methods like ENFA can be advantageous because they enable even incomplete information to be efficiently processed, that is, they make the best use of available but limited data (Elith & Leathwick, 2007; Braunisch & Suchant, 2010). Nowadays, many natural history museums are digitizing their collections, with the aim of providing online open access to researchers. Readily available, the information offers great opportunities for building preliminary, yet extended spatial models like the one developed here (Reutter et al., 2003; Rowe, 2005). However, as Phillips et al. (2009) demonstrated, such opportunistically sampled data may inherit a sampling bias towards more populated areas and/or areas where rare species are expected and where observation frequency and detection probability would be higher.
Moreover, populated areas are usually not randomly distributed in the environment, occurring mostly at low elevation close to cultivated plains and large water bodies, which may additionally lead to spurious correlations between species presence and these environmental conditions. Because *Plecotus* species roost mostly in human settlements, and bias in survey effort could not be estimated, a correction factor could not be applied. However, we accounted for that problem by choosing a sampling resolution that corresponded to the average foraging range of a roosting colony (Ashrafi, 2010). Consequently, all bat records stemming from one settlement were reduced to one presence location within a 1.3 km radius.

Braunisch & Suchant (2010) have recently demonstrated that species data collected unsystematically, if not haphazardly over a large representative region and aggregated in an ecologically reasonable way to level out the over-representation of better-surveyed areas can provide better models than data collected systematically within restricted regions. This opens new perspectives for using volunteer-based survey schemes for distribution modelling. Nevertheless, for a small sample of a rare species, it is possible that the range of inhabitable environmental conditions accounted for is incomplete, which could then lead to an underestimation of the niche breadth.

**Figure 2** Discriminant analysis of the distributions of *Plecotus auritus* (black squares), *P. austriacus* (white circles) and *P. macrobullaris* (grey triangles), calculated according to the eco-geographical variables given in Table 1. In the histograms, the relative frequency of species observations along the two discriminant factors (DF) is compared to the distribution of global conditions in the study area (light grey area: frequency of all grid cells of the study area). The scatter plot displays the species’ distributions along the two first DF. The contributions of each factor to the explained variance are given in brackets.

**Figure 3** Discriminant analysis. Correlations of eco-geographical variables (EGVs, defined in Table 1), with the first and second discriminant factors, accounting for 58% of total variance (DF1 37% and DF2 21%). The distance of an EGV from the centre indicates its importance for species discrimination.
Plecotus auritus

factors. In parentheses: variance explained by the corresponding factors is indicated. The contributions of the discriminant factors to the explained variance are given in parentheses.

Table 3 Discriminant analysis. Correlations of the eco-geographical variables with the first and second discriminant factors (DF 1 and DF 2) as well as total explained variance are indicated. In boldface, the three most important variables per factor are shown. The contributions of the discriminant factors to the explained variance are given in parentheses.

<table>
<thead>
<tr>
<th>Eco-geographical variables</th>
<th>DF 1 (37%)</th>
<th>DF 2 (21%)</th>
<th>DF 1 + DF 2 (58%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coniferous forest (≥ 90%) (fq)</td>
<td>0.13</td>
<td>−0.37</td>
<td></td>
</tr>
<tr>
<td>Mixed forest (fq)</td>
<td>0.31</td>
<td>−0.17</td>
<td></td>
</tr>
<tr>
<td>Deciduous forest (≥ 90%) (fq)</td>
<td>−0.57</td>
<td>−0.08</td>
<td></td>
</tr>
<tr>
<td>Forest border length (fq)</td>
<td>0.03</td>
<td>−0.41</td>
<td></td>
</tr>
<tr>
<td>Forest edges (fq)</td>
<td>0.16</td>
<td>−0.30</td>
<td></td>
</tr>
<tr>
<td>Open forest (fq)</td>
<td>−0.06</td>
<td>−0.43</td>
<td></td>
</tr>
<tr>
<td>Shrubbery</td>
<td>−0.01</td>
<td>0.17</td>
<td></td>
</tr>
<tr>
<td>Orchards &amp; vineyards (fq)</td>
<td>0.04</td>
<td>0.46</td>
<td></td>
</tr>
<tr>
<td>Meadows (fq)</td>
<td>0.25</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>Towns (dist)</td>
<td>0.00</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td>Rural settlements (dist)</td>
<td>0.17</td>
<td>−0.18</td>
<td></td>
</tr>
<tr>
<td>Limestone (fq)</td>
<td>−0.26</td>
<td>−0.10</td>
<td></td>
</tr>
<tr>
<td>Average summer temperature</td>
<td>−0.49</td>
<td>0.07</td>
<td></td>
</tr>
<tr>
<td>Patch richness (fq)</td>
<td>−0.35</td>
<td>−0.31</td>
<td></td>
</tr>
<tr>
<td>Patch diversity (fq)</td>
<td>0.11</td>
<td>−0.02</td>
<td></td>
</tr>
</tbody>
</table>

