



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

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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.

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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.*, 1997a; Mayer *et al.*, 2007). In 2001, a new cryptic species of long-eared bat, *Plecotus macrobullaris*, was discovered (Kiefer & Veith, 2001; Spitzenberger *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; Tvrtković *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, Gönseth *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

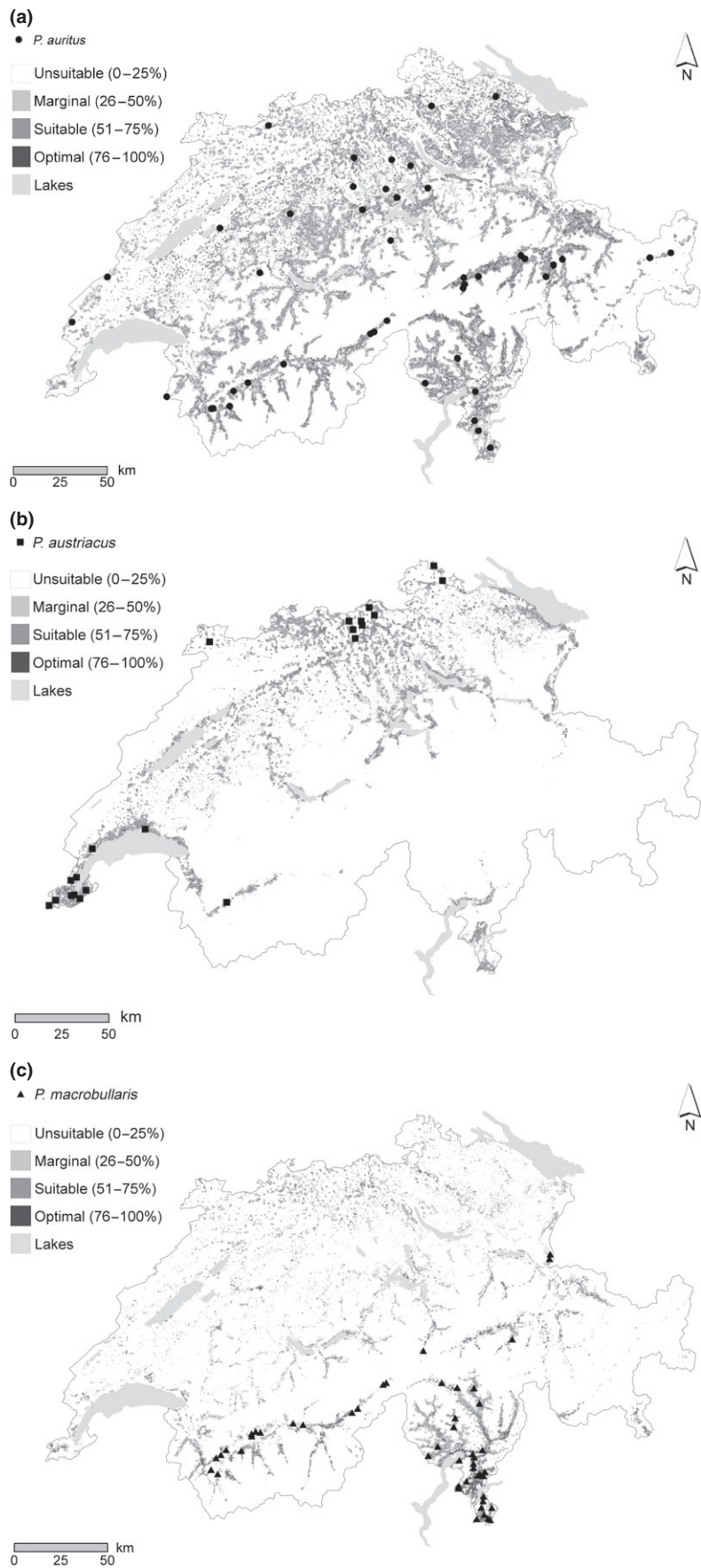


Figure 1 (a) Potential distribution of *Plecotus auritus* in Switzerland, modelled on the basis of 45 genetically ascertained locations. (b) Potential distribution of *P. austriacus* in Switzerland, modelled on the basis of 21 either genetically or morphometrically (skull) ascertained locations. (c) 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.*, 2002; Garin *et al.*, 2003; Juste *et al.*, 2004), with Ashrafi (2010) reporting foraging habitats in heterogeneous deciduous forests as well as over meadows.

