Resource partitioning in three cryptic, sympatric bat species (*Plecotus spp.*) with contrasting conservation status

Inauguraldissertation
der Philosophisch-naturwissenschaftlichen Fakultät
der Universität Bern

vorgelegt von

Sohrab Ashrafi

von Shahre-kord, Iran

Leiter der Arbeit:

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Der Dekan
Prof. Dr. U. Feller

Content

1

General introduction

1.1 Resource partitioning

In accordance to the principle of competitive exclusion (Gause´s principle) a stable coexistence of species within a community is only plausible when the limited resources are differentiated (Hutchinson 1957, 1978, Ricklefs 1990, Arlettaz *et al.* 1997, Wang, Zhang and Wang 2005). Species which live together must differ adequately in some aspects of their ecological niche to permit stable coexistence in the community (McNAb 1971, Arlettaz, Perrin and Hausser 1997). Differentiation occurs mostly along the two major dimensions, diet and habitat, which are reflected by foraging in the environment (Pianka 1969, Arlettaz 1999). When two or more similar species live in sympatry, resource partitioning is the predicted mechanism to decrease the effect of interspecific competition. According to the principle, behavioural differences must occur to permit coexistence. This is in agreement with the Lotka-Volterra model which states that a stable coexistence for actual competitors in the community occurs if the inter-specific competition is lower than intra-specific competition (Begon, 1986).

1.2 Cryptic species

Cryptic species are defined as a group of species, which are morphologically alike and hence difficult to distinguish based on external features, despite being genetically distinct (Jones 1997). These species are erroneously classified under one species name – until several species are discovered. Nowadays, using molecular methods resulted finding the discovery of many cryptic species in many taxa (e.g. in ants: Schlick-Steiner *et al*. 2006; in fish: Kon *et al*. 2006; in bats: Kiefer and Veith 2001). The confounded ecological and biogeographical aspects of cryptic species, call for a careful re-evaluation of their distribution and ecological requirements (Arlettaz 1999, Sattler *et al*. 2007). Cryptic species require special consideration in conservation planning because the existence of cryptic complexes presents at least two problems. First, species already considered as endangered or threatened might be composed of multiple species that are even rarer than previously supposed and second; the different species might require different conservation strategies (Bickford *et al.* 2006).

1.3 Long-eared bats (*Plecotus spp.*) as model species

Long-eared bats, genus *Plecotus* are widespread in the Palearctic zoogeographic zone (Spitzenberger *et al*. 2006). Several specific traits are unique to the long-eared bats from genus *Plecotus* (Horacek 1975, Swift 1998). With extraordinary long ears, which enable them to detect passively their prey, large eyes and delicate faces, they are very attractive to people. Moreover, they are morphologically easy distinguishable from other bat species due to their huge ears, but telling them apart from each other is absolutely problematic (Ashrafi *et al*. in press[1]).

Around 1960 scientists discovered the occurrence of two forms of *Plecotus* bats in Europe (Bauer 1960). Bauer described the new species as *Plecotus austriacus*, the form which previously described by Fischer in 1829. So, from then until recently, two *Plecotus* species were recognized in syntopy throughout Europe: *P. auritus* (Linnaeus, 1758) and *P. austriacus* (Fischer, 1829). With the rapid development of molecular techniques this has changed in only a short time with five European species of plecotine bats recognized today: *Plecotus auritus, P. austriacus, P. kolombatovici* Dulic, 1980, *P. macrobullaris* Kuzjakin, 1965, and the Sardinian insular endemic *P. sardus* Mucedda, Kiefer, Pidinchedda & Veith, 2002. Based on phylogenetic analysis, two major clades of plecotine bats in Europe were distinguished, the *auritus* group including *P. auritus*, *P. macrobullaris* and *P. sardus*; and the *austriacus* group, which contains *P. austriacus* and *P. kolombatovici* (Juste *et al*. 2004).

Besides the two sympatric *Plecotus* species (*Plecotus auritus* and *P. austriacus*), occurrence of a third cryptic species, *P. macrobullaris* in the alpine area of Switzerland has proven by molecular evidence (Kiefer & Veith 2001, Kiefer *et al.* 2002, Spitzenberger, Haring and Tvrtkovic 2002, Spitzenberger, Strelkov and Haring 2003). This new species is distributed in the mountainous area from the Pyrenees, along the entire alpine massif eastwards until to the Alborz Mountains in Iran (Garin *et al.* 2003, Juste *et al.* 2004, Tvrtković *et al.* 2005, Spitzenberger 2006).

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[1] Chapter 2 of this thesis, the forthcoming thesis is structured as a collection of articles which each chapter presents a separate paper.

1.4 Research topics in this thesis

1.4.1 Identification of cryptic Plecotus species

The identification and description of cryptic species have important implications for conservation (Bickford, *et al.* 2006). Up to now, identification of *Plecotus* bat species is problematic and only possible with certainty through molecular methods (Kiefer *et al.* 2002, Spitzenberger *et al*. 2002). In the **second chapter** of this thesis, we focused on the development of a field method for the identification of cryptic long-eared bats in Switzerland. We created a simple but powerful set of linear functions which can easily be applied by measuring eight external characters in the field. This identification method produced precise result (97.5% accuracy of correct identification) for data from Switzerland. Availability of such a rapid, accurate and cost-effective identification method, compared to non-instantaneous and expensive genetic methods, eases any ecological, behavioural and conservation studies on the cryptic *Plecotus* species.

1.4.2 Trophic niche

One of the major ecological niche dimensions which have been studied in several bat species is the trophic niche, one of the major niche dimensions. In the **third chapter** of this thesis, we investigated, firstly, the diet composition of long-eared bats, especially of the newly discovered cryptic species *P. macrobullaris*. Findings revealed a similar niche width and diet composition for *P. macrobullaris* and *P. austriacus*. It might indicate that these two species actually compete for the same food resources which can reflect their current parapatric distribution in the wild. In the unique mixed colony of *P. auritus* and *P. macrobullaris*, competition avoidance between *P. auritus* and *P. macrobullaris* was achieved through a mechanism of food resource partitioning. Intraspecific overlaps were larger than the interspecific overlaps, a situation which allows a stable coexistence.

1.4.3 Habitat selection

Habitat selection studies which show species-specific habitat requirements for bats are an important key of their conservation management (Walsh and Harris, 1996). As one of the main niche dimensions, we investigated habitat selection of all three *Plecotus* bat species in Switzerland, using radiotracking

(chapter four). We investigated the foraging sites of three different *Plecotus* species separately with respect to habitat characters to identify foraging habitat selection, using a generalized linear mixed model (GLMM) with binomial distribution. In spite of extreme similarities in morphology and echolocation calls (Dietrich *et al.* 2006, Ashrafi *et al*. in press) our investigations revealed distinct habitat selection for each species. We found that these species differed not only in their foraging range but undoubtedly also in their habitat selection, as predicted from niche theory for highly similar species.

1.4.4 Ecological niche modelling of three long-eared bats

In recent years, the development of predictive habitat models has been increasingly used to detect the suitable habitat of species and moreover mapping habitat predictions (Klar, N. *et al.* 2008). In the **fifth chapter,** we applied ecological niche factor analysis (ENFA) using presence-only data for all three *Plecotus* bat species from whole Switzerland to investigate the ecological niche requirements of *Plecotus* bat species at the landscape scale. Our findings show the most important eco-geographical predictors for the presence of the three *Plecotus* species in Switzerland. *P. auritus* inhabits the broadest niche, with occurrence predicted in most forested regions of Switzerland. Positive effects of orchards and vineyards but negative effects of coniferous and open forests were found for *P. austriacus.* Deciduous forests were important for *P. macrobullaris* which avoided meadows. A slightly narrower niche was predicted for *P. macrobullaris* which mainly occupied areas in the Central and Southern Alps. A very narrow niche was found for *P. austriacus* which was predicted to occur mainly in the lowlands.

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2

Multiple morphological characters needed for field identification of cryptic long-eared bat species around the Swiss Alps

Multiple morphological characters needed for field identification of cryptic long-eared bat species around the Swiss Alps

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2.1 Abstract

The identification of cryptic species may significantly change our view about their distribution, abundance, ecology and therefore conservation status. In the European Alps, molecular studies have revealed the existence of three sibling species of plecotine bats: *Plecotus auritus*, *P. austriacus* and, very recently, *P. macrobullaris.* Knowledge of the ecological niche partitioning of cryptic species is requisite to develop sound conservation policies. Yet, this requests the development of unambiguous identification methods easily applicable in the field. This study investigates the reliability of several morphological methods used for species recognition and proposes a new identification key for field workers. We captured 214 *Plecotus* bats from 29 sites in four bioregions within Switzerland, collected biopsy punches for genetic analysis, described and measured external morphological characters. All three species occurred as mono-specific colonies, except at one site where *P. auritus* and *P. macrobullaris* shared the same church attic. Qualitative traits alone did not allow reliable separation of the three species. A series of multivariate analyses conducted on external linear measurements resulted in a discriminant function enabling correct species classification with a 97.5% probability. Compared to genetic analysis, our multivariate morphological method represents a valuable, rapid and cost-effective alternative.

Keywords: *Plecotus*, sibling species, genetic identification, discriminant function analysis, mitochondrial DNA, Switzerland

2.2 Introduction

Cryptic species are a group of species which are morphologically alike and hence difficult to distinguish based on external features, despite being genetically distinct (Jones 1997). Cryptic species have been discovered in many taxa (e.g. in ants: Schlick-Steiner *et al*. 2006; in fish: Kon *et al*. 2006; in bats: Ruedi *et al*. 1990, Arlettaz *et al*. 1993, Kiefer and Veith 2001, Mayer *et al*. 2007). Before their distinction, cryptic species were biogeographically and ecologically confounded. This calls for a careful re-evaluation of their distribution and ecological requirements, especially when it comes to conservation issues (Arlettaz *et al.* 1997a, Arlettaz 1999, Sattler *et al*. 2007).

Chiroptera are one of the most speciose groups of mammals with more than 1,100 species described so far and recent discoveries of many cryptic species (Harris *et al.* 2006, Ceballos and Ehrlich 2009), which calls for investigations of species' ecological needs. With 22% of species classified as threatened worldwide, bats are amongst the most threatened vertebrates. In temperate biomes, bats are often closely associated with humans. This is due to their dependence on man-made structures for roosting and reproducing, a fact that increases their vulnerability. Currently, 42 species of bats are recognized in Europe, with 21% classified as threatened or near threatened (IUCN Red list, 2009). Bats not only require a complex network of seasonal roosting sites, but they are also very selective as regards foraging habitats (Entwistle *et al*. 1996, Arlettaz 1999, Bontadina *et al*. 1997, 2002, Popa-Lisseanu *et al.* 2009). A proper understanding of all these requisites is essential for implementing efficient conservation action.

The genus *Plecotus* (long-eared bats) is widespread in the Palearctic (Spitzenberger *et al*. 2006). It has been subjected to several studies in Europe (e.g. Entwistle *et al*. 1996, Kiefer *et al*. 2001, 2002, Juste *et al*. 2004, Dietrich *et al*. 2006, Spitzenberger *et al*. 2006). From the 1960s until recently, two sympatric *Plecotus* species were recognized in Europe: *Plecotus auritus* (Linnaeus, 1758) and *P. austriacus* (Fischer, 1829). With the continuing development of molecular techniques, five European species of plecotine bats are now recognized: *Plecotus auritus, P. austriacus, P. kolombatovici* Dulic, 1980, *P. macrobullaris* Kuzjakin, 1965, and the Sardinian insular endemic *P. sardus* Mucedda, Kiefer, Pidinchedda & Veith, 2002. Based on phylogenetic analysis, Juste *et al*. (2004) distinguished two major clades of plecotine bats in Europe, the *auritus* group including *P. auritus*, *P. macrobullaris* and *P. sardus*; and the *austriacus* group, which contains *P. austriacus* and *P. kolombatovici.* In Central Europe, the presence of the two sibling species *P. auritus* and *P. austriacus* has been known for some time. Recently, however, molecular evidence has proven the existence of a third sympatric cryptic species, *P. macrobullaris,* which occurs mostly in the Alpine region (Kiefer and Veith 2001, Kiefer *et al.* 2002, Spitzenberger *et al*. 2006). In the European Alps, *P. macrobullaris* seems to occur sympatrically with *P. auritus* and in the vicinity of *P. austriacus* (Juste *et al*. 2004).

Reliable identification of these three species is difficult, so far only possible with certainty through molecular methods (Kiefer *et al.* 2002, Spitzenberger *et al*. 2002). However, these methods are time consuming and relatively expensive, with the results not instantaneously available to field workers. Therefore, in order to complement the current distribution and conservation status of Central European *Plecotus*, an easy identification method is needed. The present study aims, firstly, to evaluate the reliability of morphological characters already proposed for species identification and, secondly, to build an up-dated, refined identification kit for field workers in Central Europe, which is a pre-requisite for any ecological and conservation studies in that area.

2.3 Materials and methods

2.3.1 Field sampling and data collection

In summer 2006 we sampled 214 *Plecotus*, which were assumed to belong to the three target species, at 29 sites in southern, northern and western Switzerland (Table 1). We captured *Plecotus* bats in four out of the six bioregions in Switzerland (Jura Mountains, Plateau, Western Central Alps and Northern Alps; Gonseth *et al*. 2001), where we expected the species to occur sympatrically. Most of the capture sites were already known as nocturnal or colonial roosts of long-eared bats (data bank of the Swiss Bat Conservation centres). Sites were not randomly chosen, but were selected in order to obtain a representative, as far as possible balanced sample including all three species. Bats were captured using mist and hand nets, typically near entrances to roosts. In a few cases, we captured individuals on the wing above ponds or in the roost vicinity.

The following eight external measurements were taken from all captured adult individuals according to Dietz and von Helversen (2004) and Tvrtkovic *et al*. (2005). We used either a dial caliper (accuracy 0.01 mm): length of forearm (FA), thumb length without claw (TH), tibia length (TIB), hind foot without claw (HF); or a steel ruler (accuracy 0.5 mm): ear length (EARL), ear width (EARW), tragus length (TRAGL) and tragus width (TRAGW) (Appendix s1, Supporting information). Additionally, a number of qualitative characters were recorded: general fur colour, especially on the back (three levels: white brown, brown, grey), colour of fur on the throat (three levels: brown, brownish grey, whitish grey), penis shape (three levels: narrowing toward the end, club shape, cylindrical pointed at the tip), density and position of hairs on hind foot (three levels: long and upright hairs on the whole hind foot, long sticking hairs at toes, short hairs on toes). The presence of a triangular pad on the lower lip (TP) as well as sex were also recorded. A biopsy punch (4 mm diameter) of wing membrane was collected from all individuals and stored in ethanol for subsequent genetic analyses.

2.3.2 Molecular species identification

Skin samples were dissolved in lysing buffer and Proteinase K at 55°C for 24 hours. Total DNA was extracted from fresh skin tissue using a high pure PCR template preparation kit (Roche Diagnostics GmbH). DNA was extracted according to the protocol suggested by the manufacturer. PCR amplification to RNA was performed with primers, using standard procedures. The 550 bp fragment of the rRNA 16s gene was obtained using the primers L 15975 and 16425 (Wilkinson and Chapman 1991). The PCR cycling procedure was as follows: denaturation step: 60 s at 95°C, 39 cycles, primer annealing for 90 s at 55°C and extension for 120 s at 72°C. PCR products were purified using the "High pure PCR product purification kit" (Boehringer, Mannheim). The amplified gene fragments were sequenced using a capillary ABI prism 377 sequencer. Then sequence alignments were carried out using DNA Sequencer and MEGA 3.1 software.

2.3.3 Morphometric species identification model

In order to identify the species based on morphological characteristics, we built a discriminant model on the prior genetically identified individuals (see Arlettaz, Ruedi, and Hausser 1991, Arlettaz *et al*. 1997a). We had to exclude four individuals from the dataset due to poor quality sequences from the molecular analysis. Therefore, we used the quantitative and qualitative morphometric measurements from 210 genetically identified individuals of all three species to create an identification model. Initially, a principal component analysis (PCA) was carried out on the eight quantitative variables to describe the overall multivariate structure of the dataset. Then we used discriminant analysis (DA) and a multinomial logit model (MNL) to group individuals based on morphological characters. The analysis was performed using the program R 2.4.1 (R Development Core Team 2006).

2.3.3.1 Discriminant analysis (DA)

We tested the assumptions of both multivariate normality and common variance-covariance matrix. Firstly, we performed a Mardia test, according to Timm (2002), for estimating multivariate normality, which revealed significant deviations from normality. We transformed the data using a Box-Cox transformation (Sokal and Rohlf 1995), which still resulted in deviation from normality. Therefore, eight outliers detected by the Mardia test were excluded from the dataset to obtain normal distribution (Tabachnik & Fidell 2001).

The assumption of a common variance-covariance matrix was tested with Box's M test, using SYSTAT 10 software. In spite of slight covariance differences, most likely due to uneven sample sizes, we used both linear and quadratic discriminant analysis (see Wahl & Kronmal 1977 and Tabachnik & Fidell 2001). The model was built using 80% of the data, with subsequent cross validation conducted with the remaining 20% data to derive a misclassification rate. We performed both linear (LDA) and quadratic (QDA) discriminant analyses, including quantitative variables, to compare error rates of the two methods for the same dataset. Finally, the outliers excluded during the first step of the modelling procedure were tested in the model (see Tabachnik 2001).

2.3.3.2 Multinomial logit model (MNL)

The model was built using all eight continuous and two categorical variables (sex and triangular pad on the lower lip) using 80% of the dataset as training data and the remaining 20% for model validation. The modelling procedure excluded missing values which caused four of the qualitative variables not being further considered. We used a stepwise search method based on AIC criterion for selecting the best model (Faraway 2006), with better performing models being those with low AIC values.

2.4 Results

2.4.1 Molecular species identification

All three *Plecotus* species were present in our sample: 104 *Plecotus macrobullaris* (84♀, 20♂*),* 64 *P. auritus* (40♀, 24♂) and 42 *P. austriacus* (25♀, 17♂) (Table 1). At 24 sites, where several individuals were caught, we have genetic evidence for the presence of mono-specific populations, except in Obergesteln (canton of Valais), where two nursery colonies of *P. macrobullaris* and *P. auritus* shared the same church attic. *P. macrobullaris* was found between 600 and 1360 m altitude in this study (Table 1). A phylogenetic analysis confirmed the identification of all three species in our sample, and showed the existence of three haplotypes of *P. auritus*, one haplotype of *P. macrobullaris* and one haplotype of *P. austriacus* (Fig. 1). All mitochondrial haplotypes except one where found in previous studies (Kiefer, 2007, Benda *et al.* 2004). Haplotype aurHT7 was recorded for the first time; it was found at four locations in three cantons (Table 1).

2.4.2 Biometric species identification

Qualitative traits did not reliably separate the three species. For example, we found overlap in fur coloration from brown to grey in all three species, possibly reflecting different age classes. Also, the majority of individuals had some long and visible hairs on the feet and around the toes, rendering this identification criterion difficult to apply. Yet, *P. macrobullaris* was the only species to bear a triangular pad on the lower lip, with almost 95% of individuals (98 out of 104) possessing this pad. Altogether, the qualitative characters did not allow a reliable species separation by applying the currently existing identification keys (Spitzenberger *et al*. 2006, Dietz, von Helversen, and Nill 2009) in the field.

Results of the principal component analysis of the eight quantitative external characters showed that the first component explained 35.2% of the overall variance. This component was mostly correlated with variables expressing body size: length of forearm (FA), tibia length (TIB), tragus length (TRAGL) and tragus width (TRAGW). The second component explained 23.6% of the total variance and correlated mainly with size of extremities, including ears: thumb length (TH), hind foot (HF), ear width (EARW) and, again, tragus width (TRAGW). The third component explained only 7% of variation and was thus disregarded. There was a large overlap between the three species in the multivariate space (Fig. 2). The low level of variance explained by the PCA further informs about weak correlations between single characters. Therefore, all eight variables were used for the discriminant analysis.

2.4.2.1 Discriminant analysis

The comparison between cross validation results of both linear and quadratic discriminant analysis showed similar error rates (Table 2). Therefore, the linear discriminant function was preferred because of its simpler form. Most of the specimens (97.5%, i.e. 39 out of 40) were correctly classified, with only one individual misclassified, which gives an error rate of 2.5% (Table 2). A scatter-plot of the scores of the linear discriminant functions 1 (LD1) and 2 (LD2) shows a clear separation of *P. austriacus* from the other two species by the first discriminant function. Yet, *P. macrobullaris* and *P. auritus* overlap, with LD2 alone not allowing a correct separation (Fig. 3). The discriminant functions in Table 3 were used for calculating classification equations for the three species (an Excel file for species determination is provided as Appendix 2 in the supporting information). Species identity can be determined based on the species-specific function (out of the three functions) yielding the largest discriminant score. The outliers were also tested with the discrimination functions, resulting in only one of the eight outliers being misclassified. There was only one actual *P. macrobullaris* identified as *P. austriacus,* most likely due to mistakes in measuring the animal or bad data recording. This outcome anyway indicates a high model performance.

2.4.2.2 Multinomial logit model

Based on AIC value, the stepwise search method elected a model including 5 variables: forearm length (FA), tragus length (TRAGL), ear width (EARW), hind foot length (HF) and presence or absence of triangular pad on the lower lip (TP) (Table 4). Cross validation results of the multinomial logit model showed a larger error rate (10%, i.e. 4 individuals out of 40) for this model compared to the discriminant analysis. Apparent and overall error rates of the three models are shown in Table 2.

2.5 Discussion

This study shows that field identification of all three species of *Plecotus* bats within and around the Swiss Alps is possible with a high accuracy by applying a simple but powerful set of linear functions. In contrast, due to intermediate or often indistinct traits, no reliable identification could be achieved using the classical external characters which had been proposed in previous studies (Tvrtkovic *et al*. 2005, Spitzenberger *et al*. 2006, and Dietz *et al*. 2009).

Our results confirm the presence of the cryptic species *P. macrobullaris* in the Swiss Alps (Kiefer and Veith 2001, Juste *et al*. 2004) and evidence the syntopic (roost sharing) occurrence of *P. macrobullaris* and *P. auritus*. While mixed colonies of *P. auritus* and *P. austriacus* are well known (e.g. Beck 1994), there is to our knowledge only one previous report of a mixed colony involving *P. macrobullaris* with another *Plecotus* species (*P. kolombatovici;* Croatia; Pavlinić 2008). According to the principle of competitive exclusion (Hutchinson 1957, 1978), sympatric distribution as well as shared nursery roosts of sibling species signify species-specific differentiation in resource utilisation, insofar as this co-existence is stable, which is very likely here (Arlettaz *et al*. 1997b, 2000, Arlettaz 1999).