Table 4 The standardized Levin’s niche breadth index (B′) of the three species calculated on the two discriminant factors (DF). In parentheses: variance explained by the corresponding factors.

<table>
<thead>
<tr>
<th></th>
<th>DF1 (37%)</th>
<th>DF2 (21%)</th>
<th>DF1 + DF2 (58%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plecotus auritus</td>
<td>0.26</td>
<td>0.37</td>
<td>0.31</td>
</tr>
<tr>
<td>Plecotus austriacus</td>
<td>0.14</td>
<td>0.22</td>
<td>0.17</td>
</tr>
<tr>
<td>Plecotus macrobullaris</td>
<td>0.31</td>
<td>0.26</td>
<td>0.30</td>
</tr>
</tbody>
</table>

Table 5 Lloyd’s asymmetric niche overlap of species X with species Y (Z_{xy}) between the three species in Switzerland, calculated on each of the two discriminant factors (DF). The variance explained by the corresponding factors is indicated in parentheses.

<table>
<thead>
<tr>
<th></th>
<th>Y</th>
<th>Plecotus auritus</th>
<th>Plecotus austriacus</th>
<th>Plecotus macrobullaris</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DF1 (37%)</td>
<td>P. auritus</td>
<td>−0.64</td>
<td>1.07</td>
<td></td>
</tr>
<tr>
<td></td>
<td>P. austriacus</td>
<td>1.38</td>
<td>−1.74</td>
<td></td>
</tr>
<tr>
<td></td>
<td>P. macrobullaris</td>
<td>0.98</td>
<td>0.74</td>
<td>−</td>
</tr>
<tr>
<td>DF2 (21%)</td>
<td>P. auritus</td>
<td>−0.42</td>
<td>1.76</td>
<td></td>
</tr>
<tr>
<td></td>
<td>P. austriacus</td>
<td>0.91</td>
<td>−1.19</td>
<td></td>
</tr>
<tr>
<td></td>
<td>P. macrobullaris</td>
<td>1.61</td>
<td>0.51</td>
<td>−</td>
</tr>
</tbody>
</table>

The predictor resolution corresponding to the average foraging range of a roosting colony entails that our models could not elucidate fine-grained resource selection patterns but addressed mostly the environmental preconditions for species presence at a landscape scale, thus representing the first step in a hierarchical approach towards disentangling the different dimensions of the niches of the sibling species.

Important eco-geographical variables

P. auritus occupied the broadest niche, with occurrence predicted in most forested regions of Switzerland. The slightly narrower niche of P. macrobullaris mainly encompassed areas in the Central and Southern Alps. P. austriacus showed a very narrow niche and was predicted to occur mainly in the lowlands. The most important eco-geographical predictors for the presence of all three species were proximity to rural settlements and high average summer temperature (the latter to a lesser extent for P. auritus which reaches higher altitudes). The positive association of all three species with villages and single buildings reflects their local roost preference, usually attics in Switzerland (Beck, 1995; Beck et al., 1995), although P. auritus is also known to roost in tree cavities and bat boxes (Fuhrmann & Seitz, 1992; Beck et al., 1995). For P. austriacus, there is no evidence for the use of tree cavities (Beck, 1995), nor for P. macrobullaris that has been found almost exclusively in buildings so far (Kiefer and von Helversen 2004, Mattei-Roesli, 2010; a single individual once occupying a tree cavity, S. Ashrafi, unpublished radiotracking data). However, as a sampling bias cannot be completely ruled out, our results regarding the importance of this variable must be treated with caution.