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.*,

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 (Geodata 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

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

2001). Moreover, its methodological approach is directly based on Hutchinson's (1957) concept of the ecological niche, delineating the multidimensional space of environmental conditions in which the species is able to persist by comparing the environmental conditions at locations with occurrence of the species with the conditions prevailing in the whole study area. It thus requires two types of input data: presence data of the three species and a set of eco-geographical variables covering the whole study area (Hirzel *et al.*, 2002). ENFA converts these partly intercorrelated variables into the same number of uncorrelated factors, which summarize the niche information into two main components, marginality and specialization (Hirzel *et al.*, 2002). Marginality, as maximized by the first factor, describes the deviation of the mean environmental conditions at the locations of the species (species distribution) from those in the study area (global distribution), thus indicating the niche position. Specialization, as maximized by the second and all subsequent factors, describes the ratio of species variance to global variance and indicates the niche breadth (Hirzel *et al.*, 2002). Overall marginality and specialization summarize the information over all eco-geographical variables (for details, see Hirzel *et al.*, 2002), with low marginality values (close to zero) characterizing species that live in 'average conditions' and high values (> 1) indicating a tendency to live in extreme habitats. Similarly, overall specialization indicates the niche breadth of a species. As the specialization values range from 1 to infinity, the inverse ($1/S = \text{tolerance}$) is used, ranging from zero to 1, with low tolerance values indicating a specialist, and high values a generalist species. It should be noted that both indices highly depend on the available conditions, so they only can be used to compare species within the same study area and in relation to the same set of variables.

For each species, we first ran an analysis including all eco-geographical variables. In order to simplify the models, we subsequently discarded the less important variables from pairs of highly intercorrelated variables (Pearson's $r > 0.75$) as well as all irrelevant variables (contribution to marginality and specialization $< |0.1|$ for all three species).

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*

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 high 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 [$c(M)$ of -0.67 , -0.56 and -0.61 for *P. auritus*, *P. austriacus* and *P. macrobullaris*, respectively] and warm summer temperature (0.25, 0.45 and 0.40, respectively) (Table 2). Concerning summer temperature, *P. auritus* showed a lower marginality and a lower specialization [$c(S) = 0.15$] than the other two species (0.39 and 0.21 for *P. austriacus* and *P. macrobullaris*, respectively). With regard to landscape diversity (patch richness and patch diversity), a high marginality existed in *P. auritus* and *P. macrobullaris* (0.29 and 0.22, 0.31 and 0.20, respectively), but not in *P. austriacus* (0.14 and 0.10).

In *P. auritus*, other important eco-geographical variables contributing to marginality were long forest border lengths

Table 2 Ecological niche factor analysis (ENFA). Contribution of the eco-geographical variables to marginality (M), explained specialization (Expl. S.) and explained information (Expl. I.; defined as $(M + \text{Expl. S.})/2$) as calculated over the number of significant factors (F) used in the ENFA models. In boldface variables contributing most to marginality and explained specialization (coefficients $> |0.2|$). Positive marginality values indicate a preference for a certain variable, and negative values indicate avoidance. Negative coefficient values for distance (dist) variables have to be interpreted as avoidance of increasing distance

Eco-geographical variables contribution to:	<i>Plecotus auritus</i>			<i>Plecotus austriacus</i>			<i>Plecotus macrobullaris</i>		
	Marginality (100%)	Expl. S. (7 F., 82%)	Expl. I. (7 F., 91%)	Marginality (100%)	Expl. S. (4 F., 95%)	Expl. I. (4 F., 97%)	Marginality (100%)	Expl. S. (3 F., 76%)	Expl. I. (3 F., 88%)
Coniferous forest (fq)	0.21	0.17	0.19	-0.34	0.33	0.33	-0.01	0.08	0.05
Mixed forest (fq)	0.12	0.19	0.15	-0.09	0.12	0.11	0.03	0.15	0.09
Deciduous forest (fq)	0.14	0.14	0.14	0.16	0.18	0.17	0.32	0.24	0.28
Forest border length (fq)	0.29	0.33	0.31	0.02	0.03	0.03	0.19	0.19	0.19
Forest edges (fq)	0.27	0.29	0.28	-0.09	0.11	0.10	0.20	0.13	0.16
Open forest (fq)	0.20	0.16	0.18	-0.28	0.26	0.27	0.20	0.16	0.18
Shrubbery (fq)	-0.18	0.14	0.16	0.01	0.02	0.02	-0.23	0.11	0.17
Orchards and vineyards (fq)	0.09	0.10	0.10	0.36	0.30	0.33	-0.01	0.04	0.03
Meadows (fq)	-0.07	0.19	0.13	-0.07	0.08	0.07	-0.24	0.16	0.20
Towns (dist)	-0.10	0.05	0.07	-0.30	0.25	0.28	-0.06	0.04	0.05
Rural settlements (dist)	-0.67	0.16	0.42	-0.56	0.45	0.51	-0.61	0.32	0.46
Limestone (fq)	-0.16	0.16	0.16	0.03	0.04	0.04	-0.08	0.19	0.13
Summer temperature (mean)	0.25	0.15	0.20	0.45	0.39	0.42	0.40	0.21	0.31
Patch richness (fq)	0.29	0.15	0.22	0.14	0.13	0.13	0.31	0.15	0.23
Patch diversity (fq)	0.22	0.21	0.22	0.10	0.13	0.11	0.20	0.14	0.17