The availability of accurate, rapid and cost-effective identification methods based on morphological characters easily assessed in the field – contrary to non-instantaneous and expensive genetic tools – is advantageous for any ecological, behavioural and conservation studies. The novel identification key proposed here for identifying *Plecotus* will facilitate the task of bat ecologists and conservationists within Switzerland and might be valid also for the rest of the European Alps. The qualitative characters proposed earlier on did not work well in our study. For instance, the fur criteria proposed by Kiefer and Veith (2001: denser, longer and whiter throat fur in *P. macrobullaris* compared to *P. austriacus* and *P. auritus*) did not perform well in our study area. The same holds true for a whitish grey fur on the throat and belly reported for *P. macrobullaris* by Spitzenberger *et al*. (2002), Tvrtkovic *et al*. (2005) and Dietz *et al*. (2009). In our sample, only 6 out of 104 individuals of *P. macrobullaris* had that character. In contrast, a triangular pad on the lower lip, another distinctive feature proposed to separate *P. macrobullaris* (e.g., Tvrtkovic *et al*. 2005, Dietz *et al*. 2009) was present in 95% of our *P. macrobullaris*. This character is thus partly discriminant: individuals harbouring a lip pad almost certainly belong to *P. macrobullaris*. However it is not always easy to appraise the presence of a lip pad in all individuals, due to variation in color and shape of the triangle. Some field experience with *Plecotus* bats is necessary in this regard. Presence of long hairs on the hind feet was also claimed to be a good criterion for species separation (e.g. Dietz *et al*. 2009) but our evaluation suggests that this character cannot confidently differentiate any of the three species, at least in our sample. In most cases longer hairs were visible in *P. auritus* and shorter hairs in *P. austriacus,* while *P. macrobullaris* had somewhat intermediate hairs, but the judgment was never definitive. Tvrtkovic *et al*. (2005) suggested the use of a bivariate scatter plot of thumb length (TH) regressed against hind foot length (HF) to separate Croatian *P. auritus* and *P. austriacus*. This did not work on our sample which shows a considerable overlap (34%) between the two species (Fig. 4). Finally, Tvrtkovic *et al*. (2005) and Dietz *et al*. (2009) have suggested penis shape as a good character for separating males of the three species (*P. macrobullaris*: cylindrical penis); *P. auritus* (penis narrowing toward the end); *P. austriacus* (club-shaped penis). The observation of the few male specimens in our sample (n = 25) confirmed this, with 96% of individuals correctly identified. However, penis shape is not very helpful for assessing the identity of nursery populations from which males are most of the time absent.

We conclude that there is no simple solution to achieve reliable identification of long-eared bats within the alpine region of Switzerland: neither qualitative characters nor bivariate graphs offer a reliable alternative to multivariate approaches. While none of the already proposed identification characters are reliable, using them singly or in combination does not render a trustworthy recognition. Based on the multilinear combination of eight characters we achieved a high probability (97.5%) of correct species classification. By checking several individuals per colony, our method represents a major improvement compared to other methods proposed earlier on. As most colonies are mono-specific, three individuals belonging to the same species increase the probability of correct identification to more than 99%. Our discriminant functions will greatly facilitate the field identification of *Plecotus* bats within and around the Swiss Alpine region. The validation of this method in the rest of the Alps and Central Europe as well has to be verified. These are crucial steps paving the way for future investigations of long-eared bats' distribution, ecology and conservation.

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2.6 References

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Table1. Study sites with coordinates, altitude, sample size (N = number of individuals), genetically identified species and haplotype (the new haplotype for *P. auritus* is in italics).

Sites (canton)[2]	Latitude	Longitude	Elevation (m)	Date	Species	N (♀, ♂)	Haplotype
Arbaz (VS)	46° 12′	7° 21′	1100	16.08.2006	*P. macrobullaris*	1 (1,0)	macHT1
Ayent (VS)	46° 16′	7° 24′	1000	18.08.2006	*P. macrobullaris*	8 (1, 7)	macHT1
Basse Nendaz (VS)	46° 11′	7° 18′	1000	9.08.2006	*P. macrobullaris*	5 (1, 4)	macHT1
Blitzingen (VS)	46° 26′	8° 11′	1300	3.08.2006	*P. auritus*	5(5, 0)	aurHT3
Col de Bretolet (VS)	46° 08′	6° 47′	1960	7.09.2006	*P. auritus*	3(2, 1)	aurHT3
Collex (GE)	46° 16′	6° 07′	450	4.09.2006	*P. austriacus*	7(4, 3)	ausHT1
Gampel (VS)	46° 18′	7° 44′	640	4.08.2006	*P. macrobullaris*	10(10, 0)	macHT1
Gouffre Cathy Arzier (VD)	46° 29′	6° 08′	1300	4.09.2006	*P. auritus*	14(4, 10)	aurHT3, *aurHT7*
Grengiols (VS)	46° 22′	8° 05′	900	3.08.2006	*P. macrobullaris*	16(15, 1)	macHT1
Grimsuat (VS)	46° 15′	7° 23′	850	17.08.2006	*P. macrobullaris*	3(2, 1)	macHT1
Isérables (VS)	46° 10′	7° 15′	1000	19.08.2006	*P. auritus*	1(0, 1)	aurHT4
Kirchrued (AG)	47° 17′	8° 5′	500	26.08.2006	*P. auritus*	7(5, 2)	*aurHT7*
Lax (VS)	46° 23′	8° 7′	1050	28.07.2006	*P. macrobullaris*	18 (15, 3)	macHT1
Lens (VS)	46° 15′	7° 23′	1100	18.08.2006	*P. macrobullaris*	3(2, 1)	macHT1
Leytron, mine (VS)	46° 11′	7° 12′	617	6.08.2006	*P. macrobullaris*	2(1, 1)	macHT1
Ittenthal (AG)	47° 31′	8° 03′	410	26.08.2006	*P. austriacus*	1(0, 1)	ausHT1
Mandach (AG)	47° 32′	8° 11′	490	22.08.2006	*P. austriacus*	27(17, 10)	ausHT1
Mönthal (AG)	47° 31′	8° 08′	500	21.08.2006	*P. austriacus*	4(1, 3)	ausHT1
Obergesteln (VS)	46° 30′	8° 19′	1360	2.08.2006	*P. auritus*	5(3, 2)	aurHT3
Obergesteln (VS)	46° 30′	8° 19′	1360	2.08.2006	*P. macrobullaris*	6(4, 2)	macHT1
Pfyn (VS)	46° 18′	7° 36′	420	7.08.2006	*P. auritus*	8(7, 1)	aurHT3, aurHT4
Poteux Cave (VS)	46° 10′	7° 10′	1000	29.07.2006	*P. macrobullaris*	12(7, 5)	macHT1
Salins (VS)	46° 12′	7° 21′	600	15.08.2006	*P. auritus*	17(11, 6)	*aurHT7*
Sembrancher (VS)	46° 04′	7° 09′	740	31.07.2006	*P. macrobullaris*	8(7, 1)	macHT1
Thalheim (AG)	47° 26′	8° 06′	460	2.08.2006	*P. austriacus*	1(0, 1)	ausHT1
Ulrichen (VS)	46° 30′	8° 18′	1340	30.07.2006	*P. macrobullaris*	2(1, 1)	macHT1
Vens, pond (VS)	46° 05′	7° 06′	1250	30.07.2006	*P. auritus*	1(1, 0)	*aurHT7*
Vens, cave (VS)	46° 05′	7° 06′	1200	08.08.2006	*P. auritus*	3(2, 1)	aurHT4, *aurHT7*
Wiler-Guttet (VS)	46° 19′	7° 40′	1260	8.08.2006	*P. macrobullaris*	14(11, 3)	macHT1
Zeihen (AG)	47° 28′	8° 05′	450	25.08.2006	*P. austriacus*	2(2, 0)	ausHT1

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[2] VS = Valais, GE = Geneva, VD = Vaud, AG = Aargau,

Table 2. Classification tables obtained from linear discriminant analysis (LDA), quadratic discriminant analysis (QDA) and multinomial logit model (MNL) for *Plecotus macrobullaris* (Pmac), *P. auritus* (Paur) and *P. austriacus* (Paus). Apparent error rates (misclassifications for each group, divided by the group sample size) and overall error rates of cross validation tests.

	Predicted species											
	LDA				QDA				MNL			
Actual species	Pmac	Paur	Paus	Error	Pmac	Paur	Paus	Error	Pmac	Paur	Paus	Error
Pmac	20	1	0	0.050	20	1	0	0.050	18	2	0	0.100
Paur	0	11	0	0.000	0	11	0	0.000	2	10	0	0.167
Paus	0	0	8	0.000	0	0	8	0.000	0	0	8	0.000
Apparent error rate				0.017				0.017				0.090
Overall error rate				0.025				0.025				0.100

Table 3. Constants and classification coefficient functions for the three *Plecotus* species. FA: length of forearm, TH: thumb length without claw, TIB: tibia length, HF: hind foot without claw, EARL: ear length, EARW: ear width, TRAG: tragus length and TRAGW: tragus width (see appendix 2 in supporting information).

	Constant	FA	TH	TIB	HF	EARL	EARW	TRAGL	TRAGW
Pmac	-1373.50	19.820	30.389	7.405	-0.737	17.685	23.308	24.605	18.204
Paur	-1266.68	18.691	32.405	7.230	2.124	17.226	22.122	22.453	14.415
Paus	-1281.60	21.937	26.501	0.949	-5.166	17.761	25.386	21.919	19.251

Table 4 . MNL model selection results with AIC, corrected AIC values and relative weights.

Model	AIC value	AICc	Delta AICc	AIC weight
Species~FA+TRAGL+EARW+HF+Factor(TP)	27.8250	29.4087	0.0000	1.0000
Species~FA+EARW+HF+factor(TP)	52.3060	53.4115	24.0027	0.0000
Species~TRAGL+EARW+HF+factor(TP)	63.0838	64.1893	34.7805	0.0000
Species~FA+TRAGL+EARW+HF	69.2416	70.3471	40.9384	0.0000
Species~FA+TRAGL+HF+factor(TP)	82.4746	83.5801	54.1714	0.0000
Species~FA+TRAGL+EARW+factor(TP)	128.6731	129.7786	100.3699	0.0000

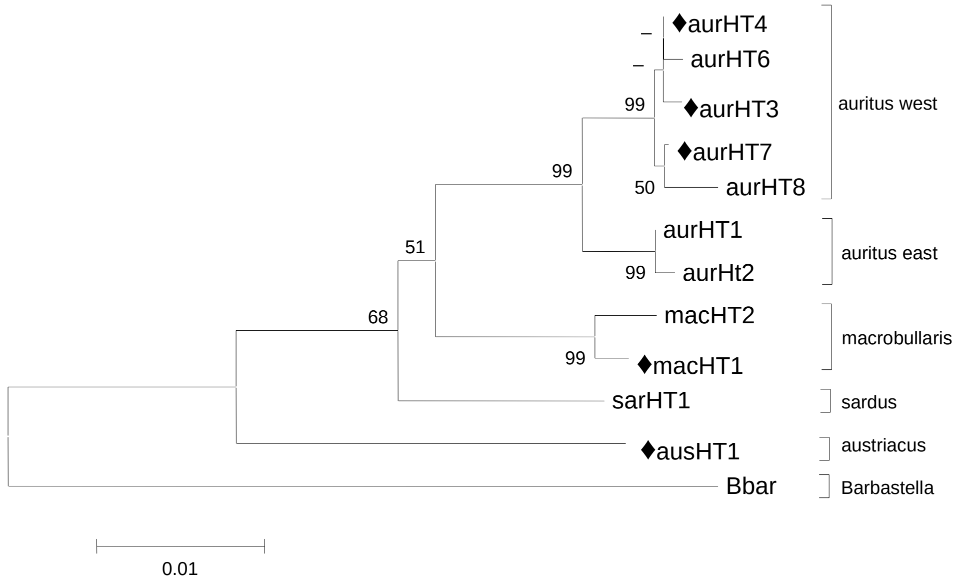

Figure 1. Phylogenetic tree drawn from published haplotypes of the genus *Plecotus* in Europe, with *Barbastella barbastellus* acting here as an outgroup. Bootstrap support values (2000 replicates) indicated for neighbour joining (NJ) and values less than 50 % are not shown. Haplotypes found in this study are depicted with a diamond. Reference numbers in Gene Bank: macHT1 (AY 531628), aurHT1 (AF 629230), aurHT3 (AF 326106), aurHT4 (AF 529229) and ausHT1 (AY 134022).

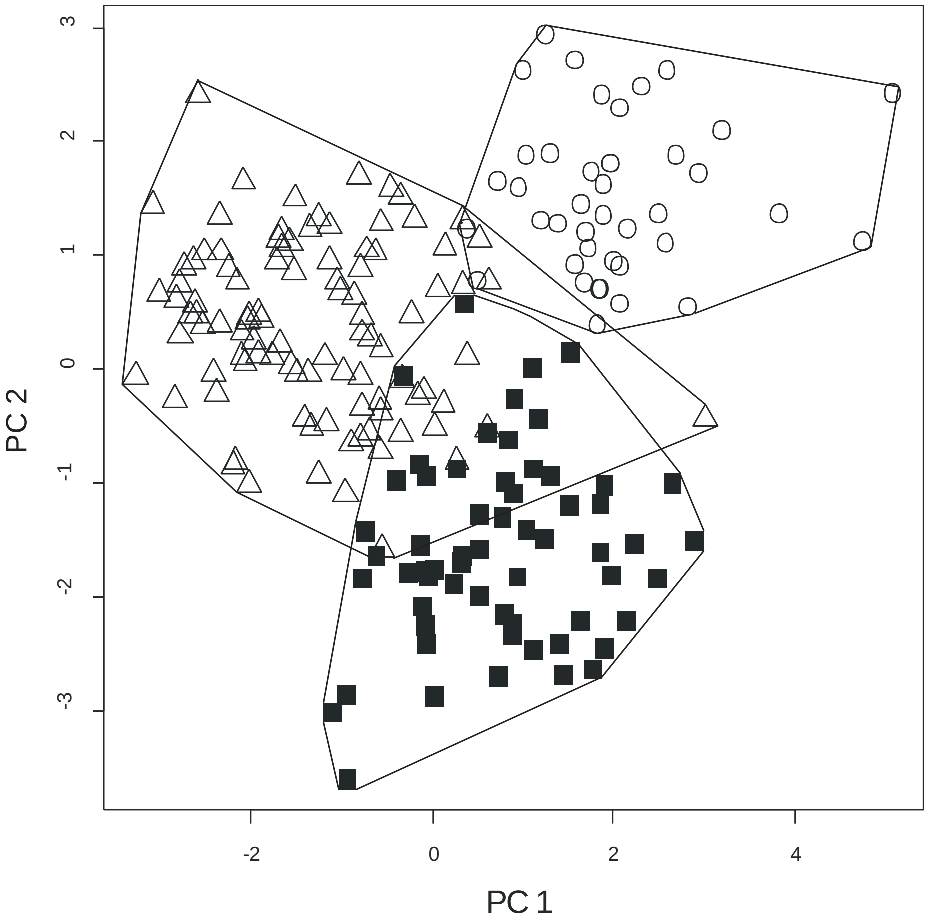

Figure 2. Relationship between first and second factors (PC) of a principal component analysis on eight external morphological characters. PC1 and PC2 explained 35.21% and 23.57% of the total variance, respectively. Untransformed data were used for the three species *P. austriacus* (○), *P. macrobullaris* (△) and *P. auritus* (■). The species groups are encompassed by polygons.

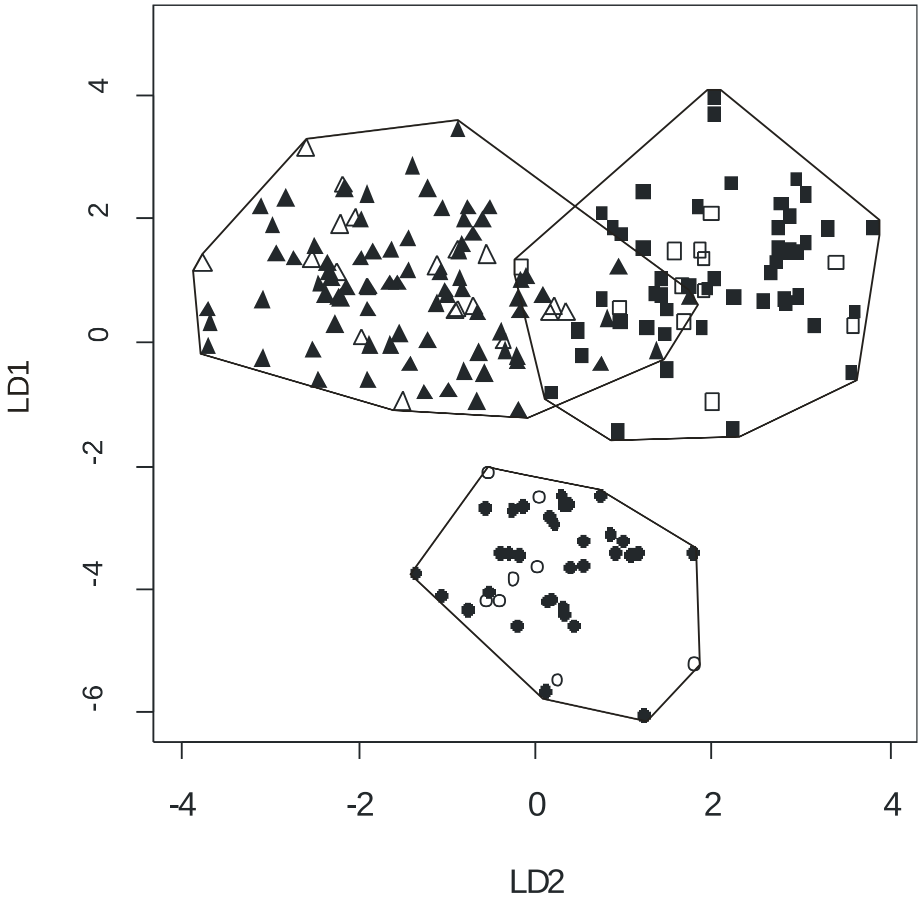

Figure 3. Scatter plot of the scores of the linear discriminant factor 1 and 2 obtained from both training data (full symbols) and validating data (empty symbols) for the three species *P. austriacus* (○), *P. macrobullaris* (△) and *P. auritus* (☐). Species groups are enclosed by a polygon.

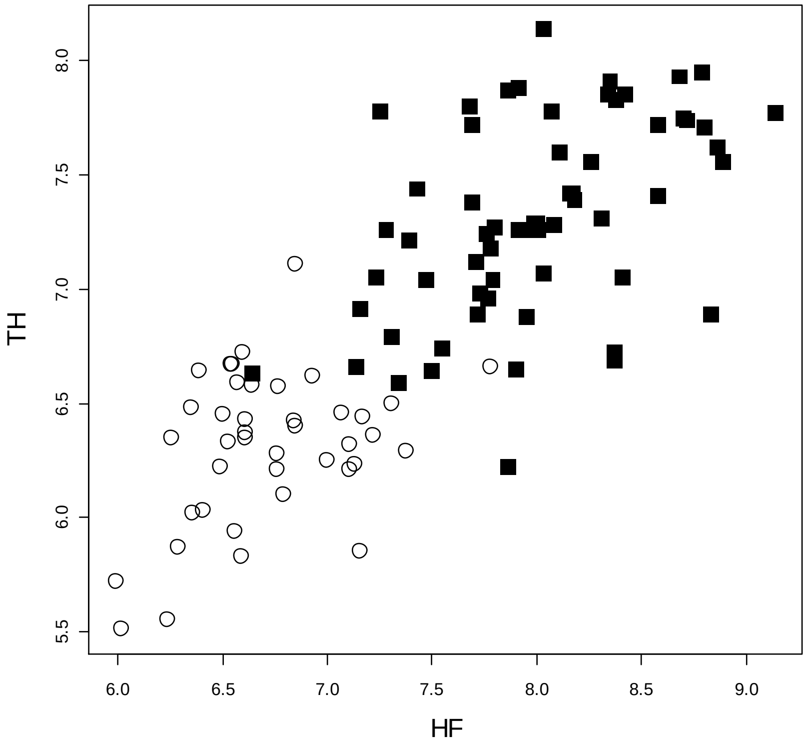

Figure 4. Scatter plot of hind foot (HF) against thumb length (TH) from *P. auritus* (■) and *P. austriacus* (○), measurements are in mm.

Appendix S1. External measurements of long-eared bats (*Plecotus spp.*) in Switzerland. Number of individuals (n), mean, standard deviation (SD), min and max for eight variables (FA: length of forearm, TH: thumb length without claw, TIB: tibia length, HF: hind foot without claw, EARL: ear length, EARW: ear width, TRAG: tragus length and TRAGW: tragus width). All measurements are in mm.

P. auritus (n=64)	Males				Females			
	mean	SD	min	max	mean	SD	min	max
FA	39.51	1.21	37.13	42.15	40.9	1.3	38.59	44.58
TH	7.38	0.39	6.66	8.14	7.27	0.46	6.22	7.95
TIB	19.86	0.62	18.68	20.96	20.11	0.65	18.85	21.29
HF	8.13	0.55	7.14	9.14	7.96	0.56	6.64	9.67
EARL	30.2	1.14	27	32	30.26	1.11	27.5	33
EARW	20.14	1.18	18	22.5	19.57	1.12	17.5	23
TRAGL	15.28	1.01	14	18.5	15.63	1.04	14	18
TRAGW	5.2	0.32	4.5	5.5	5.2	0.32	4.5	6

P. austriacus (n=42)	Males				Females			
	mean	SD	min	max	mean	SD	min	max
FA	39.77	1.22	37.15	41.6	40.66	0.78	38.59	42.2
TH	6.31	0.4	5.51	7.11	6.32	0.27	5.72	6.67
TIB	18.8	0.51	17.79	19.85	18.91	1.14	14.49	20.57
HF	6.64	0.32	6.02	7.31	6.84	0.46	5.99	7.84
EARL	30.67	0.97	29	33	31.1	0.81	30	33
EARW	20.97	0.9	19.5	23	21.04	0.67	19.5	22
TRAGL	15.44	0.62	14.5	16.5	15.56	0.37	15	16.5
TRAGW	5.65	0.27	5	6	5.65	0.28	5	6

P. macrobullaris (n=104)	Males						Females	
	mean	SD	min	max	mean	SD	min	max
FA	40.61	1.43	35.51	42.02	42.34	0.98	40.29	44.84
TH	6.89	0.45	6.09	7.72	6.94	0.34	6.11	7.77
TIB	20.14	0.71	18.47	21.57	20.95	0.61	19.17	22.13
HF	7.31	0.33	6.69	7.95	7.61	0.45	6.63	8.5
EARL	31.45	2.13	29.5	38	31.13	1.5	29	37
EARW	20	0.93	18.5	22	19.96	0.96	17.5	23
TRAGL	17.5	1.18	16	19.5	17.83	0.95	15.5	20
TRAGW	5.89	0.43	5.5	7	5.85	0.35	5	7

3

Trophic niche partitioning of cryptic species of long-eared bats in Switzerland: implications for conservation

Trophic niche partitioning of cryptic species of long-eared bats in Switzerland: implications for conservation

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Manuscript**:**

3.1 Abstract

Dietary niche partitioning is postulated to play a major role for the stable coexistence of species within a community, particularly among cryptic species. Molecular markers have recently revealed the existence of a new cryptic species of long-eared bat, *Plecotus macrobullaris,* in the European Alps, where only *P. auritus and P. austriacus* were previously known. The discovery of cryptic species calls for a re-evaluation of the conservation status of their species complex as whole, and a clarification of species-specific ecological requirements for conservation. We studied trophic niches as well as seasonal and regional variation of diet at eight colonies of the three *Plecotus* species occurring in Switzerland. Faeces were collected monthly (May-September) from individuals returning to roost after night foraging. Bat species identity was assessed with genetic markers. Twenty-one arthropod categories (orders or families) were recognized from the faeces. All three species fed predominantly on Lepidoptera, which made up 41, 87 and 88% (means across colonies) of the diet composition of *P. auritus*, *P. macrobullaris* and *P. austriacus,* respectively. The occurrence of numerous fragments of both diurnal and flightless insects in the diet of *P. auritus* (but rarely in the diet of the other two species) indicates that this species mostly gleans prey from substrates. *P. austriacus* and *P. macrobullaris* are more typical aerial feeders. The latter two species have narrow trophic niches, whilst *P. auritus* has a much broader diet. Comparison of intraspecific and interspecific niche overlaps in *P. auritus* and *P. macrobullaris* in sympatry suggests dietary niche partitioning between these two species. In contrast, the high similarity of the trophic niches of *P. austriacus* and *P. macrobullaris*, associated with a typical parapatric distribution, indicates competitive exclusion. The best conservation measures would be to preserve and restore habitats offering a high abundance of moths, the major prey of the three *Plecotus* species.