Plecotus austriacus, originally a Mediterranean species, has previously been described as thermophilous (Beck, 1995; Horáček et al., 2004). Plecotus macrobullaris was found in warmer, lower areas than P. auritus. These findings are in strong contrast to the first genetically verified locations of P. macrobullaris – the Alpine long-eared bat – that were all from locations above 800 m a.s.l. (Kiefer et al., 2002). Recent studies confirm occurrences at lower altitude: in Croatia, the majority of P. macrobullaris (19 of 28 localities) were found at altitudes lower than 800 m a.s.l. (Pavlinić & Tvrtković, 2004). This is also the case in northern Italy (Trizio et al., 2005; 693; ±341 m a.s.l.) and in the Ticino (southern Switzerland) (Mattei-Roesli, 2010, mean altitude around 450 m a.s.l.). Furthermore, according to the current state of our knowledge, the Swiss Alps represent the northernmost and consequently coldest part of the known distribution of P. macrobullaris. As the temperature-related and thus altitudinal distributions of all three species were overlapping, no exclusive altitudinal niche separation could be observed.

Our large-scale models may even reflect foraging habitat preferences of the bats at a smaller scale. High landscape diversity was important for P. auritus and to a lesser extent for P. macrobullaris. Highly important predictors for the distribution of species taken separately were forest edge frequency and forest border length, as well as coniferous forests for P. auritus, a positive effect of orchards and vineyards but a negative effect of coniferous and open forests for...
P. austriacus; deciduous forests were important for P. macrobullaris that avoided meadows and shrubbery. The results for P. auritus (Table 2) suggest a preference for forest eco-
tonal structures in general and coniferous forests in particu-
lar. A similar preference for woodland and trees in this
gleaning species has been established from radiotracking data (Beck, 1987; Anderson & Racey, 1991; Ashrafi, 2010). Accordingly, P. auritus was never found foraging regularly in the open (Swift & Racey, 1983; Fuhrmann & Seitz, 1992; Entwistle et al., 1996). The observed preference of P. austriacus for orchards and vineyards is in line with former studies (Flückiger & Beck, 1995; Kiefer & Veith, 1998; Ashrafi, 2010). An avoidance of open forests in general and conifer-
ous forests in particular, with a preference for deciduous
forests on the lower foothill slopes to the forested subalpine
areas, the distribution of this species ranges from deciduous
Alps of Switzerland; Ashrafi, 2010). In sub-Mediterranean
areas, the distribution of this species ranges from deciduous
forests on the lower foothill slopes to the forested subalpine
belt that includes coniferous trees (Pavlinič & Tvrkovič,
2004).

Are Plecotus austriacus and Plecotus macrobullaris
parapatric in Switzerland?

Based on their known distributions and currently distant
environmental niches (Tables 2 and 4; Figs 1, 3, 4), P. aus-
striacus and P. macrobullaris seem to exclude each other over
large areas in Switzerland, even though their potential distrib-
utions are overlapping. This pattern may reflect a parapatric
distribution. Our projection maps would predict P. austriacus
to occur in the lowlands of Ticino (Southern Alps), where the
species was not recorded during recent extensive surveys
(Mattei-Roesli, 2010). In our opinion, there are two main alter-
native explanations to the apparent parapatric distribu-
tion. First, the Alps may have functioned as a historical bar-
er to colonization after glaciers receded, with P. austriacus
confined to the northern external border of the Alps and P. macrobullaris in the Central and Southern Alps. In Austria,
for instance, P. austriacus has only been recorded in the north-eastern Alps (Spitzenberger 2001). In a recent survey in the
alpine massif of northern Italy (Lombardy and Trentino),
Trizio et al. (2005) found no records of P. austriacus. In con-
trast, all three species widely overlap in their distribution in
Croatia (Tvrkovič et al., 2005) where no alpine barrier is
present. However, it should be considered that resource spe-
cialization may have occurred which was not detected by the
environmental variables incorporated in the models. Second,
competitive exclusion may have led to the current, ‘mutual
avoidance’ distribution pattern. This scenario would be sub-
stantiated by recent studies of P. austriacus and P. macrobul-
laris that revealed high similarities in echolocation signals and
flight behaviour (Dietrich et al., 2006), as well as similar
moth-based diets (Ashrafi et al., 2010). The fact that only a
few areas of Switzerland seem to harbour these two species
(e.g. Geneva, M. Ruedi and C. Schönbächler, pers. comm.)
seems to underpin our view about the parapatric distribution
of P. austriacus and P. macrobullaris. A specialization on dif-
ferent, fine-grained resources that are regionally different and
not captured by the environmental variables incorporated in
our models would also fit with apparent parapatric distribu-
tions. However, such clear-cut niche segregation seems very
unlikely given the similarities of the two species regarding for-
aging behaviour and diet (Ashrafi, 2010; Ashrafi et al., 2011).

