CONTRIBUTION OF ANATOLIAN BATS TO GENETIC DIVERSITY OF WESTERN PALAEARCTIC WITH A PARTICULAR FOCUS ON KUHL'S PIPISTRELLE

by

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ABSTRACT

Identification of intraspecific conservation units and incorporating the distribution of genetic diversity into management plans are crucial requirements for assessing effective protection strategies. The first part of this study investigates the phylogeographic structures of 33 bat species present in the Near East in order to evaluate the conservation implications of their intraspecific genetic diversity. The management requirements of the identified clades and their taxonomical relations were evaluated by analysing their distributions and the levels of their genetic differentiations in mtDNA markers. In 12 species and the large *Myotis* complex, a total of 15 genetically distinct populations were identified. Comparing the phylogeographic patterns of different taxa indicates that three regions, the Balkans, the Caucasus, and the southern Anatolia, harbour genetically divergent populations and should have higher priority in conservation practices. In the second part, the response of the *Pipistrellus kuhlii* lineages to climate change was evaluated by analysing their phylogeographical patterns in association to ecological niche models (ENM). The results show that the *P. kuhlii* clades evolved in separate Pleistocene refugia located in Iberia, the North Africa, and the Middle East, and subsequently colonized Europe. These clades differ on mtDNA and microsatellites, though, are not reproductively isolated. Comparing both the current and the past predictions of ENMs with the observed genetic diversity indicate that the clades had distinct niche identities and should be analysed separately. Apparently, these differences are conserved for long periods of time and will likely to affect their response to current climate change. Nevertheless, we show that the future predictions of the 'clade only' models are consistent with the currently observed population expansions. Considering that Turkey has one of the richest bat fauna in the Mediterranean region and the Anatolian populations of various species are genetically distinct, protecting populations in Turkey is critically important for preserving the genetic diversity of the bats in the Western Palaearctic. Both regional and large-scale conservation strategies should incorporate potential differences in climate tolerance among lineages.

ÖZET

Genetik çeşitliliğin etkin bir şekilde korunabilmesi için türler içindeki farklı genetik soyların tespit edilmesi önemlidir. Bu çalışmanın ilk kısmında Orta Doğu'da dağılım gösteren 33 yarasa türünün filocoğrafik yapısı, genetik çeşitliliğin belirlenmesi amacıyla incelenmektedir. Tespit edilen mitokondriyal soyların taksonomik ilişkileri ve koruma gereksinimleri, bu popülasyonların dağılımları ve genetik farklılaşma düzeyleri kıyaslanarak araştırılmaktadır. İncelenen 12 tür ve Büyük Farekulaklı Yarasa kompleksinde genetik açıdan farklılık gösteren 15 popülasyon tespit edilmiştir. Bu popülasyonların filocoğrafik örüntülerinin karşılaştırılması, yüksek genetik çeşitlilik barındıran Balkanlar, Kafkaslar ve Güney Anadolu bölgelerine koruma uygulamalarında öncelik verilmesi gerektiğini göstermektedir. Çalışmanın ikinci kısmında *Pipistrellus kuhlii* türünün filocoğrafik örüntüleri ekolojik niş modelleriyle (ENM) bağlantılı olarak analiz edilmiş ve tür içindeki genetik soyların iklim değişikliklerine verdikleri tepkiler değerlendirilmiştir. Sonuçlar *P. kuhlii* soylarının İber Yarımadası, Kuzey Afrika ve Orta Doğu'da bulunan Pleistosen dönemi sığınaklarında birbirlerinden bağımsız olarak evrimleştiklerini ve daha sonra bu bölgelerden Avrupa'ya yayıldıklarını göstermiştir. Bu soylar mtDNA ve mikrosatelitlerde farklılık göstermekle birlikte aralarında üreme bariyerinin bulunmadığı tespit edilmiştir. Ekolojik niş modellerinin günümüz ve geçmişe yönelik tahminlerinin mevcut genetik çeşitliliğin dağılmıyla olan tutarlılıkları, bu soyların nişlerinin farklı olduğu ve bu sebepten ötürü de ayrı analiz edilmeleri gerektiği göstermektedir. Bu farklılıkların uzun zamandır korunması, soyların günümüzdeki iklim değişikliğine de farklı tepkiler vereceğine işaret etmektedir. Keza soylar temel alınarak yapılan ENM'lerinin gelecek tahminleri yakın zamanda gözlemlenen popülasyon genişlemeleri ile uyumlu sonuçlar göstermektedir. Anadolu'daki birçok türün genetik açıdan faklı popülasyonlar barındırdığı göz önünde bulundurulduğunda, bu bölgenin Batı Palaearktik yarasalarının genetik çeşitliliğinin korunması açısından oldukça önemli olduğu anlaşılmaktadır. Hem bölgesel hem de geniş ölçekli koruma stratejileri geliştirilirken genetik soyların değişen iklim koşullarına farklı düzeylerde tepki verebileceği göz önünde bulundurulmalıdır.