Keywords: cryptic species, niche partitioning, niche breadth, niche overlap, *Plecotus*, Switzerland

3.2 Introduction

The principle of competitive exclusion (Gause´s principle) is one of the most basic rules in ecology. It states that a stable co-existence of species within a community is only possible when the species-specific utilisation of limiting resources is well differentiated (Hutchinson 1957, 1978, Ricklefs 1990, Arlettaz, Perrin & Hausser 1997, Wang, Zhang & Wang 2005; but see López-Gómez, Molina-Meyer 2006, Seto & Akag 2007). Similarly, based on the Lotka-Volterra model of competition, a stable co-existence of actual competitors within a community implies that interspecific competition is lower than intraspecific competition (Begon, 1986). By corollary, comparing two types of overlap in resource utilization – the intraspecific overlap between all individuals within a single species population, and the interspecific overlap between every individual of one species and all individuals from the other species – allows testing for resource partitioning among coexisting species, assuming limiting resources (Arlettaz *et al.* 1997).

It has been found that the most important dimensions of the ecological niche are habitat and diet, with most of the ecological differentiation between species occurring along these two axes (Pianka 1969, Schoener 1986). Consequently, many ecological studies to date have focused on foraging habitats and diet composition of animals (e.g. Arlettaz *et al.* 1997, Arlettaz 1999, Vitt *et al*. 2000, Nicholls & Racey 2006). The existence of several taxonomic groups with cryptic species (species which appear morphologically identical but are genetically distinct) provides a unique opportunity to investigate the fine-grained resource partitioning mechanisms at play within animal communities (Arlettaz *et al.* 1997).

One such group of cryptic species with many recent discoveries that requires further investigation are bats, one of the most diverse mammalian taxa with more than 1100 species described globally. Bats also belong to the most endangered vertebrates in the world, with 22% of the species classified as threatened or near threatened (IUCN Red list, 2009). In the Western world, bats have undergone massive population declines since the mid 20th century. Widespread habitat loss and transformation, large-scale agricultural intensification, including pesticide application, are considered as the primary factors of bat population declines (Stebbings 1988, Ransome 1990, Walsh & Harris 1996a, b, Wickramasinge *et al*. 2004, Bontadina *et al.* 2008).

Recently, several cryptic species of bats have been discovered (e.g. Kiefer & Veith 2001, Benda *et al.* 2004, Benda, Hulva & Gaisler 2004). The taxonomic status of European long-eared bats (genus *Plecotu*s), which are widespread in the whole Palearctic zone, was scrutinized by Kiefer *et al.* (2002), Benda *et al.* (2004b) and Spitzenberger *et al*. (2006). Beside the two formerly recognized species (*Plecotus auritus* and *P. austriacus*), molecular markers could evidence the existence in the European Alps of a third cryptic species, *P. macrobullaris* (Kiefer & Veith 2001, Spitzenberger, Haring & Tvrtkovic 2002, Spitzenberger, Strelkov & Haring 2003). In Switzerland, this species mainly was known in the Alpine massif, above 600 m altitude, where it may be found in sympatry with *P. auritus* and in the vicinity of *P. austriacus* (Rutishauser *et al.* unpublished data[3], Ashrafi *et al.* in press[4]).

The three long-eared bats of Europe are not only morphologically extremely similar but also genetically closely related (Kiefer 2002, Spitzenberger *et al.* 2006): actually, there is no single external morphological character that enables full species distinction (Ashrafi *et al.* in press). The apparently stable coexistence of sympatric populations of *P. macrobullaris* and *P. auritus* in the Swiss Alps must be achieved through a process of ecological niche differentiation (Arlettaz *et al.* 1997). In contrast, there is so far no evidence for sympatric populations of *P. austriacus* and *P. macrobullaris*: although potential distributions predicted via spatial modelling overlap (Rutishauser *et al*. unpublished data), all areas checked so far harbour only one of the two species. This situation may reveal interspecific spatial exclusion (out-competition) caused by too similar ecological niches, i.e. a too large niche overlap.

Previous faecal analysis led to characterize *P. auritus* as a generalist forager (e.g. Beck, 1995). Although sympatric coexistence of *P. auritus* and *P. macrobullaris* could be achieved through a simple partitioning of foraging habitat without obligatory diet segregation (Arlettaz 1999, see chapter 5), we predict here a narrower trophic niche, i.e. a higher prey specialization in *P. macrobullaris.* This may be a way to decrease interspecific pressure in resource utilization, i.e. to achieve an acceptable balance in interspecific food resource sharing although such a mechanism would bear costs (lower survival

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[3] Chapter 5 of this thesis
[4] Chapter 2 of thesis

rates, reproductive performance and/or population density) for the species whose niche is included in that of the other (asymmetric species-specific competition coefficients; Lotka-Volterra model, Begon *et al.* 1986). In contrast, given the apparent parapatric distribution of *P. austriacus* and *P. macrobullaris*, a large dietary niche overlap between these two species would be expected. Finally, as we worked in several areas of Switzerland with varying land-use intensity, we expected narrower dietary niches in regions characterized by high-intensity agriculture, because industrial farming reduces species richness and invertebrate abundance (Britschgi, Spaar & Arlettaz 2006) in comparison with traditional, low-intensity agriculture.

To summarize, this study evaluates to which extent ecological niche specialization and differentiation occur between the three species of long-eared bats in Switzerland along one major niche dimension, the trophic axis. It further aims at identifying the ecological requirements of the three *Plecotus* species with the idea to draw recommendations for better targeted conservation management.

3.3 Materials and methods

3.3.1 Field sampling and data collection

In May-September 2007 and 2008 we sampled 899 faecal samples from 251 long-eared bats (n = 137 *P. auritus*, 41 *P. austriacus* and 73 *P. macrobullaris*) captured at 8 colonies in southern, northern and southwestern Switzerland (Table 1). All sites were already known as relatively large maternal colonies (20-40 females) from a previous study (Ashrafi *et al.* in press). Faecal samples were obtained from bats mist-netted at the entrance of their attic roost – to minimise disturbance of the colony – while returning from night foraging. The species identity of every roost had been assessed earlier on through a DNA analysis of several individuals each, in 2006 (chapter 2). During the present study, we further identify individuals based on eight external characters as suggested by Ashrafi *et al.* (in press). The bats were kept in linen bags until defecation, which took place within 30-120 min after capture. Individual faecal samples were dry-stored in paper envelopes. In the laboratory the faecal pellets were soaked in water for at least 10 min and teased apart with dissecting needles on a Petri dish under a binocular microscope (Leica MZ9.5, Leica Microsystems, Switzerland; 10-60x magnitude). Identification of arthropod remains to order or family was achieved using several identification keys (e.g. Shiel *et al*. 1997, Whitaker 1998) and a collection of arthropod remains in bat faeces (Arlettaz, Godat & Meyer 2000). The proportion (% volume) of each prey category was estimated for each faecal pellet to the nearest 5%, and then estimated for the whole individual sample.

3.3.2 Trophic niche breadth and niche overlap

Trophic niche breadths were estimated using Levin's index (Krebs 1999; Arlettaz *et al*. 1997), from the proportion p_i of all prey categories in the diet:

$$B = \frac{1}{\sum p_i^2}$$

The index B ranges from 0 to n, with n corresponding to the number of prey categories. Niche breadth was calculated at the individual level in monthly intervals in order to account for possible species-specific seasonal effects (Table 1). Moreover, individual niche breadth for colonies in the low-intensity (Upper Valais, Alps) vs. high-intensity (Lower Valais, Rhone valley) cultivated areas were compared statistically. Assumptions of normality and homogeneity were tested using Shapiro test and Leven´s test. Due to significant deviation from normality we had to rely on non-parametric tests for estimating niche breadths in either geographic (Mann-Whitney U-test) or seasonal (monthly; Kruskal-Wallis test, using *pgirmess* package) comparisons (R program; R Development Core Team 2009).

Individual niche overlaps were calculated using Freeman and Tukeys´ index according to Matusita (1955).

$$FT_{ij} = \sum_{r=1}^{k} (p_{ir} \cdot p_{jr})^{\frac{1}{2}}$$

FT_{ij} measures the niche overlap between the individuals i and j, where k is the total number of used resources (prey categories) and p_{ir} and p_{jr} are the proportions of the resource r utilized by individual i and j, respectively. The index ranges from 0 (no overlap) to 1 (complete overlap). Overlaps were measured monthly for the sole situation of sympatry (mixed colony harbouring both *P. auritus* and *P. macrobullaris*), both intraspecifically and interspecifically (through a matrix of individual samples with all possible monthly pairwise comparisons), as outlined in Arlettaz *et al.* (1997). To test for differences between the two types of overlap we used a randomization procedure which generated random series of matrices of similar size and compared their outcomes with the observed matrix (J. Goudet, unpublished, available upon request from the first author). However, too small sample sizes prevented calculations of inter- and intraspecific niche overlaps for the mixed colony at the beginning and at the end of the season.

3.4 Results

3.4.1 Diet composition

Overall, 21 prey categories were identified in the faecal samples of *P. auritus*, 15 categories in *P. macrobullaris* and 12 in *P. austriacus* (Table 2). The major prey categories in *P. auritus'* diet were Lepidoptera imagines (between colonies mean: 41.0% [of prey fragments' volume]; range: 13.0-68.0%; calculated from individual faecal samples), Coleoptera (mean: 7.0%, range: 0.6 11.3%), Diptera (16.6%, 9.6-20.7%), Dermaptera (14%, 0.6-30.0%), Arachnida (8.0%, 0.5-17.2%) and Chilopoda (4.2%, 0.0-10.0). The diet of *P. macrobullaris* comprised Lepidoptera (mean: 88.0%, range: 82.0-93.0%), Coleoptera (3.5%, 1.2-7.9%) and Diptera (6.7%, 3.8-9.0%), that of *P. austriacus* comprised Lepidoptera (87.0%, 86.0-89.0%), Coleoptera (1.3%, 0.0-2.6%), Diptera (4.8%, 3.1-6.4%) and Hymenoptera (0.8%, 0.0-1.6%). These main categories made up 90.8, 98.2 and 93.9% of the diet composition of *P. auritus*, *P. macrobullaris* and *P. austriacus*, respectively. Prey categories typically revealing a ground-foraging behaviour made up, on average, 27.8%, 0.9% and 2.7% of the diet of *P. auritus*, *P. macrobullaris* and *P. austriacus*, respectively. Fig. 1 shows the monthly variation of the main prey categories in the diet of the three species. There was no significant seasonal variation in the main prey category Lepidoptera imagines for all three species during the season (Kruskal-Wallis test, χ^2 = 3.7, 5.19 and 3.35, p = 0.085, 0.18 and 0.50 in *P. auritus*, *P. macrobullaris* and *P. austriacus,* respectively).

3.4.2 Trophic niche breadth and niche overlap

The trophic niche breadths of *P. austriacus* and *P. macrobullaris* were smaller (Levin's index of 1.22 and 1.26, respectively, Fig. 2) than that of *P. auritus* (1.90, multiple Kruskal-Wallis test, $p < 0.05$). There was no indication for a significant seasonal variation in niche breadth in all three species (Kruskal-Wallis test, $\chi^2 = 6.31$, 10.53 and 6.98; p = 0.052, 0.17, 0.13 for *P. auritus*, *P. macrobullaris* and *P. austriacus*, respectively).

A comparison of monthly interspecific vs. intraspecific (individual) niche overlaps in the sole available sympatric population (*P. auritus* and *P. macrobullaris,* Upper Valais) yielded a significant difference between intraspecific and interspecific overlaps in July and August for *P. auritus* ($p < 0.05$, randomization tests) and in August for *P. macrobullaris* ($p < 0.05$). Fig. 3). No tests could be performed in June and September due to too small sample sizes.

Comparisons of niche breadths between colonies in Upper Valais (low intensity agriculture) and Lower Valais (high intensity agriculture) revealed a much narrower prey spectrum for *P. auritus* in the intensively cultivated area (1.7 vs 2.2 for low-intensity farming; Mann-Whitney U-test, W = 288.5, $p < 0.01$) whilst no such difference existed for *P. macrobullaris* (1.2 vs 1.3, respectively, W = 161.5, p = 0.81).

3.5 Discussion

This study establishes an overall trophic specialisation on moths in all three species of long-eared bats occurring in Switzerland. Yet, *P. auritus*, the least specialized (broader niche) among the three *Plecotus* species incorporates many more Dermaptera, Diptera, Arachnida, Coleoptera and Chilopoda in its diet, which reveals a greater reliance on substrate gleaning as regards foraging tactics. In the sole sympatric population found there was evidence for a mechanism of trophic niche partitioning between *P. auritus* and *P. macrobullaris*.

A trophic specialisation of *Plecotus* on moths confirms previous European studies. The proportion recorded here for *P. auritus* (41%) is similar to the values reported by Swift & Racey (1983: 40.5%), Rydell (1989: 27.2%) and

Beck (1995: 61%). A very high proportion of moths in *P. austriacus'* diet in Beck (1995: 90%; see also Swift & Racey 1983 and Rydell 1989) is also corroborated by our findings (87.4%). The present study represents to our knowledge the first analysis of the diet of *P. macrobullaris*: with 88.0% of moths, this species appears to be as specialised on moths as *P. austriacus*. With 27.8% of diurnal and non-flying prey taxa in its diet, *P. auritus* is expected to glean many prey items from surfaces. This contrasts with the foraging behaviour of *P. austriacus* and *P. macrobullaris,* as inferred from their diet composition, which would be much more adapted to prey capture by aerial hawking*.* The similarities in morphology (Ashrafi *et al.,* in press) and echolocation calls (Dietrich *et al.* 2006) of *P. auritus* and *P. macrobullaris* had let envisioning similar gleaning foraging strategies in the two species, which is invalidated by our findings: *P. macrobullaris* had only 0.9% typical non-flying and diurnal arthropods in its diet. Hence, although it is still able to pick up insects from surfaces it mostly captures aerial prey. The same holds for *P. austriacus* with only 2.7% of flightless and diurnal taxa in its diet. Yet, the exact amount of prey consumed by gleaning is most probably largely underestimated as other prey not classified among diurnal and flightless categories can also be obtained from gleaning. The slight seasonal variation in the occurrence of moths in the diet is likely to indicate a rather constant occurrence of this food source in the environment throughout the season.

These noticeable divergences in prey spectra and niche breadths of Swiss long-eared bats are reflected in our unique niche overlap estimation for the sole situation where two out of the three species occurred in sympatry, and even in syntopy (sharing the same colonial roost): competition avoidance between *P. auritus* and *P. macrobullaris* was clearly achieved through a mechanism of food resource partitioning, with the intraspecific overlap being larger than the interspecific overlap, a situation which suffices for allowing a stable coexistence. Interestingly, this pattern of exploitative competition avoidance was apparent even in the middle of the summer, when food abundance peaks, i.e. when the basic assumption of limiting resources is less likely to be true.

The convergent diets and niche breadths of *P. austriacus* and *P. macrobullaris* may be a sign that these two species actually compete for the same food resources. The fact that *P. austriacus* inhabits exclusively lowland areas of Switzerland north of the Alps, while *P. macrobullaris* occurs mostly above 600 m altitude north of the Alps but also at low elevation in the southern Alps (Ashrafi *et al.* in press; Mattei-Roesli, in press) evokes a parapatric distribution. Predictive habitat suitability models have recently confirmed this view (Rutishauser *et al.*, unpublished data). There is thus growing evidence that the two species actually occupy the same niche and could not coexist in sympatry in a stable way.

Bats are a speciose group of mammals playing an important bioindicator role: a large portion (22%) of the world bat species are threatened or near threatened (IUCN red list 2009). The recent discovery of new cryptic species of bats poses new conservation challenges because species status and ecological requirements should be re-evaluated. In the extreme cases, the discovery of new species within a cryptic species complex may lead to the recognition that some species previously considered as threatened are indeed critically endangered if not on the brink of extinction, thus requesting immediate action (e.g. Sattler *et al*. 2007).

Like many other European Microchiroptera (Stebbings 1988, Mitchell-Jones 1995, Huston *et al*. 2001, Bontadina *et al.* 2008), long-eared bats have faced strong declines in the last decades. This is not surprising given that niche specialists are more vulnerable than generalists (Safi & Kerth 2004). Our finding that *P. auritus'* diet diversity is lower in high-intensity than in low-intensity farmland is in line with the general view that agricultural intensification has caused a dramatic impoverishment of foraging conditions for insectivorous vertebrates across temperate Europe (e.g. Wickramasinghe *et al*. 2004, Britschgi *et al.* 2006, Buckingham, Peach & Fox 2006). Our results suggest that any kind of habitat management which favours the abundance of moths would be profitable to long-eared bats.

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Table 1. Number of individual long-eared bats (n = 251; 137 *P. auritus*; 73 *P. macrobullaris;* 41 *P. austriacus)* mist-netted at the entrance to maternity roosts in six parapatric (separate roost) and two sympatric (mixed roost) populations in Valais (Swiss uplands) and Argovia (Swiss lowlands). P. aur = *P. auritus*, P. mac = *P. macrobullaris* and P. aus = *P. austriacus*.

Region	Lower Valais		Upper Valais		Upper Valais		Argovia	
Cultivation	Intensive farmland		Traditional farmland		Traditional farmland		Mixed farmland	
Colony status	2 separate colonies		2 separate colonies		1 mixed colony		2 separate colonies	
Distribution	Parapatric		Parapatric		Sympatric		Parapatric	
Species	P. aur	P. mac	P. aur	P. mac	P. aur	P. mac	P. aur	P. aus
May	6	0	8	8	0	1	9	8
June	5	0	8	6	3	2	6	8
July	10	8	7	8	10	9	8	9
August	10	5	7	10	7	7	7	9
September	6	0	7	7	8	2	5	7
May-Sept.	37	13	37	39	28	21	35	41

Table 2. Mean percentage volume of prey categories found in individual faecal samples (n= 251, totalling 899 pellets) of six sympatric and two parapatric populations of the three *Plecotus* species from different regions in northern (AG = Argovia), southern and southwestern Switzerland (VS = Valais). Major prey taxa in a given species diet are marked in bold, whereas non-flying or diurnal prey taxa are marked with an asterisk (*).

Species	Place	Lepidoptera imagines	Lepidoptera larvae *	Scarabidae	Curculionoidae *	Other Coleoptera	Tipulidae	Syrphidae *	Chironomidae	Brachycera	Other Diptera	Chyrsopidae	Hemerobiidae	Hymenoptera	Trichoptera	Dermaptera *	Hemiptera	Orthoptera	Blattodea *	Opiliones *	Other Arachnida *	Chilopoda
P. auritus	Obergesteln (Upper VS)	**32.0**	0.0	0.1	0.0	**0.5**	1.0	3.5	0.1	0.1	**16.0**	0.7	2.7	0.2	2.7	**19.0**	3.5	5.8	0.0	2.0	**8.4**	**2.6**
	Blitzingen (Upper VS)	**13.0**	0.7	1.2	0.1	**10.0**	4.7	0.0	0.0	0.0	**13.0**	0.5	0.0	0.7	0.0	**30.0**	0.2	4.4	0.0	0.2	**17.0**	**3.6**
	Salins (Lower VS)	**68.0**	0.0	0.3	10.0	**3.0**	2.3	0.0	0.0	1.2	**6.1**	2.0	0.2	0.0	0.0	**5.4**	0.9	0.3	0.1	0.1	**0.4**	**0.0**
	Kirchrued (AG)	**52.0**	8.5	0.0	0.0	**2.6**	5.1	0.3	0.0	0.0	**14.0**	0.4	0.5	0.6	0.0	**0.6**	0.1	2.0	0.0	0.0	**3.7**	**10.0**
	Average	**41.0**	2.3	0.4	2.5	**4.1**	3.3	1.0	0.0	0.3	**12.0**	0.9	0.9	0.4	0.7	**14.0**	1.2	3.1	0.0	0.6	**7.4**	**4.2**
P. macrobullaris	Obergesteln (Upper VS)	**82.0**	0.0	0.4	0.0	**7.5**	**3.7**	0.0	0.0	0.0	**5.3**	0.0	0.0	0.0	0.0	0.0	0.8	0.0	0.1	0.0	0.2	0.1
	Lax (Upper VS)	**89.0**	0.0	0.1	0.0	**1.2**	**4.7**	0.0	0.0	0.4	**2.6**	0.0	0.0	0.9	0.0	0.0	0.0	0.0	0.0	0.0	0.6	0.1
	Basse Nendaz (Lower VS)	**93.0**	0.0	0.0	0.0	**1.2**	**1.0**	0.0	0.1	0.0	**2.7**	0.0	0.0	0.1	0.0	1.8	0.0	0.0	0.0	0.0	0.1	0.2
	Average	**88.0**	0.0	0.2	0.0	**3.3**	**3.1**	0.0	0.0	0.1	**3.5**	0.0	0.0	0.3	0.0	0.6	0.3	0.0	0.0	0.0	0.3	0.1
P. austriacus	Mandach (AG)	**89.0**	0.1	0.0	0.0	**2.6**	**1.7**	0.0	0.0	0.2	**4.5**	0.2	0.0	**1.6**	0.0	0.0	0.2	0.0	0.0	0.0	0.1	0.1
	Mönthal (AG)	**86.0**	0.0	0.0	0.0	**0.0**	**0.8**	0.0	0.0	0.0	**2.3**	0.0	0.0	**0.0**	0.0	0.0	5.8	0.0	0.0	0.0	5.0	0.0
	Average	**87.0**	0.1	0.0	0.0	**1.3**	**1.3**	0.0	0.0	0.1	**3.4**	0.1	0.0	**0.8**	0.0	0.0	3.0	0.0	0.0	0.0	2.6	0.1

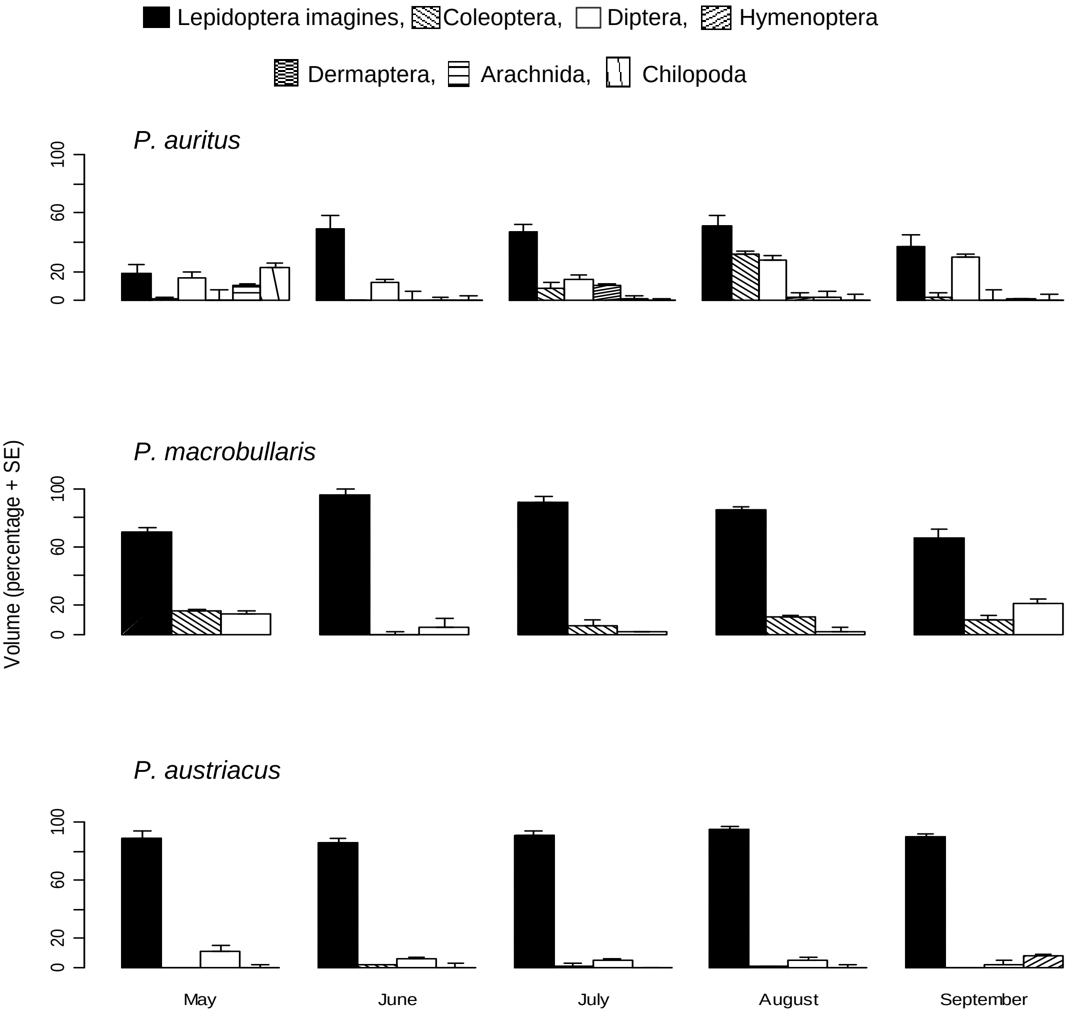

Figure 1. Seasonal variation of the main prey taxa (mean + SE) encountered in the diet of the three *Plecotus* bat species from May to September.