CONCLUSIONS AND IMPLICATIONS FOR CONSERVATION

Before the discovery of P. macrobullaris, its sibling species
have been classified in the Swiss red list (Duell, 1994) as
dangered (P. auritus) and potentially endangered (P. aus-
triacus). The high reciprocal environmental niche overlap of
P. auritus and P. macrobullaris shown by the Lloyd’s index
(Tab. 5) may actually indicate a stable coexistence of these
two species in sympatry, whereas the apparent parapatric dis-
persion of P. austriacus and P. macrobullaris may either
reflect different post-glacial recolonization patterns or com-
petitive exclusion, suggesting that these two species utilize
fairly similar resources.

P. auritus is able to exploit a broad range of environmental
conditions and, based on our models, the species is pre-
dicted to occur all over Switzerland. However, in our
opinion, its currently wide distribution questions the validity
of its classification as endangered. For P. austriacus, many
bat biologists were already aware in the 1980s of the exis-
tence of a different form of Plecotus in the Valais Alps (e.g.
Arlettaz et al., 1997c). As these bat workers were reluctant to
identify live specimens to species, databases include hundreds
of live records of Plecotus sp. that a posteriori relate either to
P. austriacus or to P. macrobullaris, which are particularly
difficult to differentiate, which represents a typical conserva-
tion puzzle (Schönrogge et al. 2002). Already considered as
rare in Switzerland before the recognition of P. macrobullaris
(Weck, 1995), the status of P. austriacus is more critical today
than ever before. In addition to having a narrow niche, P. austriacus is mainly restricted to the lowlands where human activities concentrate, rendering it especially vulnera-
ble. Therefore, we propose to upgrade P. austriacus’ status to
species of high conservation concern, which requires close
monitoring and specific conservation measures that have still
to be defined. Plecotus macrobullaris has not been allocated a
Red List status yet, but it seems quite common in the Alpine
arch; nevertheless, more investigations are needed to accu-
rately describe its status and distribution. Our predictive
maps will be useful to this latter task.

This study exemplifies the possible changes in bioge-
ographical, ecological and conservation knowledge that may
be revealed by the discovery of a single new species in a
cryptic taxonomic complex (Schönrogge et al., 2002; Sattler
et al., 2007). It stresses the importance of an early recognition of the existence of sibling species and their ecological requirements to provide the necessary guidance for conservation management.

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REFERENCES


**SUPPORTING INFORMATION**

Additional Supporting Information may be found in the online version of this article:

**Figure S1** Genetically ascertained records of *Plecotus auritus* (*N* = 45), *P. austriacus* (*N* = 21) and *P. macrobullaris* (*N* = 49) in Switzerland.

**Table S1** Origin of *Plecotus* specimens used in this study.

**Table S2** Ecological niche factor analysis (ENFA).

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**BIOSKETCH**

Our research team focuses on the conservation biology of endangered species, mostly within farmland and Alpine ecosystems, with the objective to provide guidance for evidence-based habitat management and restoration. The discovery of a new cryptic *Plecotus* species in Switzerland led us to launch a research programme to develop new identification methods, and study the distribution and ecological requirements of the three species in order to re-assess their conservation status and guide conservation management. *Website: www.cb.iee.unibe.ch.*

Author contributions: F.B. and R.A. conceived the study; M.D.R., S.A. and R.A. collected the data; M.D.R. and V.B. analysed the data; and M.D.R., V.B., F.B. and R.A. led the writing.

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