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1. MOTIVATION AND OBJECTIVES

My first experience with bats dates back to 1999 when I volunteered in a bat conservation project in Boğaziçi University. The study was coordinated by two graduate students, Arpat Özgül and Raşit Bilgin, from the Institute of Environmental Sciences, and supervised by Andrzej Furman. Back then, I was an 'indifferent' undergraduate in chemical engineering department of the same university and actually was more a caver. Indeed that is how I knew Arpat and Raşit, from Boğaziçi University Speleological Society (a.k.a. BÜMAK).

The project aimed to investigate population distribution and conservation status of cave-dwelling bats in Bosphorus area and it was one of the initial attempts of bat protection in Turkey. Many other studies followed it and they together provided the first systematic population data of bats in Turkey (Furman and Özgül, 2002; 2004). I attended to most of these projects as a field assistant and started to learn identifying bats. However, it took me few more years to understand that identifying bats was not an easy task at all, but even more difficult in Turkey.

First of all, as bats are active at dark, they generally lack visual traits, which can help identifying them. Closely related species generally look quite similar and without additional information, such as their echolocation calls, it can be difficult to discriminate them. Nonetheless, one of the most common bat species in Europe, *Pipistrellus pipistrellus*, was found to be represented by two distinct taxa, which were first realized by their distinct echolocation calls and later confirmed by genetics (Barratt et al., 1997). Nevertheless, with the use of molecular markers, the taxonomy of many species groups in Europe have been revised (e.g. Juste et al., 2004; Spitzenberger et al., 2006) and many other new species have been proposed, just in the last two decades (Mayer and von Helversen, 2001; Ibáñez et al., 2006; Mayer et al., 2007).

In 2004, I enrolled at the Institute of Environmental Sciences, and with Andrzej Furman, we started up a molecular ecology lab. Meanwhile, Raşit Bilgin was doing his

Ph.D. at Columbia University in population genetics and together we started to use molecular techniques to compare the Anatolian bats with their European conspecifics. In the following years, we investigated the phylogeography of various Anatolian bats and identified that several of them have genetically distinct populations in Turkey (Bilgin et al., 2006; Bilgin et al., 2008; Bilgin et al., 2009; Furman, Öztunç, and Çoraman, 2010; Furman et al., 2011). Some of these exhibit such high levels of divergences; that they might represent cryptic species.

Most of the well-defined mammalian species has less than 2% intraspecific variation in mitochondrial cytochrome b (Cytb) gene, and a higher level merits additional study concerning possible undescribed taxa (Baker and Bradley, 2006). However, as mtDNA is inherited maternally, the observed differences do not necessarily mean that the identified forms are reproductively isolated. With evidence from biparentally inherited markers, such as nuclear genes, the taxonomic relation of genetically diverged populations can be clarified.

Identifying cryptic species is important not only for understanding evolutionary and ecological mechanisms but also crucial for assessing accurate conservation plans. Currently, conservation strategies are mostly based on species level planning, and ecological needs of taxa define their protection requirements. Apparently, finding out a ‘generalist’ species that is composed of various ‘specialist’ forms will require a reassessment in conservation strategies. In order to evaluate the possible implications of genetically diverged Anatolian populations, we investigated some of them in more detail by utilizing mitochondrial and nuclear markers.