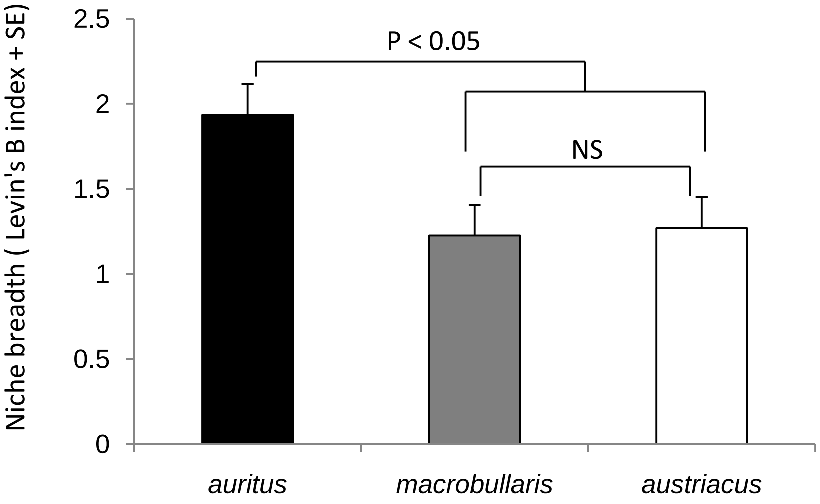

Figure 2. Mean (+ SE) trophic niche breadth (Levin´s index) computed for the three long-eared bat species from May to September. The significance of pairwise comparisons is indicated (multiple Kruskal–Wallis test, $p < 0.05$; NS = non significant).

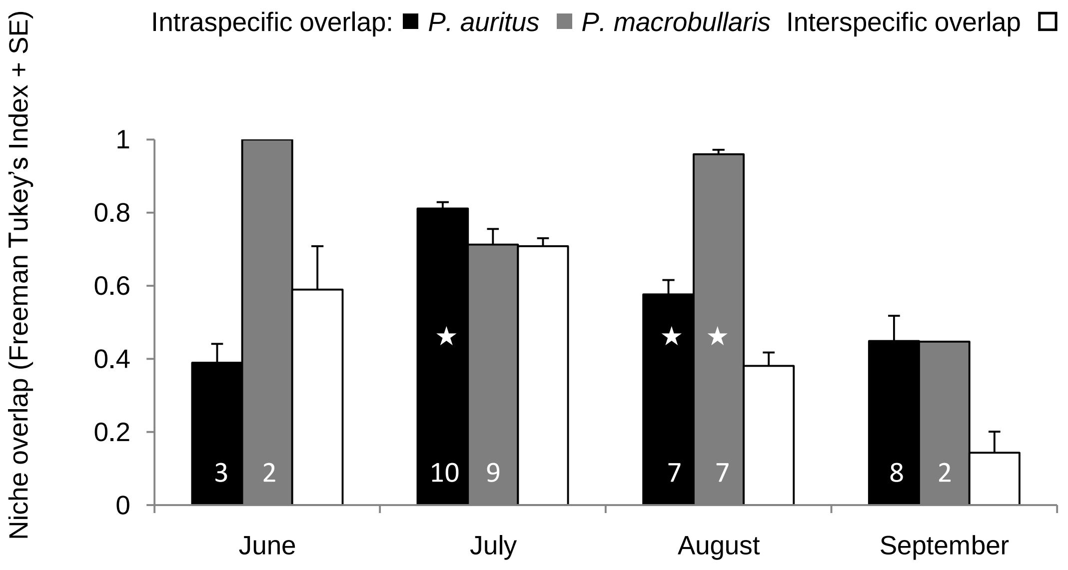

Figure 3.

Monthly niche overlaps (Freeman-Turkey's index + SE, sample size given in columns) in a sympatric population of *P. auritus* and *P. macrobullaris*. Stars show significant intraspecific-interspecific pairwise comparisons (randomization tests, $p < 0.05$).

4

Habitat selection of three cryptic *Plecotus* bat species in the European Alps discloses distinct implications for conservation

Habitat selection of three cryptic *Plecotus* bat species in the European Alps discloses distinct implications for conservation

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4.1. Abstract

Assessing the ecological requirements of species coexisting within a community is an essential requisite for developing sound conservation action. A particularly interesting question is what are the mechanisms that govern the stable coexistence of cryptic species within a community, i.e. species that are almost impossible to distinguish. Resource partitioning theory predicts cryptic species, as other sympatric forms, to occupy different ecological niches, which has been widely inferred from eco-morphological studies. The recent discovery of the cryptic species *Plecotus macrobullaris* in the long-eared bat complex in the European Alps, with even evidence for a few mixed colonies, caused many troubles to bat ecologists when it came to envision conservation measures beyond roost protection. We tested whether foraging habitat segregation occurred among the three cryptic Plecotus bat species existing in Switzerland. We radiotracked 24 breeding female bats (eight of each species) at 6 sites across the country. We compared habitat features at used (visited by a bat) vs. random locations within home ranges, relying on habitat mapping and GIS data, and conducting mixed effects logistic regression. Distinct, species-specific habitat preferences could be revealed. *P. auritus* foraged mostly within traditional orchards in roost vicinity, showing a high preference for habitat heterogeneity. *P. austriacus* was foraging up to 4.7 km from the roost, selecting mostly fruit tree plantations, hedges and tree lines. *P. macrobullaris* preferred patchy deciduous and mixed forests surrounded by grassland. These habitat preferences can orient future conservation programmes.

Keywords: cryptic species, heterogeneity, *Plecotus*, radiotracking, resource partitioning, Switzerland

4.2. Introduction

Ecologists are interested in the ecological requirements of morphologically similar species and the mechanisms that enable coexistence of these species in the community (Schoener 1974, Abrams 1998, Arlettaz 1999). Based on the principle of exclusion (Gause's principle), stable co-existence of species within a community is only conceivable when the species-specific utilisation of limited resources is well differentiated (Hutchinson 1978, Ricklefs 1990, Arlettaz, Perrin & Hausser 1997, Wang, Zhang & Wang 2005). Previous evidence suggests that the three most important niche dimensions, in decreasing order of relevance, are habitat, food, and time of foraging (Pianka 1969, Schoener 1986). This signifies that most frequently different preferences in habitat selection are the major mechanism, which allows species to coexist in the community (Arlettaz, 1999).

Bats are the second largest order of mammals with more than 1100 species and a high proportion of threatened species worldwide (IUCN red list 2009). Bat populations are declining as a result of many factors, including habitat transformation, habitat loss, extensive use of pesticides and large-scale agri- and silvicultural intensification which influence quality and diversity of available habitats and therewith prey abundance (Stebbings 1988, Ransome 1990, Walsh & Harris 1996a, b, Wickramasinge *et al*. 2004, Bontadina, *et al.* 2008). Habitat selection studies which clarify the species-specific habitat requirements for bats are therefore an important key for their preservation and successful management (Walsh and Harris, 1996a). Consequently, in recent years, large body of literature has been devoted to habitat preferences (Kunz & Parsons 2009), including some especially focused on morphologically similar or phylogenetically closely related species in the wild (see Arlettaz, 1999, Nicholls & Racey 2006, Davidson-Watts, Walls & Jones 2006).

One of the taxa that occur widespread in the whole Palearctic is long-eared bats (genus *Plecotus* spp., Swift & Racey 1983, Spitzenberger, *et al*. 2006). In addition to two sympatric *Plecotus* species (*Plecotus auritus* and *P. austriacus*), the existence of a third "cryptic species" ("two or more distinct species that are erroneously classified under one species name", Bickford *et al.* 2007), *P. macrobullaris* in the alpine area of central Europe has been proven mainly by molecular markers (Kiefer & Veith 2001, Kiefer *et al.* 2002, Spitzenberger, Strelkov and Haring 2003, Ashrafi *et al.*, in press[5]). These cryptic species overlap in most morphometric characters (Ashrafi *et al.* in press) and therefore are expected to show extreme similarity in flight performance (speed and manoeuvrability). In addition, it has been stated that echolocation signals of these cryptic bat species are very similar, all with characteristics belonging to the group of narrow space gleaning foragers (Schnitzler & Kalko 2001, Dietrich *et al.* 2006). Although gleaning behaviour in *P. austriacus* and *P. macrobullaris* has been rarely found, Lepidoptera are major food components in all three species (see diet composition results, chapter 3). Thus, species with similar morphology and comparable echolocation signals are expected to exploit their foraging habitat in a similar way.

Existence of cryptic and partly sympatric three species of long-eared bats in Switzerland (Ashrafi *et al.*, in press), provides a remarkable model system to investigate the resource exploitation in the case of species with extreme similarities. To our knowledge there is no published study regarding resource partitioning of long-eared bats in Europe so far. Based on previous single-species studies (Swift & Racey 1983, Entwistle, Racey, & Speakman 1996) and based on models of potential distribution of *Plecotus* bat species in Switzerland (Rutishauser, *et al.* unpublished data)[6], we derived the following expectations of foraging resource partitioning: *P. auritus* forages associated with forests in heterogeneous landscapes, *P. austriacus* in more semi-open areas like orchards, while *P. macrobullaris* is expected to exhibit a different habitat selection pattern compared to the two other species.

As there is almost no information regarding habitat preferences of *P. macrobullaris*, we are generally interested to identify key habitat types in the foraging niche of this species. It is stated that structurally complex habitats may provide more niches and different ways to exploit the environmental resources (Bazzaz 1975). In addition, positive relationships between vegetation habitat heterogeneity and animal species diversity are well documented on both local

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[5] Chapter 2 of this thesis

[6] Chapter 5 of this thesis

and regional scales (Davidowitz & Rosenweig 1998). Thus, we expect a positive effect of heterogeneity in the habitat selection of *Plecotus* bats in their foraging areas. Based on our previous investigation on the trophic niche of these bats and of models of predicted distribution (chapters 3 and 5 of this thesis), we expect the preference of more heterogeneous foraging habitat for *P. auritus* as a generalist compared to other *Plecotus* bat species, which are more specialists. Additionally, we hypothesise that *P. austriacus* and *P. macrobullaris* (the more specialised species) commute more distantly to reach suitable isolated foraging sites and consequently have larger home ranges compared to *P. auritus*.

In this study, we aimed to investigate habitat selection of all three *Plecotus* bat species in Switzerland, using radiotracking. We study habitat selection of three *Plecotus* bat species, assuming the preferences would be indicative of habitat partitioning. If indeed foraging habitat segregation occurs among these cryptic species, then separate, targeted conservation strategies would be needed for the conservation of the species

4.3. Materials and methods

4.3.1 Study sites and colonies

This study was conducted at six *Plecotus* colonies, two for each species, in north and south-western Switzerland (Cantons of Aargau and Valais) during the summers of 2008 and 2009. The bat colonies were located in church attics of three bioregions of Switzerland (Jura Mountains, Plateau and Western Central Alps; Gonseth *et al*. 2001). The sites were already known as relatively large maternal colonies (20-40 individuals) from our previous study (Ashrafi *et al.* in press) where species affiliation was confirmed by DNA.

4.3.2 Radio-locations and random points

Eight individual bats of the three species (total N = 24) were captured between June and September 2008. Bats were caught using mist and hand nets, typically near entrances of their day roosts before they emerged for feeding. In a few cases, we captured individuals in the roost using hand nets. All animals were sexed and weighted. To ensure proper species identification we also applied the

Plecotus species' recognition model (Ashrafi *et al.* in press). Adult female bats in the breeding stage (mostly lactating), when energy demand is at its peak (Kurta *et al*. 1989) and they use foraging habitats in the vicinity of their roosts, were chosen for radiotracking. Two types of radio tags were used (Holohil Systems Ltd., Carp, Ontario, Canada, BD-2N, 0.44g and Biotrack, Wareham, Dorset, England, Pip 31, 0.45 g). Transmitter weight did not exceed 6% of bat body weight, therefore should not have adverse effects on the flight behaviour (Bontadina *et al*. 2002). To fix the transmitters the fur between the scapulae was trimmed and the radio transmitters were attached using Torbot liquid bonding cement (Cranston, Rhode Island, USA). The bats were tracked with Australis (26k Scanning Receiver, Titley Electronics, Australia) and Wildlife receivers (TRX-1000S, Wildlife Materials, USA) using hand-held Yagi-antennae (Titley Electronics, Australia) to locate the bats, applying the triangulation technique (White & Garrott 1990, Bontadina *et al.* 2002). Bats first were tracked from a car to locate their foraging places which were up to 4.7 km in aerial distance from the roost. Then, radiotracking of an individual bat was performed on foot by a team of two persons (focal animal method, White & Garrott 1990), otherwise the alpine foraging grounds with complex topography were not accessible at night. An observer team coordinated their work by walkie-talkies and timer watches to record simultaneously location data in five minutes intervals. At every interval the time, observer positions, bearings to the bat signal and estimated accuracy of the location of the bat was recorded on a dictaphone in the field. We attributed each bat location to one of three categories of accuracy. The accuracy classes high, medium and low were attributed if the radius of the error range was estimated to be smaller than 50, 100 or 250m, respectively (Bontadina & Naef-Daenzer 1996). Most of the observations (> 90%) had high to medium accuracy and only these were retained for further analysis, so that the error polygon mostly was not larger than one hectare. In order to account for location errors we buffered locations for analysis by a radius of 50m.

Home ranges were estimated as the 100% Minimum Convex Polygon (MCP, White & Garrott 1990) in a geographic information system (GIS), encompassing all (50m buffered) radio-locations during foraging of a given bat.

For every bat equal numbers of random locations as actual visited locations were generated within the buffered MCP to estimate habitat availability. Random locations were uniformly distributed within the home range. All spatial calculations were done in ArcView GIS 3.3 using the Animal Movement Extension (Hooge, Eichenloub & Solomon 1999). Moreover, to compare activity ranges' area (MCPs) and maximum flight distances from roosts, we applied multiple Kruskal-Wallis tests (using *pgirmess* package in R 2.10.0, R Development Core Team 2009).

4.3.3 Habitat variables

In summer 2009, habitat variables were mapped at the buffered visited and random points. We grouped the variables in two main categories, habitat cover and heterogeneity (Appendix S1). The percentage cover of each habitat type was estimated visually in the field with an accuracy of 5%. The category of heterogeneity included variables which describe habitat incongruity in horizontal and vertical dimensions. As indicator of horizontal heterogeneity we used Simpson's index of diversity (Krebs 1999) with the percentage cover of each habitat type at the buffered location. Vertical heterogeneity was estimated using LIDAR data. In this method digital surface models of Switzerland are used to describe the elevation of the earth's surface including vertical structures, using airborne laser scanning methods (Artuso, Bovet & Streilein 2003). These data are available in cell sizes of 2.5 x 2.5m and have a vertical accuracy of ±0.5 m in open terrain. By contrasting this digital surface model with the ground surface, the height of the vegetation layer was calculated. Therewith we obtained five different height classes of vegetation covers (Appendix S1). Then we extracted two types of variables indicative for the vertical structure: percentage cover giving the amount of vegetation in the buffers with radius of 50m; as measure of variance we used the average of aggregations of 9 LIDAR pixels, calculated in a moving window all over the circle, and standard deviation within each aggregated height class was computed too. Finally, weighted mean number of each height class' pixels occurring in the buffered points was calculated as a single variable. The first three variable types were calculated for each vertical height class 1 to 5 (from ground to top, see Appendix S1 for details) and in addition vertical heterogeneity variable, overall 16 variables resulted. The spatial averages were improved by using weighted averages (quartic kernels, see Wing & Tynon 2006). Overall our initial data set contained 30 predictors.

4.3.4 Data preparation

We first, excluded the habitat descriptors that occurred marginally or at very low densities (< 1% in average) for a given species. Then we removed infrastructure variables which were not in the main focus of this study and occurred at low densities as well. Second, we assessed the correlation between continuous predictors using the conservative Spearman rank correlation test. To avoid collinearity among the first main group predictors (habitat cover) we excluded one variable of a pair if their correlation coefficient was > |0.7| (Hosmer & Lemeshow 1989). We then re-grouped the variables of the main habitat cover category into two subcategories: first, farmland and grassland; including, steppe, arable, vineyard, extensive grassland, intensive grassland, traditional orchard and fruit tree plantation, second, forest and hedges which include deciduous forest, conifer forest, mixed forest and hedgerow. We used the term “extensive grassland” as grassland managed at low intensity and “intensive grassland” as those managed at high intensity.

In the second main group (heterogeneity) where spearman’s correlation tests indicated high correlations among variables, except horizontal heterogeneity, we applied principal component analysis (PCA) to decrease the number of variables. Then first and second principal components of vertical heterogeneity (VH1 and VH2) describing 77% of total variance were used in the modelling procedure. These two components describe the variance of habitat features along the vertical dimension in the circle of buffered locations. This data preparation procedure resulted in the exclusion of the variables vineyard for all species and steppe for *P. auritus* and *P. austriacus* (reason: low utilisation density).

All retained continuous variables were scaled using Z-standardization procedure to achieve a set of unit-free predictors which are directly comparable and allow easier interpretation.

$$Z = \frac{X - M}{SD}$$

Z is the standardised value of the variable X with M being the mean and SD the standard deviation of X. Z values have therefore by definition a mean of zero and SD of 1.

4.3.5 Modelling procedure

We modelled the use of habitat characters for the three *Plecotus* species separately, using generalized linear mixed model (GLMM) with binomial distribution and inclusion of the random effects. Mixed effect models enabled the analysis of all radiotracked individual bats together, with the individuals being treated as random effects, accounting for variability among individuals and habitat predictors as fixed effects simultaneously (Johnson *et al.* 2005). Additionally, to control for large-scale variation across the sites, a variable coded as site were implemented as a fixed effect in all models according to Whittingham *et al.* 2005. We applied a priori defined sets of candidate models to avoid difficulties of stepwise regression (Whittingham *et al.* 2006). After excluding the infrastructure variables, we had three sets of variables: I. grassland and farmland, II. forest and hedges and III. heterogeneity (Fig. 1). Models were built by different combinations of covariates with all variables of each set combined with single variables of the other sets and vice versa (Fig. 1). Thus, we constructed 180, 180 and 276 a priori defined models for *P. auritus*, *P. austriacus* and *P. macrobullaris*, respectively. Different numbers of models were used because differences in the number of variables relevant and used occurred for each species.

Logistic Mixed Effects Regressions (lmer) in the Lme4 package of the software R 2.10.0 were used to model habitat use of *Plecotus* bat species (visited vs. random locations within activity range). Adapted models were ordered according to their best fit to data using the Akaike Information Criterion (AIC) and Akaike weight. We used the top set of candidate models contributing >= 90% of AIC weights to get more robust estimates (Burnham & Anderson 2002). With these we applied model averaging, which resulted in a weighted coefficient estimate for each variable. Finally we ranked the explanatory variables based on their relative importance, using the coefficients of estimated standardised variables. All variables were already scaled, therefore the coefficients indicate the change from complete absence of one habitat to its maximal presence within in the activity range.

4.4. Results

Altogether 24 females, eight per species originating from two study sites each, were successfully radiotracked (Table 1). In total, we mapped 368 radio locations, on average (± SD) 46 ± 20 locations per bat for *P. auritus*, 355 radio locations, on average 41 ± 1.3 per bat for *P. austriacus* and 312 points, on average 39 ± 1.9 per bat for *P. macrobullaris*. Average areas of home ranges were (± SD), 51.8 ± 33.8 ha (range: 5.2 – 103.2 ha) for *P. auritus*, 295.5 ± 296 ha (range: 12.9 – 804.0 ha) for *P. austriacus* and 239.5 ± 284 ha (range: 64.0 – 797.0 ha) for *P. macrobullaris* (Table 1). The result of two separate Multiple Kruskal-Wallis tests shows smaller home ranges and shorter flight distances for *P. auritus* compared to the other two species ($p < 0.05$) but no significant difference is found between *P. austriacus* and *P. macrobullaris* ($p > 0.05$). Moreover, the variable site made no substantial difference to the modelling results in all three species.