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).

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 [$c(M)$ of -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 [$c(M)$ of -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; Pavlinić & Tvrtković, 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_{cont}) was comparatively closer to one and had a small standard deviation ($B_{\text{cont}} = 0.77 \pm 0.10$, mean \pm standard deviation). The B_{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.

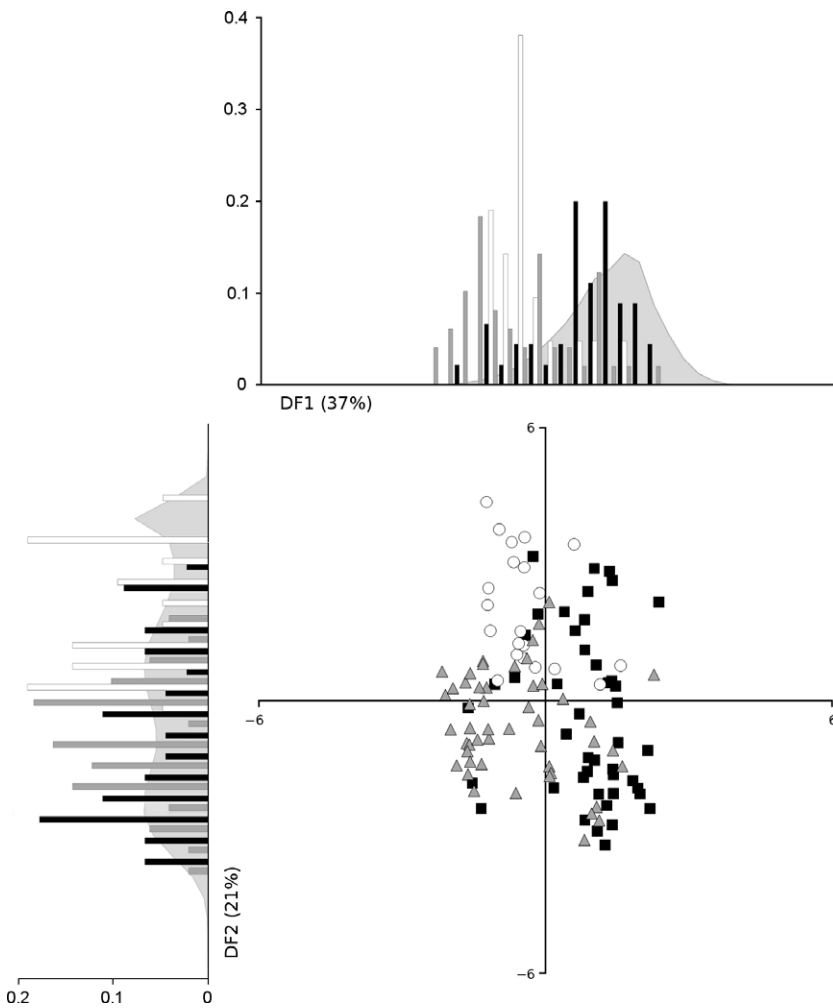


Figure 2 Discriminant analysis of the distributions of *Plecotus auritus* (black squares), *P. austriacus* (white circles) and *P. macrobullaris* (grey triangles), calculated according to the eco-geographical variables given in Table 1. In the histograms, the relative frequency of species observations 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.