Our studies about bent-winged bat, *Miniopterus schreibersii,* demonstrate such an example, which started with identification of a genetically distinct group, that was later on accepted as a distinct species. First, in 2006, we identified that *M. schreibersii* in Anatolia consisted of two genetically distinct lineages (Bilgin et al., 2006). These lineages, which were mainly regarded as subspecies, had a contact zone in Anatolia; *M. s. schreibersii* inhabiting Europe and the coastal regions of Anatolia and *M. s. pallidus* inhabiting Central Anatolia and the Middle East (Bilgin et al., 2006; Furman et al., 2009). Comparing the

level of their genetic divergence with reference to other *Miniopterus* taxa, we suggested that they might represent distinct biological species (Furman, Öztunç, and Çoraman, 2010). Nevertheless, using microsatellite markers, we identified that the mitochondrial lineages also diverged on nuclear DNA, indicating that they are reproductively isolated (Furman, Postawa, et al., 2010). We also showed that the lineages differ slightly in their size, wing shape, and echolocation call parameters. Although, these differences were not sufficient to fully diagnose individuals, they were discriminative between the lineages.

Based on these evidences, *M. pallidus* is currently regarded as a distinct species (Benda et al., 2011; Šrámek et al., 2013) and has been recently added to the Annex of European species list of United Nations Environment Programme's Eurobats Agreement. Apparently, designating eastern populations as a distinct species will require an update in the conservation status of the nominate form, and also a new status for *M. pallidus*. *Miniopterus schreibersii* is listed as 'Near Threatened' in the IUCN Red List of Threatened Species. However, significant population declines and range contractions recorded in Europe almost qualified it as 'Vulnerable' (Hutson et al., 2014). Considering that most of the stable populations are in Turkey and some of these are represented by *M. pallidus*, obviously justifies a higher protection status for *M. schreibersii*.

The similarity of genetic discontinuity patterns among different species suggest that they are affected by similar evolutionary mechanisms. Nevertheless, not only bats but various other species groups exhibit comparable structures in Anatolia (see Bilgin, 2011). The levels of divergences show that most of these populations diverged around the same time period in the Pleistocene. During that era, there have been many ice ages and populations fluctuated drastically throughout these glacial cycles. For instance, in the Last Glacial Maximum, most of Northern Europe was covered with ice sheets and the populations were pushed into the southern glacial refugia located in the Iberian Peninsula, Italy, and the Balkans. These populations evolved in long term isolation and once the glacial period was over, they recolonized Europe. Hence most of the genetical lineages we identified evolved in the glacial refugia in or around Anatolia and then expanded to the neighbouring regions.

Various studies showed that Anatolia had a crucial role in the evolutionary history of species; both as a glacial refugium and as a dispersal path in the interglacial periods (Bilgin, 2011). In some species, it was not possible to assess their taxonomic relations without investigating the Anatolian populations. For example, the taxonomic relationship of *Myotis myotis* and *M. blythii* have been thoroughly investigated in the last few decades but mainly remained unclear.

Both of these species occur in sympatry in Europe and Asia Minor; *M. blythii* extending further to Central Asia, whereas *M. myotis* reaching its eastern border of distribution in Eastern Anatolia (Arlettaz et al., 1997; Benda et al., 2006; Dietz et al., 2009; Bachanek and Postawa, 2010; Furman et al., 2013). Although they considerably differ in their ecology and morphological characters, their identification, especially in Anatolia, is difficult because of their overlapping traits. But more interestingly, despite their diversification in nuclear DNA, these forms share same mtDNA haplotypes (Castella et al., 2000; Berthier et al., 2006). These contrasting patterns on mitochondrial and nuclear markers coupled with their morphological similarities rise questions about their taxonomic status.

Berthier et al. (2006) explained the observed discordance by the recurrent replacement of the mtDNA through hybridization. They suggested that *M. blythii* was originated in Central Asia and expanded to Europe through Anatolia. During this expansion, they got into contact with *M. myotis* and because of the gene flow between them, *M. blythii* lost their mtDNA signature. Based on this hypothesis, the Anatolian populations should also harbour haplotypes that are similar to those found in Central Asia. However, we found that these species also share the same mtDNA lineage in Anatolia; even in areas that are outside the distributional range of *M. myotis* (Furman et al., 2011; Furman et al., 2013).