4.4.1. Habitat selection

Plecotus auritus

The result of averaging the seven best models explained 91% of AIC weights (Appendix S2, Table A). The ranking of the coefficients of the eight variables in the final models suggested by model averaging is (negative relations are indicated by a minus sign) : –(intensive grassland) > traditional orchard > horizontal heterogeneity > - (extensive grassland) > - (VH2) > - (arable) > VH1 > - (fruit tree plantation) (Table 2A).

The optimum model (average of the top ranked models) shows that occurrence probability of *P. auritus* is high in traditional orchards. Horizontal heterogeneity has a positive impact on the probability of occurrence too (Fig. 2A & B). Incidence of these bats is affected negatively by arable and grasslands i.e. extensive and intensive grasslands (Fig. 2C). Fruit tree plantation influenced the occurrence of this species slightly in the negative direction. Finally, vertical heterogeneity indicated by VH1 and VH2, had slight effects on the occurrence probability. The optimum model reveals very small coefficients and relative high uncertainty for these two variables as well as for fruit tree plantation.

Plecotus austriacus

The result of averaging the nine best models explained 90% of AIC weights (Appendix S2, Table B). The ranking of the coefficients of the nine variables in the model suggested by model averaging are - (deciduous forest) > - (arable) > - (intensive grassland) > - (mixed forest) > - (extensive grassland) > fruit tree plantation > hedgerow > - (traditional orchard) > conifer forest (Table 2B).

The optimum model shows that occurrence probability of *P. austriacus* is positively influenced by fruit tree plantation and hedgerows. They avoided deciduous forests, arable lands, mixed forests and grasslands; i.e. extensive and intensive grasslands (Fig. 3).

Plecotus macrobullaris

The result of averaging the nine best models explained 90% of AIC weights (Appendix S2, Table C). The ranking of the coefficients of the variables in the model suggested by model averaging is – (steppe) > deciduous forest > VH2 > intensive grassland > - (conifer forest) > mixed forest > extensive grassland > horizontal heterogeneity > arable > hedgerow > VH1 > fruit tree plantation > traditional orchard (Table 2C).

The optimum model shows that occurrence probability of *P. macrobullaris* is high in deciduous forests and intensive grasslands (Fig. 4A & B). They avoided steppes and conifer forests. Finally, vertical heterogeneity, indicated by VH2, is positively associated with the occurrence probability (Fig. 4C). This principal component was mostly correlated with variables expressing occurrence of higher vegetation (height classes 2, 3 and 5, see Table S1). In addition, horizontal heterogeneity slightly positive related to the occurrence probability of *P. macrobullaris*.

4.5. Discussion

In this paper we inspected habitat segregation in three cryptic *Plecotus* bat species. We found that these species differed not only in their foraging range but undoubtedly also in their habitat selection - as predicted from niche theory for highly similar species. Beside the selection of characteristic species-specific habitat types, traditional orchard (Fig. 5) for *P. auritus*, fruit tree plantations and hedgerow for *P. austriacus*, and deciduous, mixed forest and grasslands for *P. macrobullaris*, Habitat heterogeneity was favoured by the first and last species, respectively.

4.5.1. Foraging behaviour

Mean foraging distance from the roost was more then twofold the distance in *P. austriacus* and *P. macrobullaris* compared to *P. auritus* (2.9 ± 1.5, 2.5 ± 1.6 and 1.2 ± 0.6 km for *P. austriacus*, *P. macrobullaris* and *P. auritus* respectively). Their suitable foraging sites typically were isolated and dispersed in the landscape. This suggests that they used hedges and other linear structures like forest edges or forest roads as commuting routes to reach their preferred foraging sites. *P. austriacus* showed a preference for fruit tree plantation, hedges and in contrast, strong avoidance of farmland and grasslands as well as deciduous forests. We observed during field work *P. austriacus* individuals commuting far from the roost (up to 4.7 km) in short time to reach patches of fruit tree plantations. There they captured in foraging bouts of several hours on the fly for arthropods which accumulated under the hail protection nets covering the plantations (Fig. 6). Similarly, radio-tracked individuals of *P. macrobullaris* were found in patches of deciduous and mixed forests in greater distance to the roosts (4.6 km). We observed individuals of *P. macrobullaris* which crossed open meadows in fast and low flight to reach their foraging areas, semi-open woodlands (Fig. 7).

Our comparison of mean activity areas (MCPs) reflects the differences in foraging ranges in the three species. The larger polygons in *P. austriacus* and *P. macrobullaris* result from the longer commuting flights to reach suitable foraging sites. It has been demonstrated in a previous study (Ashrafi *et al.* unpublished data)[7], that *P. austriacus* and *P. macrobullaris* have a narrower trophic niche compared to *P. auritus*. We suggest that a higher specialisation (higher selectivity of specific habitats) results in the longer flight distance to reach their suitable foraging sites. In contrast *P. auritus* which is a more generalist in dietary pattern seems to find a wider range of potential foraging sites in the vicinity of their roosts.

4.5.2. Resource partitioning by cryptic Plecotus species

Our study is the first attempt to test habitat selection simultaneously in three cryptic *Plecotus* bat species of the Alps. We have focused on female bats during pregnancy and lactation when they are energetically stressed and restrict their foraging habitats to the vicinity of their maternity roost. Although these cryptic *Plecotus* species are so similar that they overlap in all morphometric characteristics (Ashrafi *et al.* in press) as well as their echolocation calls (Dietrich *et al.* 2006), our study revealed differential foraging habitat selection.

A possible case of spatial competition is given in the case *P. auritus* and *P. macrobullaris* which occupy roosts next to each other or even share a common roost (Ashrafi, *et al.* in press). However, our result evidences a different habitat selection for these two species. *P. auritus* select traditional orchard over all other habitat types, whereas *P. macrobullaris* selects mainly deciduous forest and intensive grassland. Both species selected heterogeneity, in horizontal dimension and *P. macrobullaris* selected vertical heterogeneity as well (Tables 2A, B). A preference for heterogeneous habitat types is in accordance to the wider foraging niche breadth of *P. auritus* compared to other two species (see results of niche breadth in chapter three). Apparently heterogeneous habitat types produce more

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[7] Chapter 3 of this thesis

diverse food due to their influence on the distribution and interactions of species (Tews *et al.* 2004). *P. macrobullaris* select heterogeneity of vertical habitat structures, indicating that this species might exploit the vertical structure, e.g. that foraging is not restricted to the ground level. For *P. auritus* it is surprising that forest habitat types did not appear in the best models, although in the raw data, the average frequency of forest types in visiting points are larger compared to the random points (e.g. in 6 of 8 individuals, Appendix S3), although this might be blurred be variable interactions. Previous studies (Stebbing 1982, Swift & Racey 1983, Entwistle, Racey, & Speakman 1996) found this species selected forest. However, it must be considered that habitat selection does strongly depend on the scale it was measured (Whittingham, *et al.* 2005). Our result do not indicate any preference of forest types in *P. auritus* at the local scale (foraging sites within home ranges) which is different to the results obtained from distribution models (landscape scale with a resolution of km^2 squares, see chapter 5).

P. auritus and *P. austriacus* avoided arable habitats for foraging. This is comparable to previous studies (Wickramasinghe, *et al.* 2004, Britschgi, Spaar & Arlettaz 2006) and in accordance to our findings (chapter 3), which demonstrated a lower diversity of arthropods in intensified farmlands.

4.5.3. Implications for conservation

The need of special considerations while building conservation plans for cryptic species was highlighted by Schonrogge *et al.* 2002 and Bickford *et al.* 2006. Our results confirm habitat segregation as a major mechanism for resource partitioning among the complex of *Plecotus* bat species.

Distinct foraging habitat niches for each of the *Plecotus* species demonstrate the need of targeted strategies for an effective conservation management. Because *P. austriacus* and *P. macrobullaris* search for suitable foraging patches scattered in their home ranges, it is important to preserve not only these key habitat patches but also linear structures to connect the suitable foraging sites. Suitable commuting structures are possibly hedges, tree lines and woodland corridors (Russo, *et al.* 2002, Nicholls & Racey 2006). The increase of agricultural intensification across Europe amplifies the problem of fragmentation of structured habitat, therefore accessibility to suitable foraging sites and arthropod prey availability are two important issues for the preservation of bat populations. Particularly the long-eared bats who select for heterogeneous habitats might strongly suffer from habitat simplification and intensified farmland. Our results find notably *P. auritus* at risk, but also the other two *Plecotus* species are affected because of their selection for patchy vegetation structures.

P. austriacus' occurrence at the landscape scale and their roosts were clearly associated with lower and warmer areas (see Ashrafi *et al*. in press & chapter 5 of this thesis). Therefore, we suggest targeted conservation plans to achieve correct management of preferred sites mainly below 500 m a.s.l. Additionally maintaining structural connectivity among these areas appears important to preserve the remained populations of this endangered species.

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Table 1. Radiotracking of *Plecotus* females carried out in summer 2008.

Species	Individual	Reproductive status	Colony	Date of capture	Number of nights[1]	Number of locations[2]	Home range[3] (MCP, ha)	Max. distance from roost (km)
P. auritus	1	Pregnant	Kirchrued (AG)	17.06.2008	1	35	43.35	0.78
	2	Not pregnant	Kirchrued (AG)	17.06.2008	3	17	81.84	0.47
	3	Not lactating	Kirchrued (AG)	22.09.2008	4	56	103.25	2.3
	4	Not pregnant	Kirchrued (AG)	17.06.2008	2	84	33.65	1.16
	5	Lactating	Blitzingen(VS)	18.07.2008	3	44	33.15	1.34
	6	Not lactating	Blitzingen(VS)	21.07.2008	3	44	5.21	0.74
	7	Post lactating	Blitzingen(VS)	24.08.2008	3	57	29.63	1.37
	8	Lactating	Blitzingen(VS)	21.07.2008	2	31	84.32	1.6
P. austriacus	1	Lactating	Mandach (AG)	21.06.2008	9	36	12.9	0.63
	2	Lactating	Mandach (AG)	21.06.2008	4	45	623.94	4.36
	3	Not lactating	Mandach (AG)	07.09.2208	3	45	148.93	4.7
	4	Lactating	Mandach (AG)	21.06.2008	3	37	113.49	3.9
	5	Lactating	Mönthal (AG)	03.07.2008	3	27	803.96	4.2
	6	Lactating	Mönthal (AG)	27.06.2208	5	35	63.89	1.55
	7	Post lactating	Mönthal (AG)	10.08.2008	3	48	470.18	2.56
	8	Post lactating	Mönthal (AG)	15.09.2008	7	62	126.43	2.03
P. macrobullaris	1	Not lactating	Obergesteln (VS)	16.07.2008	5	62	75.64	1.42
	2	Not lactating	Obergesteln (VS)	16.07.2008	5	30	64.08	1.08
	3	Not lactating	Obergesteln (VS)	16.07.2008	4	32	72.5	1.19
	4	Not lactating	Obergesteln (VS)	27.08.2008	2	53	87.46	1.5
	5	Lactating	Sembrancher (VS)	09.07.2008	4	34	797.02	4.65
	6	Not lactating	Sembrancher (VS)	29.07.2008	7	35	581.91	4.61
	7	Post lactating	Sembrancher (VS)	29.07.2008	6	14	113.92	1.87
	8	Post lactating	Sembrancher (VS)	01.09.2008	2	52	123.48	4.41
Total	24				93	1015		

[1] night with successful radio-monitoring, data collected from dusk to dawn.

[2] bearings with medium and high accuracy

[3] foraging home range (activity range)

Table 2. Estimated coefficients and standard errors for the variables of the optimum model for A) *P. auritus*, B) *P. austriacus* and C) *P. macrobullaris*. Absolute values of coefficients are sorted in decreasing order of importance.

A) *P. auritus*

Variable	Estimate	Standard error
Intensive grassland	-0.822	0.158
Traditional orchard	0.632	0.119
Horizontal heterogeneity	0.468	0.107
Extensive grassland	-0.346	0.125
VH2	-0.087	0.094
Arable	-0.056	0.090
VH1	0.018	0.108
Fruit tree plantation	-0.013	0.050

B) *P. austriacus*

Variable	Estimate	Standard error
Deciduous forest	-0.879	0.218
Arable	-0.611	0.200
Intensive grassland	-0.566	0.174
Mixed forest	-0.531	0.166
Extensive grassland	-0.441	0.188
Fruit tree plantation	0.274	0.188
Hedgerow	0.114	0.107
Traditional orchard	-0.027	0.080
Conifer forest	0.013	0.098

C) *P. macrobullaris*

Variable	Estimate	Standard error
Steppe	-0.438	0.154
Deciduous forest	0.288	0.101
VH2	0.276	0.159
Intensive grassland	0.230	0.111
Conifer forest	-0.203	0.191
Mixed forest	0.135	0.146
Extensive grassland	0.080	0.134
Horizontal heterogeneity	0.064	0.093
Arable	0.055	0.091
Hedgerow	0.036	0.086
VH1	0.036	0.079
Fruit tree plantation	0.032	0.058
Traditional orchard	0.011	0.027

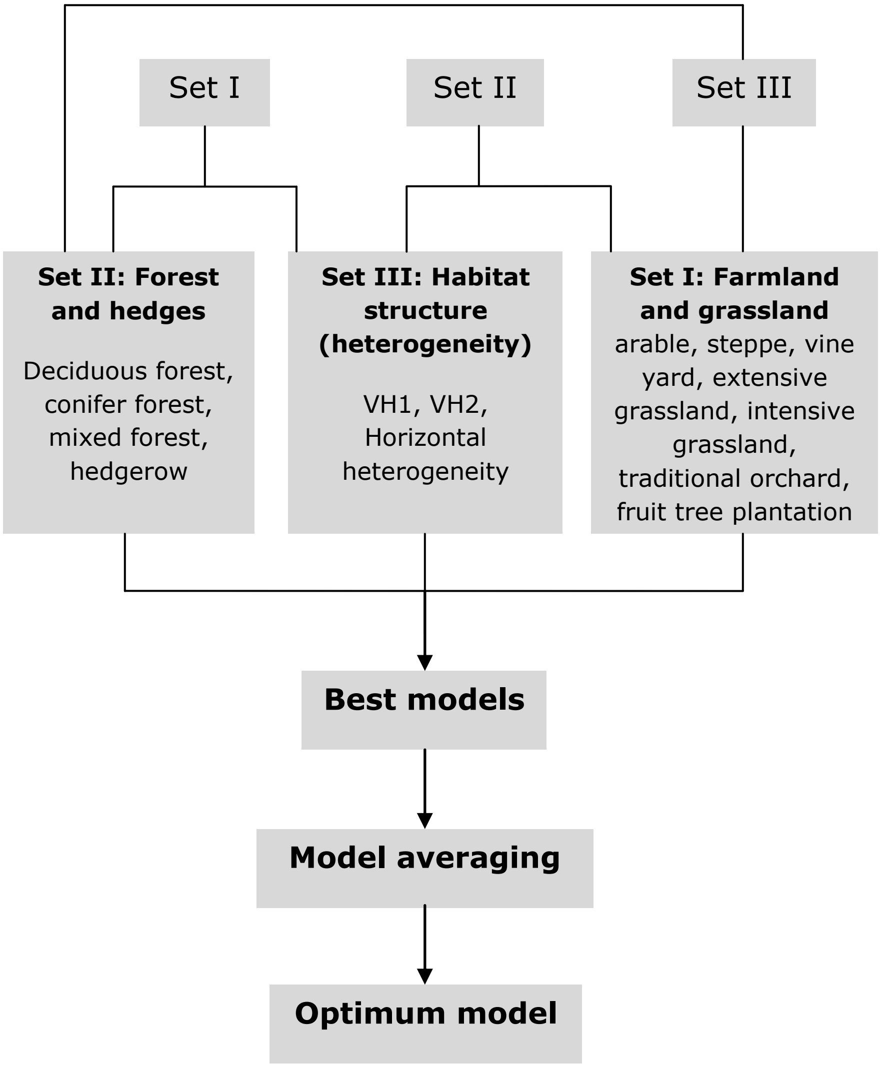

Fig. 1. Design of model selection approach. Sub-categories contained variables from grassland and farmland (set I), forest and hedges (set II) and habitat heterogeneity (set III). All predictors of each set are listed in the boxes (variable details in Appendix S1). Models were constructed by all variables of each set combined with single variables of the other sets and all combinations of covariates.

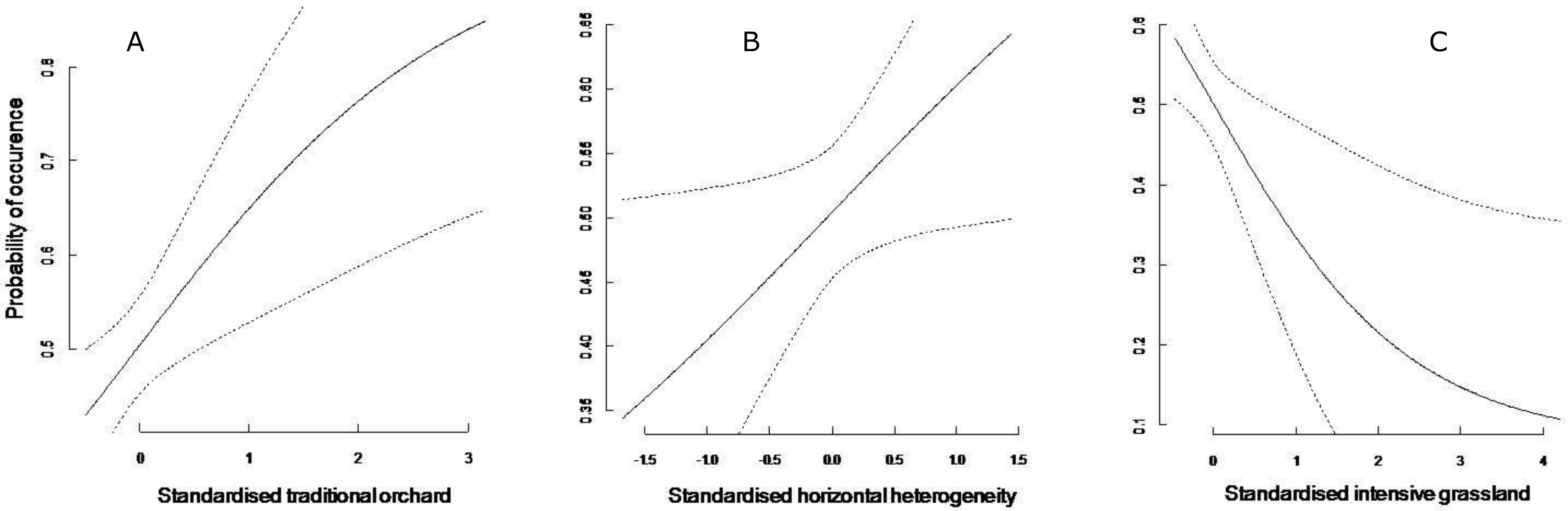

Fig. 2. *P. auritus* parameter estimates of habitat variables included in the averaging model, derived from a Generalised Linear Mixed Model (GLMM) procedure: Estimate of occurrence probability in relation to (A) traditional orchard; (B) horizontal heterogeneity; (C) intensive grassland. Broken lines show 95% confidence intervals.

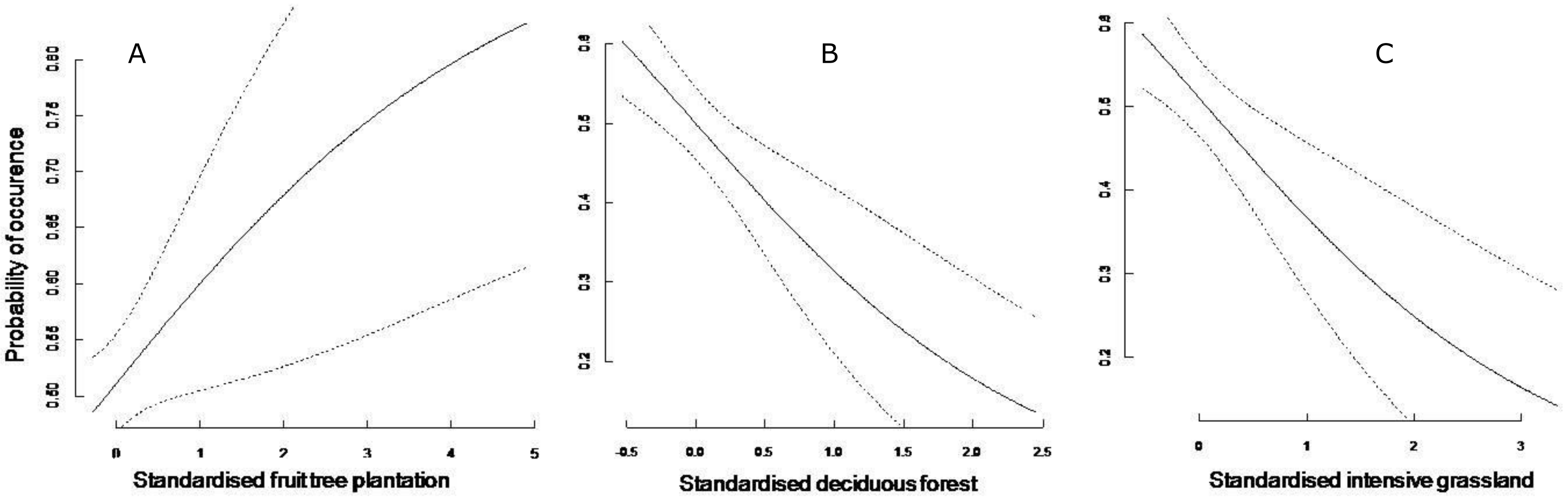

Fig. 3. *P. austriacus* parameter estimates of habitat variables included in the averaging model: Estimate of occurrence probability in relation to (A) fruit tree plantation; (B) deciduous forest; (C) intensive grassland. Broken lines show 95% confidence intervals.