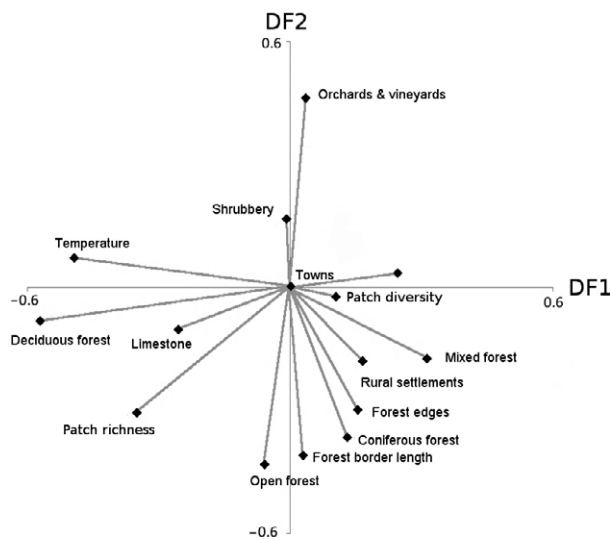


Figure 3 Discriminant analysis. Correlations of eco-geographical variables (EGVs, defined in Table 1), with the first and second discriminant factors, accounting for 58% of total variance (DF1 37% and DF2 21%). The distance of an EGV from the centre indicates its importance for species discrimination.

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.

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

Eco-geographical variables	DF 1 (37%)	DF 2 (21%)
Coniferous forest ($\geq 90\%$) (fq)	0.13	-0.37
Mixed forest (fq)	0.31	-0.17
Deciduous forest ($\geq 90\%$) (fq)	-0.57	-0.08
Forest border length (fq)	0.03	-0.41
Forest edges (fq)	0.16	-0.30
Open forest (fq)	-0.06	-0.43
Shrubbery	-0.01	0.17
Orchards & vineyards (fq)	0.04	0.46
Meadows (fq)	0.25	0.04
Towns (dist)	0.00	0.00
Rural settlements (dist)	0.17	-0.18
Limestone (fq)	-0.26	-0.10
Average summer temperature	-0.49	0.07
Patch richness (fq)	-0.35	-0.31
Patch diversity (fq)	0.11	-0.02

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.

	DF1 (37%)	DF2 (21%)	DF1 + DF2 (58%)
<i>Plecotus auritus</i>	0.26	0.37	0.31
<i>Plecotus austriacus</i>	0.14	0.22	0.17
<i>Plecotus macrobullaris</i>	0.31	0.26	0.30

Table 5 Lloyd's asymmetric niche overlap of species X with species Y ($Z_{x(y)}$) 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.

X		Y		
		<i>Plecotus auritus</i>	<i>Plecotus austriacus</i>	<i>Plecotus macrobullaris</i>
DF1 (37%)	<i>P. auritus</i>	—	0.64	1.07
	<i>P. austriacus</i>	1.38	—	1.74
	<i>P. macrobullaris</i>	0.98	0.74	—
DF2 (21%)	<i>P. auritus</i>	—	0.42	1.76
	<i>P. austriacus</i>	0.91	—	1.19
	<i>P. macrobullaris</i>	1.61	0.51	—

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 ecological structures in general and coniferous forests in particular. 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 coniferous forests in particular, with a preference for deciduous forests in this species [$c(M) = 0.16$, Table 2], indicates that foraging often takes place in dense deciduous woodland (Kiefer & Veith, 1998). The preference of *P. macrobullaris* for deciduous forests in this study is supported by local-scale radiotracking surveys conducted in Valais (Western Central 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 (Pavlinić & Tvrtković, 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. austriacus* and *P. macrobullaris* seem to exclude each other over large areas in Switzerland, even though their potential distributions 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 alternative explanations to the apparent parapatric distribution. First, the Alps may have functioned as a historical barrier 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 contrast, all three species widely overlap in their distribution in Croatia (Tvrtković *et al.*, 2005) where no alpine barrier is present. However, it should be considered that resource specialization 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 substantiated by recent studies of *P. austriacus* and *P. macrobullaris* 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 different, fine-grained resources that are regionally different and not captured by the environmental variables incorporated in our models would also fit with apparent parapatric distributions. However, such clear-cut niche segregation seems very unlikely given the similarities of the two species regarding foraging 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 (Duelli, 1994) as endangered (*P. auritus*) and potentially endangered (*P. austriacus*). 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 distribution of *P. austriacus* and *P. macrobullaris* may either reflect different post-glacial recolonization patterns or competitive 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 predicted 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 existence of a different form of *Plecotus* in the Valais Alps (e.g. Arletaz *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 conservation puzzle (Schönrogge *et al.* 2002). Already considered as rare in Switzerland before the recognition of *P. macrobullaris* (Beck, 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 vulnerable. 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 accurately describe its status and distribution. Our predictive maps will be useful to this latter task.

This study exemplifies the possible changes in biogeographical, 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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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.iew.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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