On the other hand, Bogdanowicz et al. (2009) proposed that the Central Asian populations were unrelated to European ones, and *M. myotis* and *M. blythii* are distinct only at the subspecies level. They suggested that the current discordance is the result of incomplete lineage sorting, which initiated in the early Middle Pleistocene. However, this

proposed scenario could not explain the differentiation of sympatric populations on nuclear markers.

Recently, by including samples from Anatolian populations, we showed that the cytonuclear discordance in this species complex results from a combination of past vicariance and dispersal events (Furman et al., 2014). These two species probably originated in different glacial refugia, *M. myotis* in the east and *M. blythii* in the west; and in the subsequent periods dispersed into each other's ranges. During these expansions, their mtDNA was introgressed; *M. myotis* getting the mtDNA of *M. blythii* in the east and *M. blythii* getting the mtDNA of *M. myotis* in the west. As most of these introgression events occurred in and around Anatolia, it would be difficult to assess their taxonomic relations without investigating the populations in Turkey.

Recent studies highlight the importance of populations in the east, which might also have significantly contributed to the high levels of genetic variability observed in Europe. Indeed, relatively few studies investigate these regions, yet their results show that the populations in the Middle East and the Caucasus represent significant centres of genetic diversity (Cooper et al., 1995; Hewitt, 1999; Hampe et al., 2003; Rokas et al., 2003; Dubey et al., 2006; Rossiter et al., 2007). Considering putative glacial refugia identified in western Georgia (Hewitt, 1999; Krebs et al., 2004; Connor et al., 2007) and Anatolia (Médail and Diadema, 2009), it is likely that these eastern ranges harbour high levels of genetic diversity.

The first part of the dissertation, titled "Contribution of Anatolian bats to the genetic diversity of Western Palaeartic", aims to assess the genetic diversity in Turkey. We use mtDNA markers to investigate the phylogeographical patterns of Anatolians bats in comparison to European populations. The main objectives are i) to detect genetically distinct Anatolian lineages, ii) to identify areas harbouring high genetic diversity; and iii) to evaluate the conservation implications of observed phylogeographical patterns.

Frequent identification of distinct lineages also brought new concepts for conservation biology. Based on the assumption that genetically distinct lineages which

evolved in long term isolation are likely to adapt to different ecological environments, it was suggested that they require different protection strategies (Moritz, 1994a; Crandall et al., 2000). This opinion has been widely accepted and nowadays, numerous studies evaluate phylogeographic structure of species in order to assess evolutionary distinct units.

In the second part of the study, we analyse one of the species complex in greater detail to examine the assumption of 'genetically distinct lineages are likely to adapt to different ecological environments.' Nevertheless, most of the current studies rely only on molecular data and assume that genetic units have distinct 'evolutionary potentials' (Crandall et al., 2000). However, relationship between genetic divergence and ecological adaptations are rarely studied. In order to evaluate this assumption, we investigate the phylogeography of Kuhl's pipistrelle, *Pipistrellus kuhlii*, in relation to its response to climate change, both the past and the current. We evaluate the possible niche divergences of the *P. kuhlii* lineages by comparing their phylogeographical patterns to species distribution models, and also quantify their niche identities by using relatively new ecological modeling techniques.

2. CONTRIBUTION OF ANATOLIAN BATS TO THE GENETIC DIVERSITY OF WESTERN PALAEASCTIC

This chapter was published in "Conservation Genetics" journal almost as it is, titled as "Phylogeographic analysis of Anatolian bats highlights the importance of the region for preserving the Chiropteran mitochondrial genetic diversity in the Western Palaearctic" (Çoraman et al., 2013).