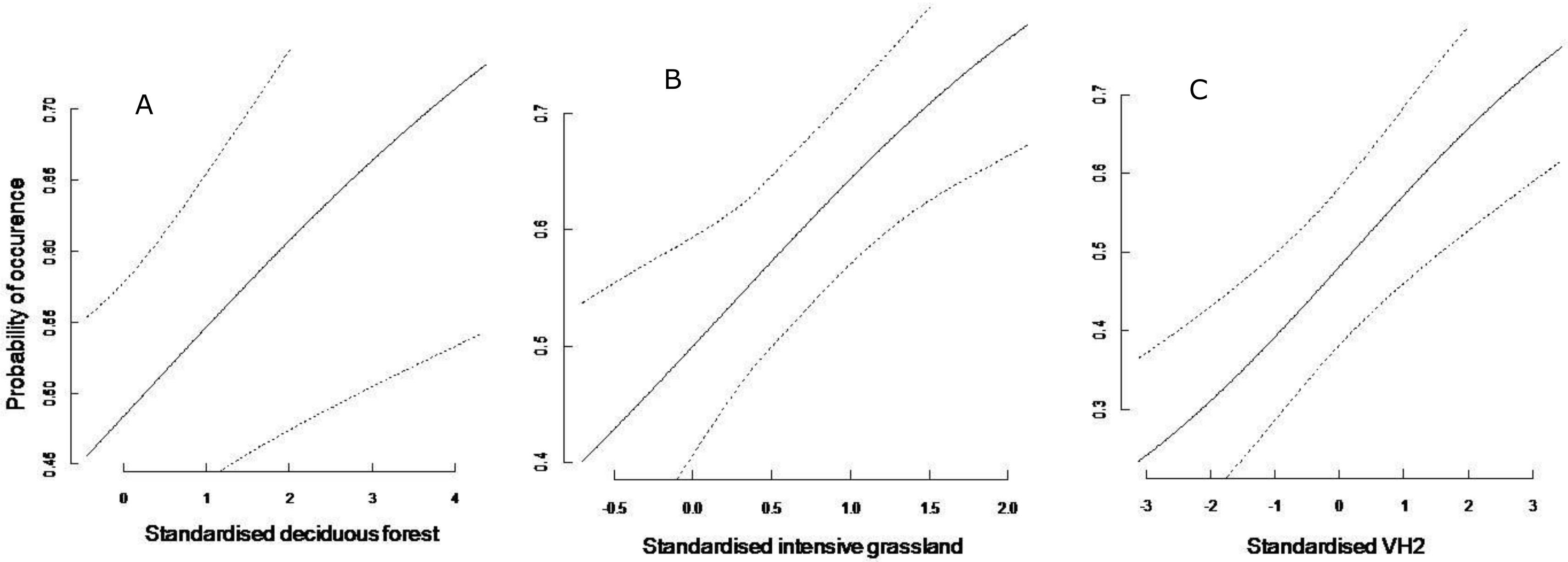

Fig. 4. *P. macrobullaris* parameter estimates of microhabitat variables included in the averaging model: Estimate of occurrence probability in relation to (A) deciduous forest; (B) intensive grassland; (C) VH2. Broken lines show 95% confidence intervals.

Fig. 5. Preferred foraging habitats selected by *P. auritus*: traditional orchard

Fig. 6. Preferred foraging habitats selected by *P. austriacus*: fruit tree plantation

Fig. 7. Preferred foraging habitats selected by *P. macrobullaris* deciduous and mixed forest.

Appendix S1. Variables recorded at visited (radiotracking) and random locations.

Main category	sub-category	Variable	Definition
Habitat cover	**I.**	Steppe	% cover
		Arable	% cover
		Vineyard	% cover
		Extensive grassland	% cover
		Intensive grassland	% cover
		Traditional orchard	% cover
		Fruit tree plantation	% cover
	II.	Deciduous forest	% cover
		Conifer forest	% cover
		Mixed forest	% cover
		Hedgerow	% cover of hedges and tree lines
		Settlement	% cover
		Road	% cover
Heterogeneity	**III.**	Horizontal heterogeneity	Simpson's index
		veg1wp	% of HC1 (0-1.5 m)
		veg2wp	% of HC2 (1.5-3 m)
		veg3wp	% of HC3 (3-10 m)
		veg4wp	% of HC4 (10-20 m)
		veg5wp	% of HC5 (>20 m)
		cp1wp	% aggregation HC1
		cp2wp	% aggregation HC2
		cp3wp	% aggregation HC3
		cp4wp	% aggregation HC4
		cp5wp	% aggregation HC5
		cp1wsd	SD aggregation HC1
		cp2wsd	SD aggregation HC2
		cp3wsd	SD aggregation HC3
		cp4wsd	SD aggregation HC4
		cp5wsd	SD aggregation HC5
		Vertical complexity	weighted mean number of unique vegetation height classes (in the 50 m buffer)

Appendix S2. The top best GLMM models explaining occurrence of *P. auritus* (A), *p. austriacus* (B) *and P. macrobullaris* (C) based on Akaike's Information Criterion (AIC). For each model, the number of estimated parameters (K), the difference of the AIC between that model and the best model (ΔAIC), and the Akaike weight are shown.

A *P. auritus*

Model No.	Variables	K	ΔAIC	Akaike weight
151	Horizontal heterogeneity+VH1+ VH2+Intensive grassland + Extensive grassland +Traditional orchard	8	0.000	0.256
49	Arable + Intensive grassland + Extensive grassland + Fruit tree plantation +Traditional orchard + Horizontal heterogeneity	8	0.787	0.173
56	Arable + Intensive grassland + Extensive grassland + Fruit tree plantation +Traditional orchard + Horizontal heterogeneity + VH2	9	1.282	0.135
155	Horizontal heterogeneity+ VH1+ VH2+Arable+Intensive grassland + Extensive grassland + Traditional orchard	9	1.391	0.128
158	Horizontal heterogeneity+ VH1+ VH2+Intensive grassland + Extensive grassland + Fruit tree plantation + Traditional orchard	9	1.888	0.100
55	Arable + Intensive grassland + Extensive grassland + Fruit tree plantation +Traditional orchard + Horizontal heterogeneity + VH1	9	2.706	0.066
59	Arable + Intensive grassland + Extensive grassland + Fruit tree plantation +Traditional orchard + Horizontal heterogeneity + VH1 + VH2	10	3.273	0.050

B *P. austriacus*

Model	Variables	K	ΔAIC	Akaike weight
115	Deciduous forest + Conifer forest + Mixed forest + Hedgerow + Arable + Intensive grassland + Extensive grassland + Fruit tree plantation	10	0.000	0.256

40	Arable + Intensive grassland + Extensive grassland + Fruit tree plantation +Traditional orchard + Deciduous forest +Mixed forest + Hedgerow	10	0.038	0.173
22	Arable + Intensive grassland + Extensive grassland + Fruit tree plantation +Traditional orchard + Deciduous forest +Mixed forest	9	0.542	0.135
44	Arable + Intensive grassland + Extensive grassland + Fruit tree plantation +Traditional orchard + Deciduous forest + Conifer forest + Mixed forest + Hedgerow	11	1.982	0.128
100	Deciduous forest + Conifer forest + Mixed forest + Hedgerow +Arable + Intensive grassland + Extensive grassland	9	2.095	0.100
37	Arable + Intensive grassland + Extensive grassland + Fruit tree plantation +Traditional orchard + Deciduous forest + Conifer forest + Mixed forest	10	2.420	0.066
116	Deciduous forest + Conifer forest + Mixed forest + Hedgerow + Arable + Intensive grassland + Extensive grassland + Traditional orchard	10	2.808	0.050
101	Deciduous forest + Conifer forest + Mixed forest + Hedgerow + Arable + Intensive grassland + Fruit tree plantation	9	3.636	0.050
117	Deciduous forest + Conifer forest + Mixed forest + Hedgerow + Arable + Intensive grassland + Fruit tree plantation + Traditional orchard	10	4.586	0.050

C *P. macrobullaris*

Model	Variables	K	ΔAIC	Akaike weight
189	Deciduous forest + Conifer forest + Mixed forest + Hedgerow + Horizontal heterogeneity + Steppe+ Horizontal heterogeneity + VH2	10	0.000	0.25
187	Deciduous forest + Conifer forest + Mixed forest +Hedgerow + Steppe+ VH2	8	0.230	0.223

191	Deciduous forest + Conifer forest + Mixed forest + Hedgerow + Horizontal heterogeneity + Steppe + VH1 + VH2	10	1.334	0.128
190	Deciduous forest + Conifer forest + Mixed forest + Hedgerow + Steppe + VH1+ VH2	9	1.634	0.11
22	Arable + Intensive grassland + Extensive grassland + Fruit tree plantation + Traditional orchard + Steppe + Deciduous forest + Mixed forest	10	2.818	0.061
178	Deciduous forest + Conifer forest + Mixed forest +Hedgerow+ Arable+ Intensive grassland +Extensive grassland + Fruit tree plantation + Steppe	11	3.558	0.042
37	Arable + Intensive grassland + Extensive grassland + Fruit tree plantation + Traditional orchard + Steppe + Deciduous forest + Conifer forest + Mixed forest	11	3.755	0.038
40	Arable + Intensive grassland + Extensive grassland + Fruit tree plantation + Traditional orchard + Steppe + Deciduous forest + Mixed forest + Hedgerow	11	4.521	0.026
44	Arable + Intensive grassland + Extensive grassland + Fruit tree plantation + Traditional orchard + Steppe + Deciduous forest + Conifer forest + Mixed forest + Hedgerow	12	4.719	0.024

Appendix S3. Observed frequency of habitat variables for each individual bat of all three *Plecotus* bat species. Radiotracking points are indicated by visited vs. random points.

Species, ID	Point type	Steppe	Arable	Vineyard	Extensive grassland	Intensive grassland	Traditional orchard	Fruit tree plantation	Deciduous forest	Conifer forest	Mixed forest	Hedge row	Infra structure
P. auritus, 1	visited	0.0	0.3	0.0	25.9	0.7	0.0	0.0	1.7	42.9	17.3	1.2	9.9
P. auritus, 2	visited	0.0	5.9	0.0	36.6	5.6	1.6	0.0	17.8	0.0	23.5	3.5	5.4
P. auritus, 3	visited	0.0	0.0	0.0	18.8	2.5	1.3	0.3	43.1	0.0	17.8	1.9	14.4
P. auritus, 4	visited	0.0	18.7	0.0	13.5	12.3	13.2	0.0	1.6	0.0	1.4	9.2	30.1
P. auritus, 5	visited	0.0	0.3	0.0	36.0	4.8	0.0	0.0	9.8	23.2	10.5	3.3	12.0
P. auritus, 6	visited	0.0	0.0	0.0	31.7	4.5	0.0	0.0	17.7	15.3	10.9	3.7	16.1
P. auritus, 7	visited	0.0	0.0	0.0	31.2	0.2	0.0	0.0	1.6	51.0	4.4	0.8	10.7
P. auritus, 8	visited	0.0	12.7	0.0	36.5	3.3	6.7	0.0	20.5	0.0	2.5	0.0	17.8
	average	0.0	4.8	0.0	28.8	4.2	2.8	0.0	14.2	16.5	11.1	3.0	14.5
P. auritus, 1	random	0.0	0.1	0.0	37.1	3.4	0.1	0.0	3.3	36.5	3.5	5.8	10.2
P. auritus, 2	random	0.0	6.5	0.0	24.0	18.5	0.6	0.1	22.1	0.0	17.4	1.8	9.1
P. auritus, 3	random	0.0	10.6	0.0	16.9	23.4	2.2	0.0	38.1	0.0	1.3	0.9	6.6
P. auritus, 4	random	0.0	7.4	0.0	32.7	25.2	2.1	0.0	4.9	0.0	23.9	1.2	2.5
P. auritus, 5	random	0.0	0.0	0.0	30.8	5.2	0.0	0.0	9.0	28.0	6.8	5.3	14.8
P. auritus, 6	random	0.0	0.0	0.0	38.7	12.2	0.0	0.0	5.2	24.5	7.9	4.3	7.3
P. auritus, 7	random	0.0	0.7	0.0	29.9	0.0	0.0	0.0	0.0	59.2	3.4	1.4	5.5
P. auritus, 8	random	0.0	19.9	0.0	26.2	22.6	3.5	0.0	12.8	0.0	6.0	1.0	8.0
	average	0.0	5.7	0.0	29.5	13.8	1.1	0.0	11.9	18.5	8.8	2.7	8.0
P. austriacus, 1	visited	0.0	28.5	0.0	15.4	27.9	1.5	0.0	11.0	6.2	0.0	1.9	7.7
P. austriacus, 2	visited	0.0	2.0	0.0	13.6	1.4	2.4	32.7	8.3	18.3	14.0	0.9	6.4
P. austriacus, 3	visited	0.0	24.4	0.0	22.7	0.6	1.4	0.0	29.1	0.0	11.1	0.4	10.1
P. austriacus, 4	visited	0.0	9.2	0.0	0.2	2.4	0.6	0.0	39.8	25.7	16.7	0.0	5.3
P. austriacus, 5	visited	0.0	36.3	0.0	27.3	6.8	5.1	0.1	4.7	0.0	0.0	5.2	14.6
P. austriacus, 6	visited	0.0	4.2	0.0	20.6	5.7	5.7	46.3	1.5	0.0	6.7	0.6	8.9
P. austriacus, 7	visited	0.0	13.6	0.0	31.4	32.6	7.3	0.1	0.2	0.0	0.0	1.6	13.2
P. austriacus, 8	visited	0.0	35.5	0.0	19.9	21.9	3.0	0.0	5.2	0.0	0.0	2.6	12.0
	average	0.0	19.2	0.0	18.9	12.4	3.4	9.9	12.5	6.3	6.1	1.6	9.8
P. austriacus, 1	random	0.0	14.4	0.0	17.3	18.5	2.3	0.0	39.8	0.0	3.8	0.4	3.5
P. austriacus, 2	random	0.0	16.3	0.0	13.1	17.9	2.9	2.9	26.1	6.9	9.6	0.4	4.0
P. austriacus, 3	random	0.0	31.1	0.0	9.1	10.3	1.0	0.0	34.7	0.0	6.6	0.4	6.7
P. austriacus, 4	random	0.0	18.7	5.8	12.6	10.6	5.5	0.0	26.8	7.7	5.7	0.9	5.8
P. austriacus, 5	random	0.0	33.2	0.0	21.9	10.7	1.5	0.2	23.6	0.0	2.9	2.4	3.8
P. austriacus, 6	random	0.0	3.3	0.0	17.8	12.6	5.1	9.0	8.1	3.1	30.0	0.8	10.1

P. austriacus, 7	random	0.0	33.4	1.5	14.9	38.5	2.6	0.0	2.2	0.0	0.0	0.9	6.0
P. austriacus, 8	random	0.0	16.1	0.4	18.6	6.2	0.4	0.0	33.2	0.0	20.9	0.7	3.6
	average	0.0	20.8	1.0	15.7	15.7	2.7	1.5	24.3	2.2	9.9	0.9	5.4
P. macrobullaris, 1	visited	0.0	2.6	0.0	10.8	29.8	0.0	0.0	11.6	32.1	0.0	2.3	10.8
P. macrobullaris, 2	visited	0.0	0.0	0.0	7.7	58.1	0.2	2.6	4.8	15.3	2.3	0.9	8.0
P. macrobullaris, 3	visited	0.0	25.5	0.0	30.5	28.5	0.5	0.0	1.8	0.0	0.0	2.3	11.1
P. macrobullaris, 4	visited	0.0	0.3	0.0	44.1	4.3	0.9	0.9	4.9	5.9	33.1	1.9	3.8
P. macrobullaris, 5	visited	0.7	0.3	0.0	29.7	29.8	0.0	0.0	6.3	10.3	9.7	1.2	12.0
P. macrobullaris, 6	visited	0.0	0.8	0.0	44.2	0.0	0.4	0.0	9.6	20.4	16.2	2.7	5.8
P. macrobullaris, 7	visited	0.0	13.3	0.0	10.4	29.2	0.1	0.0	35.2	0.0	4.7	2.8	4.2
P. macrobullaris, 8	visited	0.5	1.0	0.0	35.0	18.8	0.0	0.0	5.9	8.0	20.1	1.0	9.8
	average	0.1	5.5	0.0	26.5	24.8	0.2	0.4	10.0	11.5	10.8	1.9	8.2
P. macrobullaris, 1	random	0.0	3.4	0.0	10.8	38.7	0.0	0.0	5.5	28.2	0.0	1.6	11.8
P. macrobullaris, 2	random	0.9	0.0	0.0	18.3	36.4	0.0	0.0	10.3	21.4	5.3	1.2	6.1
P. macrobullaris, 3	random	0.0	30.9	0.0	34.4	19.8	0.0	0.0	3.6	0.0	0.0	3.3	7.9
P. macrobullaris, 4	random	0.0	0.4	0.0	22.9	6.5	0.6	0.0	2.9	37.1	16.2	1.8	11.6
P. macrobullaris, 5	random	2.2	0.2	0.0	22.2	37.3	0.0	0.0	5.8	5.8	12.7	1.3	12.5
P. macrobullaris, 6	random	0.0	0.4	0.0	47.3	3.5	1.5	0.0	9.2	13.1	11.5	3.8	9.6
P. macrobullaris, 7	random	0.0	0.8	0.0	17.5	4.5	0.1	0.0	6.5	30.0	15.0	0.6	25.0
P. macrobullaris, 8	random	4.2	0.9	0.0	36.2	11.2	0.0	1.3	10.0	13.3	14.8	1.3	7.0
	average	0.9	4.6	0.0	26.2	19.7	0.3	0.2	6.7	18.6	9.4	1.9	11.4

5

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

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

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5.1 Abstract

The discovery of cryptic species poses new challenges for conservation. Species distribution and status have to be re-evaluated and the ecological requirements within the species complex as a whole have to be re-assessed to suggest adequate conservation guidelines. The recent discovery of the cryptic bat species *Plecotus macrobullaris* in Switzerland calls for a novel appraisal of the distribution and conservation status of the three indigenous *Plecotus* species.

We investigated the environmental niches (Ecological Niche Factor Analysis; ENFA) of the three long-eared bat species at the landscape scale and modelled their potential distributions using DNA-assessed records. Discriminant analysis was used for interspecific comparisons of niches. The occurrence of all three species was explained by proximity to rural settlements, probably due to a higher roosts offer in buildings, and by warm summer temperature. The distribution of *P. auritus* was positively associated with forest ecotonal structures and coniferous woodlands in heterogeneous landscape matrices. *P. austriacus* preferred orchards and vineyards; it avoided open and coniferous forests, but deciduous forests were marginally beneficial. *P. macrobullaris'* presence was linked mostly to deciduous forests, with an avoidance of shrubbery and meadowland. *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 to occur mainly in the lowlands, with its habitat requirements overlapping with those of *P. macrobullaris*. Although the potential distribution areas of these two species overlapped, current observations suggest a parapatric distribution in Switzerland, possibly evoked by interspecific competition over similar resources.

The projected distribution of *P. auritus* confirms previous knowledge, whereas our results shed new light on the distributions of the two other species*.* In contrast to the newly discovered *P. macrobullaris*, which is actually quite widespread and common in the Central and Southern Swiss Alps, *P. austriacus* is restricted to warmer cultivated lowlands and thus may suffer from recent major land-use changes. We suggest reclassifying *P. austriacus* to a higher conservation status. Modelled distributions can serve for new geographic range assessments, to specify regional status and to orient finer field surveys and ecological research.

Keywords: Chiroptera, conservation status, Ecological niche factor analysis (ENFA), discriminant analysis, niche characteristics, *Plecotus*, potential distribution, Switzerland

5.2 Introduction

The recognition of cryptic species, i.e. species that were originally not distinguished from others due to high morphological similarities (Bickford *et al.* 2007), contributes to a wide extent to the discovery of new species (Ceballos and Ehrlich 2009), partly due to the increasing application of molecular genetic techniques. In mammals for instance, the worldwide number of known species increased by 10% within the last 15 years, with 60% of the new discoveries being cryptic species (Ceballos and Ehrlich 2009). The discovery of cryptic species implies that the previously known species belonging to the cryptic species complex are in fact rarer than was realised. The acquired knowledge about the previously known species has thus to be questioned and, consequently, the potential distribution and ecology of all the species within the cryptic complex have to be re-evaluated in order to decide if conservation action is necessary.

Chiroptera species represent 21% of the 5487 known mammalian species (Schipper *et al.* 2008). Since 1993, 94 new bat species have been described worldwide (Ceballos and Ehrlich 2009) and even in a well studied area as Europe many new species have been identified since the introduction of protein and DNA screening (e.g. Arlettaz *et al.* 1997b, Mayer *et al.* 2007). In 2001, a new cryptic species of long-eared bats, *Plecotus macrobullaris*, was discovered (Kiefer and Veith 2001; Spitzenberger *et al.* 2002; Spitzenberger *et al.* 2003). Its morphological characteristics overlap considerably with its sibling species, *P. auritus* and *P. austriacus* (Kiefer and Veith 2001, Pavlinić and Tvrtković 2004, Ashrafi *et al.* in press), 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 woodlands up to the timberline (Beck *et al.* 1995) whereas *P. austriacus* was mainly restricted to the lowlands (Beck 1995). Due to the discovery of *P. macrobullaris* the hitherto knowledge is questionable. Therefore, distribution and ecological niche requirements of all three *Plecotus* species, especially in the Alps, should be reassessed.

This study explores the environmental niches, mostly habitat requirements (hereafter often referred to as «niche» for simplicity) and predicts the potential distributions of all three sibling species as a basis for reassessment of their conservation status in Switzerland. We applied a presence-only species distribution model using genetically verified data, supplemented by properly re-identified museum specimens (skull morphometric) in the case of the rarer *P. austriacus*. We further interpreted the distribution patterns of the three species and identified differences in their realised niches by determining the most important eco-geographical predictors for occurrence at the landscape scale. Niche breadth and niche overlap were quantified and areas of potential occurrence in Switzerland determined in order to prioritize areas for future research and conservation actions.

5.3 Material and methods

5.3.1 Study site

The study was carried out in Switzerland, Central Europe. The country covers a total area of 41,293 km^2 subdivided into six biogeographical regions (Fig. 1, Gonseth *et al.* 2001). 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 and more extensively as meadowland or pastureland. There the slopes are covered with predominantly coniferous forests up to 2300 m a.s.l., although grassland, crops and vineyards occur on some foothills. The southern part of the Alps, Valais (Western Central Alps) and Ticino (Southern Alps) are characterised by a mild climate with deciduous forests prevailing in the Ticino valleys. The Jura, a calcareous hill range, consists of predominantly large coniferous woodlands and pastures. Between these two mountain ranges lies the lower Midland, which is densely populated, intensively cultivated and characterised by relatively small and fragmented forests.

5.3.2 Species data

We used published DNA-verified species records (Juste *et al.* 2004, Ashrafi *et al.* in press, Mattei-Roesli *et al.* 2010, Kiefer, A. unpublished data). In addition, DNA samples (faeces and tissues) collected by regional bat workers were analysed by the A. Kiefer group, Germany (protocol in Ashrafi *et al.,* in press). Because the knowledge of current distribution patterns is of major interest for conservation, we focussed mainly on DNA-verified specimens sampled within the last 25 years (since 1984; Fig. 1). However, to enlarge our dataset for the rare *P. austriacus* we added 11 skull-measured specimens from 1948-1992 (I. Pavlinić, unpublished data). To obtain independence between samples we avoided multiple records from the same colony and applied a minimum distance of 1300 m between samples, which resembles the average foraging distance from the roost, as measured for all three species (chapter 4 of this thesis).

5.3.3 Eco-geographical Variables (EGVs)

Based on multiple literature reviews on ecological requirements (e.g. Entwistle *et al.* 1996, Flückinger and Beck 1995, Fuhrmann and Seitz 1992, Kiefer and Veith 1998, Swift and Racey 1983) and on information obtained from a radio tracking study of the three *Plecotus* species (chapter 4), variables which were expected to be relevant for at least one of the three species were preselected for the analyses (Table 1). All EGVs were prepared as raster maps with a 100 x 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 the feature's 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 (chapter 4). In FRAGSTATS (McGarigal *et al.* 2002) we computed two landscape diversity metrics based on four landscape types (settlement, forest, shrubbery, meadows): Patch richness (PR) measures the number of different landscape types present within a predefined radius around each grid cell. Patch diversity (Simpson's diversity index, SIDI) additionally includes the proportional distribution of area among landscape types. These index values were calculated and averaged within a circular moving window (r = 1300 m). Before applying the ENFA, all eco-geographical variables (EGVs) were normalised by Box-Cox transformation (Box and Cox 1964).

5.3.4 Data analysis

An Ecological Niche Factor Analysis (ENFA, Hirzel *et al.* 2002) was performed to investigate the species' 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 for elusive bat species, or when absences are considered unreliable as in cryptic species complexes (Hirzel *et al.* 2001). Based on Hutchinson's (1957) concept of the ecological niche as the multi-dimensional space of environmental conditions in which the species is able to persist, ENFA compares the environmental conditions at locations used by the species with those prevailing in the study area. It thus requires two types of input data: presence data of the three species and a set of eco-geographical variables (EGVs) 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 specialisation (Hirzel *et al.* 2002). Marginality, as maximised by the first factor, describes the deviation of the mean environmental conditions at the species' locations (species distribution) from those in the study area (global distribution), thus indicating the niche position. Specialisation, as maximised 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). For each species, we first ran an analysis including all EGVs. In order to simplify the models, we subsequently discarded from the less important from pairs of highly intercorrelated variables (Pearson's r > 0.75) the less important from pairs as well as all irrelevant variables (contribution to marginality and specialisation < 0.1 for all three species).

5.3.4.1 Potential distribution:

Based on the resulting predictor set, maps of potential species' distributions were calculated to identify potentially suitable and non-suitable areas for the three species. Habitats suitability (HS) maps were computed from the number of factors containing a significant amount of information as determined according to MacArthur's broken stick heuristics (Mc Arthur 1960, Hirzel *et al.* 2002). 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. It assigns a partial HS-value to each cell in the study area, which is proportional to the cells' distance 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 (Hirzel *et al.* 2002).

5.3.4.2 Model validation

Models were evaluated by means of a five-fold cross-validation (integrated in BIOMAPPER 4.0, Hirzel *et al*. 2007). As an evaluation measure we calculated the continuous Boyce index ranging from -1 to 1, with 0 indicating a random model (Hirzel *et al*. 2006).

5.3.4.3 Niche differentiation

The species' niches were compared by discriminant analyses (Legendre and Legendre 1998) based on the same set of eco-geographical variables. Discriminant factors were computed so as to maximise the interspecific variance between the three species by minimising the intraspecific variance at the same time. The correlations of the EGVs with the discriminant factors indicate along which variable the species' distributions differ most. An analysis of the species' frequency distributions along the discriminant factors permits comparison of niche breaths and niche overlaps between species. The standardised Levin's index (B') (Colwell and Futuyma 1971) with values ranging from 0 (narrow niche) to 1 (broad niche) was employed to measure niche breadth (Arlettaz *et al.* 1997c). 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 nonreciprocal. All calculations are integrated in BIOMAPPER 4.0 (Hirzel *et al.* 2007).

5.4 Results

5.4.1 Presence data

We collected 115 *Plecotus* records from Switzerland (Fig. 1). The samples of *P. auritus* (N = 45) were distributed over all six bioregions in Switzerland (Fig. 1 and 2). *P. austriacus* (N = 21) was found in Northern (lower Midland and Jura) and Western Switzerland, with only one record at low altitude in the Alps (Western Central Alps; Fig. 1 and 3). *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. 1 and 4).

5.4.2 Environmental niche characteristics and potential distributions

The models revealed the lowest overall marginality of 1.03 and a high tolerance of 0.67 for *P. auritus,* indicating that this species is the most generalist among the tree. The high overall marginality in *P. austriacus* (1.40) and *P. macrobullaris* (1.56) indicates that the occurrences of these species depend 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 specialisation (S), with coefficients c(M) and c(S) > |0.2|. Additional results can be inferred from table 2. Among the EGVs 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 (above) and a lower specialisation (c(S) = 0.15) than the other two species (0.39 and 0.21 for *P. austriacus* and *P. macrobullaris*, respectively). A high marginality regarding landscape diversity (patch richness and patch diversity) 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 EGVs contributing to marginality were long forest border length (0.29) and edges (0.29 and 0.27, both also showing high contributions to specialisations of 0.33 and 0.29, respectively) and coniferous forests (0.21). The potential distribution for *P. auritus* was computed using the first seven factors, which accounted for 91% of the explained information. Potentially suitable areas for this species are present throughout Switzerland (Fig. 2).

P. austriacus preferred short distances to towns (-0.30) as well as a high frequency of orchards and vineyards (c(M) = 0.36), and avoided coniferous and open forests (-0.34 and -0.28), with at the same time showing a low tolerance towards deviations from optimal conditions in these variables (c(S) 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 explaining 97% of the information. The model indicated potentially suitable areas in the Midland 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. 3).

In *P. macrobullaris* an important EGV contributing both marginality and specialisation (Table 2) was a high frequency of deciduous forest (c(M) = 0.32; c(S)= 0.24). Meadows and shrubbery (-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 on three factors explaining 88% of the information. The most suitable areas for this species were situated in the Western Centrals Alps and Ticino (Southern Alps), lower Alpine valleys and some small scattered areas in the Jura and the Midland (Fig. 4).

Being a surrogate for temperature and thus highly correlated with this variable, altitude *per se* was not included in our models. The resulting maps, however, predicted, *P. auritus* to occur up to the timberline (ca 2000 m a.s.l.), *P. macrobullaris* up to moderate elevations (ca 1500 m) whereas *P. austriacus* was restricted to low elevations (below 600 m).

5.4.3 Model evaluation

The model evaluation revealed the highest predictive power for the distribution model of *P. auritus.* For this species the continuous Boyce index, B_{cont} was comparatively closer to one and had a small standard deviation (B_{cont} = 0.77 ± 0.10, mean ± 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.

5.4.4 Niche differentiation

In the discriminant analysis the first two factors accounted for 58% of the total variance (first factor: 37%, second factor: 21%) indicating a moderate level of discrimination between the three species, at least as expressed by our model, as well as extensive overlaps of their niches (Fig. 5). The first discriminant factor maximised the partitioning between *P. auritus* and *P. macrobullaris*, with *P. austriacus* taking an intermediate position closer to *P. macrobullaris* (Fig. 5). 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. 6, Table 3). The species frequency distributions along the second discriminant axis showed a separation of *P. austriacus* from the other two species which largely overlapped (Fig. 5). Along this axis, *P. austriacus* was situated more towards the pole of orchards and vineyards, whereas the other two species located more towards forests (open and coniferous), forest border length and edges (Fig. 6, Table 3).

The standardised Levin's niche breath 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) and *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 in the niches of the two other 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*. Along the second discriminant factor *P. auritus* was again overlapping with *P. macrobullaris* and *vice versa* (see also Fig. 5).

5.5 Discussion

Spatial distribution models are useful for setting conservation priorities for both species and habitats (Chefaoui and Lobe 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 are advantageous because they allow efficiently processing even incomplete information, i.e. making best use of available but limited data (Elith and Leathwick 2007, Braunisch & Suchant 2010). Nowadays, many natural history museums are digitalizing their collections, with the idea to provide online open access to researchers. Readily available, the information offers great opportunities for building preliminary, but extended spatial models like the one developed here (Reutter *et al.* 2003, Rowe 2005). If the appropriate modelling approaches are applied, even biases inherent to sampling (e.g. higher density in better surveyed areas) would not represent a major obstacle (Elith and Leathwick 2007, Hausser *et al.* 1995). Braunisch & Suchant (2010) have recently demonstrated that species data collected unsystematically over a large representative region can provide better models than data collected systematically at smaller scales, which opens new perspectives in spatial conservation modelling. We accounted for potential deficiencies in spatial precision of the collated *Plecotus* data by choosing a sampling and predictor resolution that corresponds to the average foraging range of a colony. Consequently, our models cannot elucidate fine-grained resource selection patterns but address the environmental preconditions for species presence at a landscape scale, thus representing the first step in a hierarchical approach of disentangling the different dimensions of the sibling species' niches.

5.5.1 *Important eco-geographical variables*

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). High landscape diversity was also important for *P. auritus,* and to a lesser extent for *P. macrobullaris*. Highly important predictors for the distribution of single species were: forest edges and 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* which avoided meadows and shrubbery. *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.

5.5.1.1 Rural settlements

The positive association of all three species to 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 and Seitz 1992; Beck *et al.* 1995). For *P. austriacus* there is no evidence for the use of tree cavities (Beck 1995), similar to *P. macrobullaris* which has been found almost exclusively in buildings so far (Kiefer and von Helversen 2004, Mattei-Roesli 2010; only one radio-tracked individual occupying a tree cavity, S. Ashrafi, unpublished data). However, as presence data were not sampled systematically, our results may also be affected by sampling bias because bats are more easily found in populated areas.

5.5.1.2 Temperature and elevation –

P. austriacus, an originally Mediterranean species, has previously been described as thermophilous (Horaček *et al.* 2004; Beck 1995). *P. 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 show the same trends: in Croatia the majority of *P. macrobullaris* (19 out of 28 localities) were found at altitudes lower than 800 m a.s.l. (Pavlinić and Tvrtković 2004). This is also the case in Northern Italy (Trizio *et al.* 2005; 693±341 m a.s.l.) and Ticino (Southern Switzerland) (Mattei-Roesli 2010, mean altitude around 450 m a.s.l.). Furthermore, the Swiss Alps represent according to our current knowledge 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.

5.5.1.3 Foraging habitats

Our large-scale models provide some information about possible foraging habitat preferences of the bats at a smaller scale. Our results (Table 2) suggest for *P. auritus* a preference for forest ecotonal 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 and Racey 1991). There were also no reports of foraging of radiotracked *P. auritus* over meadows (Entwistle *et al.* 1996;

Fuhrmann and Seitz 1992; Swift and Racey 1983). The observed preference of *P. austriacus* for orchards and vineyards is in line with former studies (Flückinger and Beck 1995; Kiefer and Veith 1998). An avoidance of open forests in general and coniferous forests in particular, with a marginal contribution (c(M) = 0.16) of deciduous forests in this study (Table 2), supports the view that foraging often takes place in dense deciduous woodlands (Kiefer and Veith 1998). The preference of *P. macrobullaris* for deciduous forests in this study is supported by radiotracking surveys conducted in Valais (Western Central Alps of Switzerland; chapter 4 of thesis). In Submediterranean areas, the distribution of this species ranges from deciduous forests on the lower foothill slopes to the forested subalpine belt which includes coniferous trees (Pavlinić and Tvrtković 2004).

5.5.2 Are P. austriacus and P. macrobullaris parapatric in Switzerland?

Based on their known distributions and currently distant environmental niches (Tables 2 and 4; Figs. 1, 3 and 4), *P. austriacus* and *P. macrobullaris* seem to exclude each other over large areas in Switzerland, even though their potential distributions are somewhat 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 surveys (Mattei-Roesli 2010). Areas where apparent suitable habitat is not occupied are sometimes referred to as «non-equilibrium distributions» (Chefaoui and Lobo 2007). In our case there are two main alternative explanations to the apparent parapatric distribution. First, the Alps may have functioned as an historical barrier to colonization after glaciers receded. In Austria, *P. austriacus* was only recorded in the northeastern Alps (Spitzenberger 2001). In Lombardy, Northern Italy, there are no records south of the Alps (Trizio *et al.* 2005). In contrast, all three species widely overlap in their distribution in Croatia (Tvrtković *et al.* 2005) where the alpine barrier is not present. Second, competitive exclusion may have led to the current, «mutual avoidance» distribution pattern. This scenario is 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 extremely similar moth-based diets (chapter 3 of thesis). Further research is necessary to elucidate the reasons for the observed parapatric distribution.

5.5.4 Conclusions and implications for conservation

Our three model species are classified (Duelli 1994) as endangered (*P. auritus*), potentially endangered (*P. austriacus)* and not yet categorised (*P. macrobullaris)* in the Swiss red list. The high reciprocal environmental niche overlap of *P. auritus* and *P. macrobullaris* shown by the Lloyd 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* suggests that these two species may compete for 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, its currently wide distribution questions in our opinion the validity of its classification as endangered. Due to uncertainties in the occurrence of *P. austriacus*, which were largely due to the inability of local bat workers to identify Alpine long-eared bats in the past (e.g. Arlettaz *et al.* 1997a), due also to missing systematic surveys of population trends (Beck 1995), *P. austriacus* represents a typical conservation problem around cryptic species (Schönrogge *et al.* 2001). 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* to a species of high conservation concern which requires tight monitoring and specific conservation measures. *P. macrobullaris* has not been allocated a red list status yet. It seems quite common in the Alpine arch, but more investigations are needed to specify its distribution. Our predictive maps will be useful to this task. This study highlights the possible changes in biogeographical, ecological and conservation knowledge evoked by the discovery of novel species in cryptic complexes (Sattler *et al.* 2007, Schönrogge *et al.* 2002), stressing the importance of an early recognition of cryptic species and their habitat requirements for conservation management.

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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 follwing reasons: 1 = contribution to marginality and specialisation <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).

Variable category	EGVs	Description	Data source	Survey period
Forest	Coniferous forest (≥90%) (fq)	90-100% conifers	Geostat	1990/92
	Mixed forest (fq)	> 10% and < 90% conifers	Geostat	1990/92
	Deciduous forest (≥ 90%) (fq)	90-100% deciduous trees	Geostat	1990/92
Semiopen 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 *et al.* 2002) on forest, shrubbery, meadows and settlements.	Swisstopo	2004
	Patch diversity (fq)		Swisstopo	2004

Table 2. Ecological Niche Factor Analysis (ENFA). Contribution of the eco-geographical variables (EGVs) to marginality (M), explained specialisation (Expl. S.) and explained information (Expl. I.; defined as (M + Expl.S.)/2)) as calculated over the number of significant factors (F.) used in the ENFA models. In bold variables contributing most to marginality and explained specialisation (coefficients > |0.2|). Positive marginality values indicate a preference for a certain variable, negative values indicate avoidance. Negative coefficient values for distance (dist) variables have to be interpreted as avoidance of increasing distance.

EGVS Contribution to:	*P. auritus*			*P. austriacus*			*P. macrobullaris*		
	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

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

Eco-geographical variables	DF 1 (37%)	DF 2 (21%)
Coniferous forest (≥90%) (fq)	0.13	**-0.37**
Mixed forest (fq)	0.31	-0.17
Deciduous forest (≥ 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. Niche breath. The standardised Levin's niche breath 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%)
P. auritus	0.26	0.37	0.31
P. austriacus	0.14	0.22	0.17
P. macrobullaris	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). In parentheses: variance explained by the corresponding factors.

		Y		
	X	*P. auritus*	*P. austriacus*	*P. macrobullaris*
DF1 (37%)	*P. auritus*	–	0.64	1.07
	P. austriacus	1.38	–	1.74
	P. macrobullaris	0.98	0.74	–
DF2 (21%)	*P. auritus*	–	0.42	1.76
	P. austriacus	0.91	–	1.19
	P. macrobullaris	1.61	0.51	–

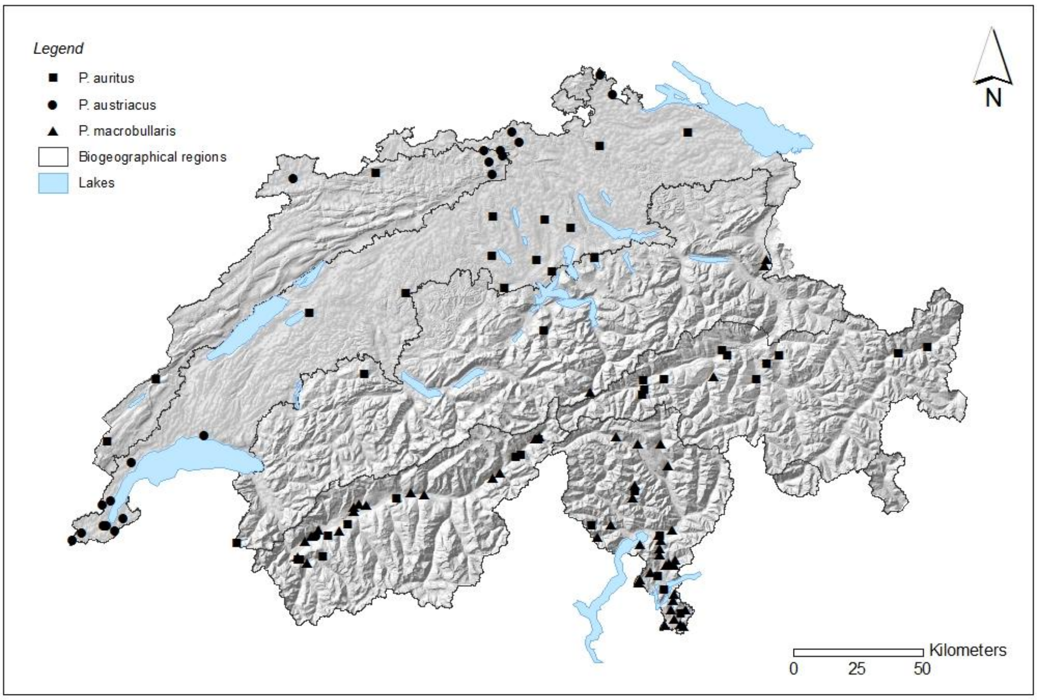

Figure 1. Genetically ascertained records of *Plecotus auritus* (N = 45), *P. austriacus* (N = 21) and *P. macrobullaris* (N = 49) in Switzerland. The six biogeographical regions of Switzerland according to Gonseth *et al.* (2001) are delimited by black lines.

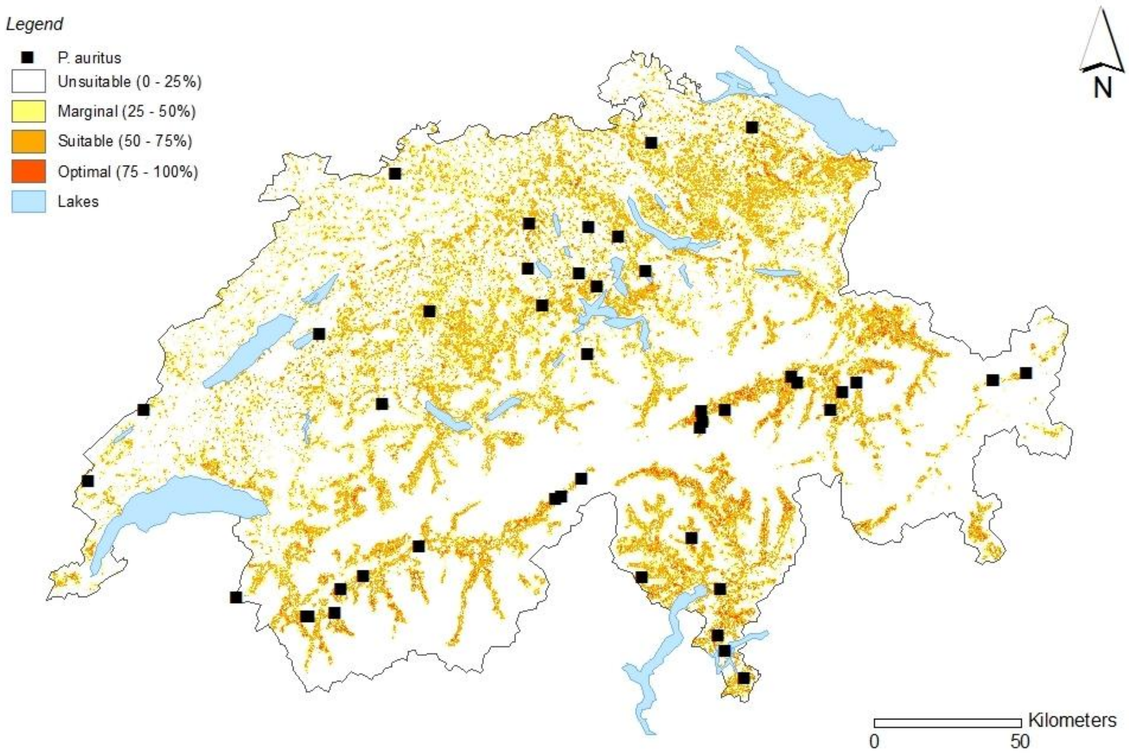

Figure 2. Potential distribution of *Plecotus auritus* in Switzerland, modelled on the basis of N = 45 genetically ascertained locations.

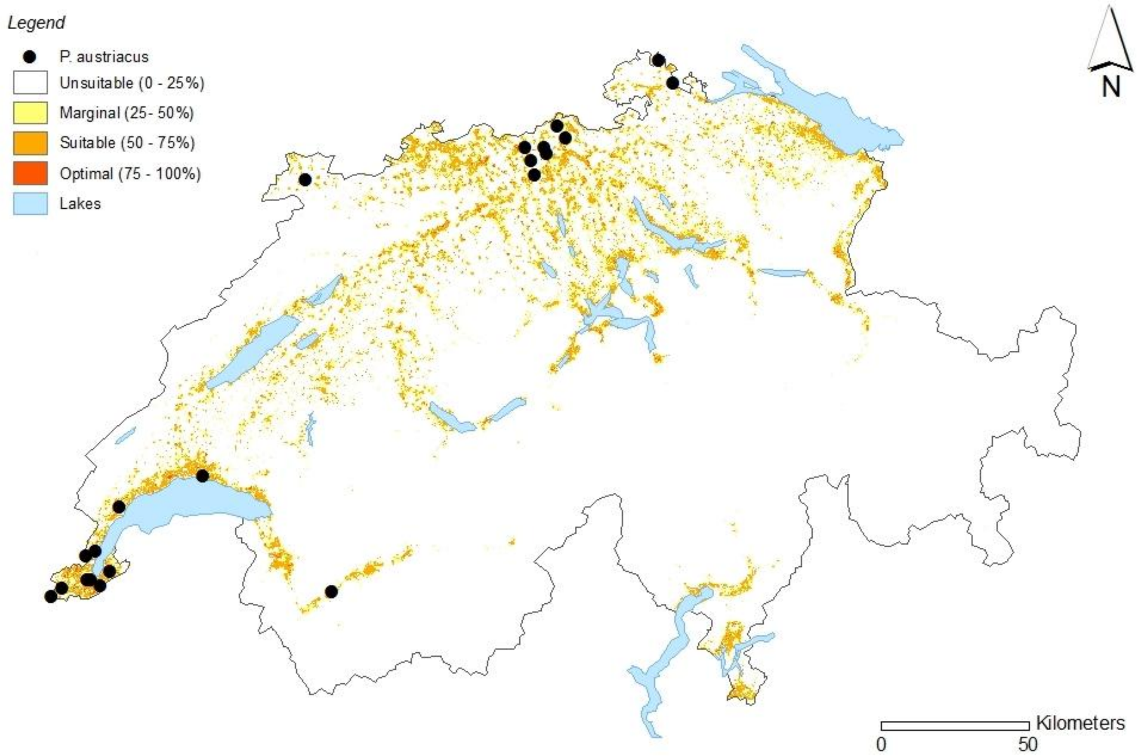

Figure 3. Potential distribution of *P. austriacus* in Switzerland, modelled on the basis of N = 21 genetically and morphometrically (skull) ascertained locations.

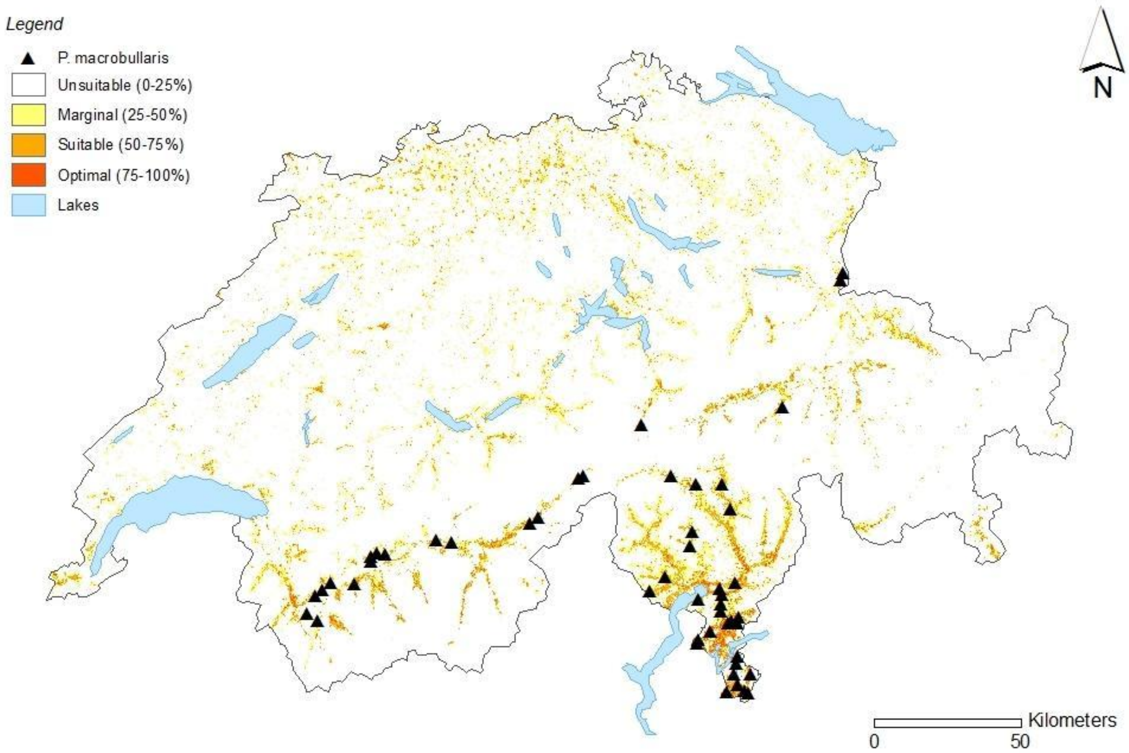

Figure 4. Potential distribution of *P. macrobullaris* in Switzerland modelled on the basis of N = 49 genetically ascertained locations.

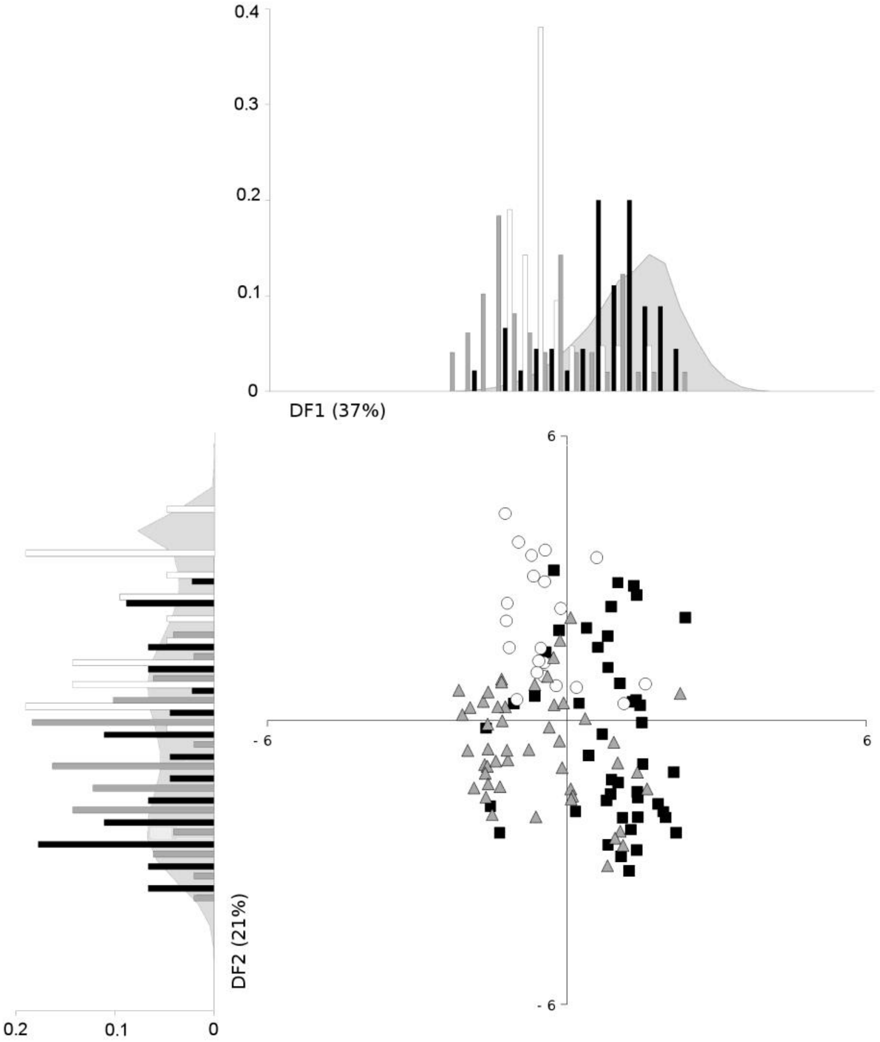

Figure 5. Discriminant analysis of the distributions of *P. auritus* (black), *P. austriacus* (white) and *P. macrobullaris* (grey), calculated on the eco-geographical variables given in Table 1. In the histograms the relative frequency of species observations along the two discriminant factors (DF) 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 on the two first DF. The contributions of each factor to the explained variance are given in brackets.

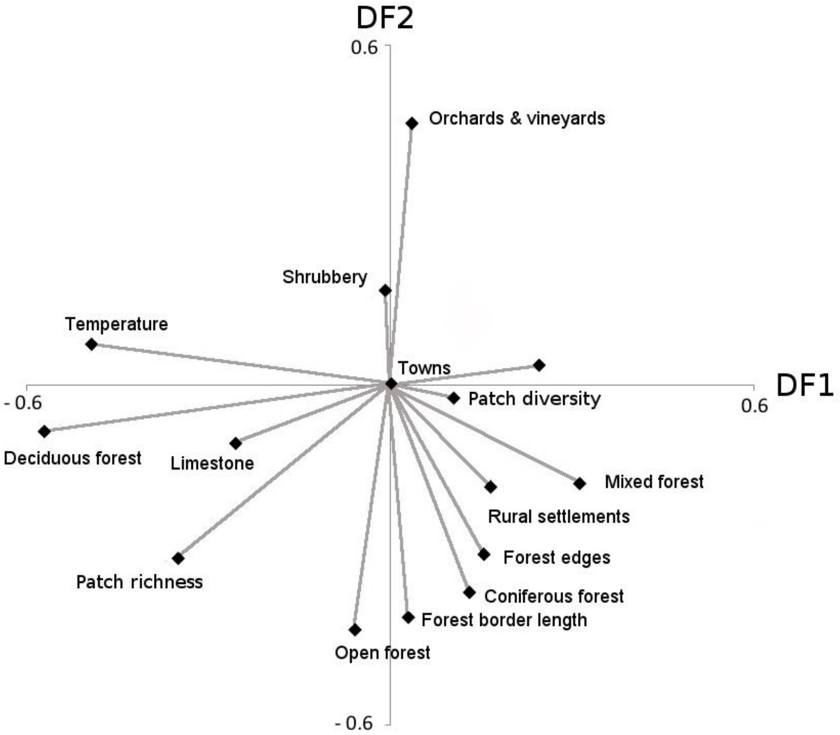

Figure 6. Discriminant analysis. Correlations of eco-geographical variables (EGVs, defined in Table 1), with the first and second discriminant factor. The distance of an EGV from the centre indicates its importance for species discrimination.

Appendix 1 Ecological Niche Factor Analysis (ENFA). Contribution of the eco-geographical variables (EGVs) to the marginality (M) and specialisation factors (F), to overall explained specialisation (Expl. S.) and explained information (Expl. I.). Coefficient values are provided for the significant factors of the ENFA models for *P. auritus* (N = 45), *P. austriacus* (N = 21) and *P. macrobullaris* (N = 49).

P. auritus

Variable Category	EGVs	M + F1 (14%)	F2 (20%)	F3 (18%)	F4 (11%)	F5 (8%)	F6 (7%)	F7 (6%)	Expl. S. (82%)	Expl. I. (91%)
Closed habitats	Coniferous forest (≥90%) (fq)	0.21	0.03	-0.18	0.42	0.14	-0.51	-0.32	0.17	0.19
	Mixed forest (fq)	0.12	0.24	-0.33	-0.23	0.32	0.23	-0.05	0.19	0.15
	Deciduous forest (≥ 90%) (fq)	0.14	0.16	0.21	-0.02	-0.32	-0.29	-0.14	0.14	0.14
Semiopen habitats	Forest border length (fq)	0.29	-0.38	-0.57	0.32	-0.31	0.67	-0.16	0.33	0.31
	Forest edges (fq)	0.27	0.63	0.18	-0.38	-0.17	0.03	0.70	0.29	0.28
	Open forest (fq)	0.20	-0.07	0.39	-0.23	-0.17	0.08	0.06	0.16	0.18
	Shrubbery (fq)	-0.18	0.00	-0.27	-0.21	-0.41	-0.15	0.12	0.14	0.16
	Orchards and vineyards (fq)	0.09	0.00	0.18	-0.30	-0.14	-0.15	-0.03	0.10	0.10
Open habitats	Meadows (fq)	-0.07	0.46	0.29	0.15	-0.08	0.01	0.31	0.19	0.13
Settlements	Towns (dist)	-0.10	-0.04	-0.01	-0.07	0.14	-0.04	0.06	0.05	0.07
	Rural settlements (dist)	-0.67	0.11	-0.13	-0.09	0.15	0.04	-0.06	0.16	0.42
Geology	Limestone (fq)	-0.16	0.21	0.12	0.18	-0.39	-0.08	-0.31	0.16	0.16
Climate	Average summer temperature	0.25	0.05	-0.12	0.35	0.33	-0.25	0.09	0.15	0.20
Landscape and habitats*	Patch richness (fq)	0.29	-0.17	-0.16	-0.16	-0.09	0.03	-0.33	0.15	0.22
	Patch diversity (fq)	0.22	0.28	0.22	-0.35	0.35	-0.18	-0.16	0.21	0.22

P. austriacus

Category	EGVs	M + F1 (80%)	F2 (8%)	F3 (4%)	F4 (2%)	Expl. S. (95%)	Expl. I. (97%)
Closed habitats	Coniferous forest (≥90%) (fq)	-0.34	-0.51	0.12	-0.49	0.33	0.33
	Mixed forest (fq)	-0.09	0.11	-0.55	0.57	0.12	0.11
	Deciduous forest (≥ 90%) (fq)	0.16	-0.45	-0.29	-0.19	0.18	0.17
Semiopen habitats	Forest border length (fq)	0.02	0.06	0.15	0.24	0.03	0.03
	Forest edges (fq)	-0.09	0.27	0.46	-0.14	0.11	0.10
	Open Forest (fq)	-0.28	-0.17	0.25	0.35	0.26	0.27
	Shrubbery (fq)	0.01	0.00	0.13	0.27	0.02	0.02
	Orchards and vineyards (fq)	0.36	0.14	0.13	0.08	0.30	0.33
Open habitats	Meadows (fq)	-0.07	-0.16	-0.29	0.03	0.08	0.07
Settlements	Towns (dist)	-0.30	0.12	0.04	0.02	0.25	0.28
	Rural settlements (dist)	-0.56	0.00	-0.07	0.16	0.45	0.51
Geology	Limestone (fq)	0.03	-0.10	0.19	-0.21	0.04	0.04
Climate	Average summer temperature	0.45	-0.38	-0.01	0.08	0.39	0.42

Landscape and habitats*	Patch richness (fq)	0.14	-0.08	0.09	0.13	0.13	0.13
	Patch diversity (fq)	0.10	0.45	0.36	0.16	0.13	0.11
P. macrobullaris							
Category	EGVs	M + F1 (38%)	F2 (23%)	F3 (15%)		Expl. S. (76%)	Expl. I. (88%)
Closed habitats	Coniferous forest (≥90%) (fq)	-0.01	0.19	0.19		0.08	0.05
	Mixed forest (fq)	0.03	0.59	0.05		0.15	0.09
	Deciduous forest (≥ 90%) (fq)	0.32	0.36	-0.25		0.24	0.28
Semiopen habitats	Forest border length (fq)	0.19	0.36	0.23		0.19	0.19
	Forest edges (fq)	0.20	0.05	0.28		0.13	0.16
	Open Forest (fq)	0.20	0.14	-0.34		0.16	0.18
	Shrubbery (fq)	-0.23	0.04	-0.09		0.11	0.17
	Orchards and vineyards (fq)	-0.01	-0.01	0.20		0.04	0.03
Open habitats	Meadows (fq)	-0.24	0.01	0.46		0.16	0.20
Settlements	Towns (dist)	-0.06	-0.02	-0.06		0.04	0.05
	Rural settlements (dist)	-0.61	0.31	-0.15		0.32	0.46
Geology	Limestone (fq)	-0.08	0.46	-0.36		0.19	0.13
Climate	Average summer temperature	0.40	0.09	0.27		0.21	0.31
Landscape and habitats*	Patch richness (fq)	0.31	-0.13	-0.03		0.15	0.23
	Patch diversity (fq)	0.20	0.00	-0.40		0.14	0.17

The first factor explains 100% of the marginality (M), the contribution to the explained specialisation of each specialisation factor (F) is given in brackets. Positive coefficient values on the marginality factor indicate preference for a certain variable, negative values indicate avoidance. Negative distance (dist) values to an EGV have to be interpreted as preference for proximity. For the specialisation factors the sign is irrelevant. In the last two columns the variables' contributions to overall explained specialisation (Expl. S.) and explained information (Expl. I = (M. + Expl.S.)/2) over the significant factors are indicated

6

General conclusion

In this work I studied resource partitioning of three cryptic and partly sympatric *Plecotus* bat species in Switzerland from a biological, ecological and conservation perspective. Like many other species of insectivorous bats, long-eared bats experienced strong declines over the last few decades in Central Europe, including Switzerland. Extensive habitat loss and transformation, large-scale agricultural intensification, including pesticide application, are considered as primary factors of bat population declines.

6.1 Conclusions

Most investigations on the ecology of *Plecotus* bat species have been done before the discovery of the new cryptic *P. macrobullaris* in Europe. We have to face the fact that all previous information about their distribution, abundance and ecology should be carefully re-evaluated. To complement the current distribution and conservation status of Central European *Plecotus*, an easy identification method is essential. Thus, in the first step of this study we provided a new field identification approach to recognize these species by morphological characteristics. The proposed identification key of this study presented precise results (97.5% accuracy of identification) on independent data from Switzerland. Collecting and applying new data from other parts of Europe might contribute to further improve this identification key which is recommended as a rapid and inexpensive replacement to the molecular methods.

Our investigations on habitat selection show preference of heterogeneous habitats and avoidance of open arable lands (chapter 4) for these species, reflecting their sensitivity to the habitat simplification and intensification of farmlands around their roosts. Additionally, this result is confirmed by the evidence of low arthropod diversity found in the intensified farmlands (chapter 3). Their preferences for habitats which are under acute pressure of landscape transformations demonstrate the importance of further monitoring to detect negative changes of the populations. To find the response of studied species to habitat changes, further research on trophic niches of these cryptic bats, including the availability of food biomass, is advisable. After the discovery of the new species *P. macrobullaris* and based on predicted distribution models for long-eared bats (chapter 5), we suggest the re-evaluation of the current conservation status of long-eared bats in Switzerland.

6.2 Implications for conservation

The need of special considerations while building conservation plans for cryptic species was highlighted by Schonrogge *et al.* 2002 and Bickford *et al.* 2006. Our three studied species are classified as endangered (*P. auritus*), potentially endangered (*P. austriacus*) and not yet categorised (*P. macrobullaris*) in the Swiss red list (Duelli 1994). In this study we found that all three long-eared bats are Lepidoptera specialists in their diet use (chapter 3), suggesting that all kinds of habitat managements which favour the abundance of moths would be beneficial to them. It has been shown by previous studies that managing farmlands to maximize key insect families, by maintaining structurally diverse habitats and reducing agrochemical use, would indeed benefit bat populations (Wickramasinghe *et al*. 2004). Intensification on one hand decreases the abundance of those insect families most commonly eaten by bats and on the other hand amplifies the detrimental effects by increasing the fragmentation of structured habitats. *Plecotus* species are affected notably because of their selection for heterogeneous and patchy vegetation structures (see results of chapter 4). Therefore, maintaining structural connectivity among these areas needs more attention to preserve the remained populations of this endangered species. Our findings on the commuting distances between roosts to foraging sites should be considered in the management programs to protect the foraging habitats within a range of 5km. Specially *P. austriacus* and *P. macrobullaris* which they commute more distantly from roost to reach the suitable foraging patches maybe benefit more from habitat enhancement programs, respecting connectivity structures. Many studies stated that long-eared bats especially *P. austriacus* and *P. macrobullaris* are related mainly to man-made structures for roosting (Entwistle, Racey & Speakman 1997, Horáček, Bogdanowicz & Đulić 2004, Ashrafi *et al*. in press). Therefore recognition and detailed monitoring programs are vital elements to protect and enhance maternity roosts.

6.3 Relevance for conservation

This study exemplifies for the first time the evidence of resource partitioning among three cryptic *Plecotus* bat species of Europe. In the absence of a reliable species identification based on single qualitative or univariate measures we elaborated multivariate functions that serve as valuable and instantaneous identification tool for field workers. Albeit of the morphological similarity in the three species, niche separation takes effect at least in the trophic and the habitat dimensions of resource use. Additionally, we suggest the occurrence of competitive exclusion between two species at the landscape scale, an effect putative resulting in a parapatric distribution. Different species-specific needs reflect that separate conservation and monitoring programs adapted to the preferences of each species are essential for a successful long-term conservation of these species.

6.4 References

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Erklärung

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Matrikelnummer: **5-104-187**

Studiengang: **Phil.-nat. Zoologie**

Bachelor ☐ Master ☐ Dissertation ☒

Titel der Arbeit: **Resource partitioning in three cryptic, sympatric bat species (*Plecotus spp.*) with contrasting conservation status**

Leiter der Arbeit: **Prof. Dr. R. Arlettaz**
Dr. F. Bontadina

Ich erkläre hiermit, dass ich diese Arbeit selbständig verfasst und keine anderen als die angegebenen Quellen benutzt habe. Alle Stellen, die wörtlich oder sinngemäss aus Quellen entnommen wurden, habe ich als solche gekennzeichnet. Mir ist bekannt, dass andernfalls der Senat gemäss Artikel 36 Absatz 1 Buchstabe o des Gesetztes vom 5. September 1996 über die Universität zum Entzug des auf Grund dieser Arbeit verliehenen Titels berechtigt ist.

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Curriculum Vitae

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Personal information

Date of birth	29 Dec 1972
Place of birth	Shahre kord, Iran
Citizenship	Iranian
Gender	Male
Marital status	Married

Education

2005 - 2010	University of Bern, *PhD thesis* "Resource partitioning in three cryptic, sympatric bat species (*Plecotus spp.*) with contrasting conservation status"
1999 – 2001	University of Tehran, Iran, MSC in Environmental Sciences
1995 – 1999	University of Gorgan, Iran, BSC in Environmental sciences

Employment history

2001 – 2005	Assistant Professor and lecturer at University of Tehran and Bo-Ali Sina University of Hamedan, Iran
2000 – 2001	Wildlife's expert at department of Environment Preservation, Chaharmahal Va Bakhtyari, Iran

Teaching

2006 – 2009	Practical for beginners, Conservation Biology, Institute of Ecology and Evolution, University of Bern, teaching and field assistant

2006 – 2009	Excursion, Cave fauna: hibernating bats and arthropods, Conservation Biology, Institute of Ecology and Evolution, University of Bern, teaching and field assistant
2007 – 2008	Practical in zoology: Morphology and anatomy of the rat, Institute of Ecology and Evolution, University of Bern, teaching assistant
2001 – 2005	Teaching courses of "Game Biology" and "Wildlife Ecology" at University of Tehran and Bo-Ali Sina University in Hamedan

Publications

	Ashrafi S., Bontadina F., Kiefer A., Pavlinic I., Arlettaz R. (2010) Multiple morphological characters needed for field identification of cryptic long-eared bat species around the Swiss Alps, Journal of Zoology, in press
2008	Sattler T., Ashrafi S., Bontadina F. 2008. Langzeit-Monitorings sind möglich. Fledermaus-Anzeiger 87, 5.

Presentation of work at conferences

2009	Ashrafi, S., Bontadina, F., Beck, A., Arlettaz, R. Food habits of long-eared bats: the Swiss menu and its conservation implications. Annual meeting of the Swiss Zoological, Botanic, Mycological and Systematic Societies 12-13 February 2009, University of Bern, Switzerland
	Ashrafi, S., Bontadina, F., Arlettaz, R. (2009) Niche partitioning of long-eared bats in Switzerland, Seminar in Environmental Science (IFU) 22 April 2009, Institute of Environmental Science, University of Zurich, Switzerland
	Rutishauser M., Bontadina F., Kiefer A., Braunisch V., Ashrafi S., Arlettaz R. Predicted distribution of long-eared bats in Switzerland. Annual meeting of the Swiss Zoological, Botanic, Mycological and Systematic Societies 12-13 February 2009, University of Bern, Switzerland
2008	Ashrafi, S., Bontadina, F., Kiefer, A., Pavlinic, I., Arlettaz, R. The first European conference of Iranian scientists in agriculture & natural sciences (ECISAN 2008), University of Paris 11, 8-9 October 2008, France
	Ashrafi, S., Bontadina, F., Kiefer, A., Pavlinic, I., Arlettaz, R. Multiple morphological characters needed to identify

	cryptic species in the field. XIth European Bat Symposium, 18-22 August 2008, Cluj-Napoca, Romania
2007	Ashrafi, S., Bontadina, F., Kiefer, A., Pavlinic, I., Arlettaz, R. Presence of a third *Plecotus* bat species in the Swiss alps confirmed by morphology and DNA, The annual meeting of the Swiss Zoological, Botanical and Mycological Societies, 15 – 16 February 2007, WSL, Switzerland.
2002	Ashrafi, S., Studying and identifying bats at Sarab Cave in Chaharmahal Va Bakhtyari province, 1st Conference of science and biodiversity of Iran, University of Kerman
2000	Ashrafi, S., Shir Abad Cave bats, 9th Biology conference, University of Tehran, Iran

Skills

Computer:

Microsoft office
Special software's: Statistical and GIS software's

Language Proficiency:

Persian (Farsi): mother tongue
English: Fluent
German: Rudimentary
Arabic: Rudimentary

Awarded grants

2005 – 2009	Scholarship by the Ministry of Science, research and Technology of Iran

Interest and achievements

Reading, Calligraphy, Photography, Hiking, Playing basketball