2.1. Introduction

Phylogeographic studies investigating the intraspecific genetic variability of co-distributed taxa can be utilized to assess the evolutionary conservation value of areas (Moritz, 1994a; Moritz and Faith, 1998). Comparative phylogeographical analyses revealed that species present in the same geographical areas frequently consist of several similarly distributed and genetically divergent populations, indicating that particular biogeographical regions have played a critical role in shaping the evolutionary history of those species (Avise, 2009). In the Western Palaearctic, for instance, phylogeographic patterns of various species highlight the importance of protecting populations from the areas of the Quaternary refugia (Taberlet and Cheddadi, 2002; Schmitt, 2007). Major glacial refugia located in Iberia, Italy, and the Balkans harbour genetically distinct intraspecific clades and the populations in these regions hold most of the extant genetic variability in Europe (Taberlet et al., 1998; Hewitt, 1999; Hampe and Petit, 2005). As such biogeographical regions provided conditions needed for the survival of diverse regional biota in the past, protecting populations in these areas can also sustain the species in the future.

Recent studies highlight the importance of populations in the east, which might also have significantly contributed to the high levels of genetic variability observed in Europe. Indeed, relatively few studies that evaluated the eastern distributional ranges concluded that the populations in the Middle East and the Caucasus represent significant centres of genetic diversity and the potential areas of origin of populations now occupying Europe

(Cooper et al., 1995; Hewitt, 1999; Hampe et al., 2003; Rokas et al., 2003; Dubey et al., 2006; Rossiter et al., 2007). The distribution of many taxa present in Europe extends to West Asia and putative glacial refugia identified in these eastern ranges, such as in western Georgia (Hewitt, 1999; Krebs et al., 2004; Connor et al., 2007) and Anatolia (Médail and Diadema, 2009), also suggest that these areas might also harbour genetically distinct lineages.

Phylogeographic analyses of bats in the Western Palaearctic revealed similar patterns to other European biota, suggesting that glacial periods also played a major role in shaping their population genetic structure. Various chiropteran species consist of genetically distinct lineages, which originated in multiple glacial refugia with the genetic variability of the populations in southern latitudes generally being higher than that in the north (Ruedi and Castella, 2003; Juste et al., 2004; Ibáñez et al., 2006; Rossiter et al., 2007). Again in the east, genetically distinct lineages found in some of the Anatolian bat species, suggest that the populations in the eastern ranges might harbour multiple ESUs (Bilgin et al., 2006; Bilgin et al., 2009; Bilgin, 2012), some of which might even represent cryptic species (Furman, Öztunç, and Çoraman, 2010). In these regards, investigating the genetic variability of the eastern populations, including bats, has critical importance for developing more effective species protection strategies and evaluating the conservation value of these relatively understudied geographic regions.

In this study, we investigate the mitochondrial genetic variability within 33 microchiropteran taxa in Turkish Thrace and Anatolia. Our primary aim is to identify genetically distinct intraspecific clades. In order to assess the intraspecific evolutionary distinct units and to interpret their taxonomical relations, we evaluated the levels of mtDNA divergences along with their geographic distributions. Based on the identified phylogeographic patterns, we discuss the implications of the intraspecific genetic diversity on species protection strategies and evaluate the evolutionary conservation value of Anatolia. Considering that Turkey's biodiversity is threatened by the current developmental practices (Şekercioğlu et al., 2011), documenting the genetic variability of Anatolian populations is crucial both for highlighting its importance for species conservation strategies and also for prioritizing local protection efforts.

2.2. Material and Methods

2.2.1. Sample Collection and Species Identifications

To investigate the genetic variability of Anatolian bats, we used tissue samples from the collections of Niğde University Department of Zoology and Boğaziçi University Institute of Environmental Sciences. In the analyses, we included one to 29 samples from each taxon, which were collected from different geographical regions of Turkey and from neighbouring countries (Armenia, Georgia, Greece, Iran, and Syria). The final set consisted of 235 samples representing 33 species (Table A.1). In principle, the bats were identified with reference to their morphological features. Nevertheless, the identity of 13 samples was uncertain. Species identification of ten samples from *Myotis aurascens*/*M. mystacinus* complex, and three samples from *Myotis nattereri*/*M. schaubi* complex were based on the genetic markers. Because *Myotis myotis* and *M. blythii* are morphologically similar in Anatolia and also share the same mtDNA haplotypes (Furman et al., 2011; Furman et al., 2013), their sequences were analysed together. Specimens from Niğde University were collected between 1999 and 2010 and tissue samples were kept in 80% ethanol after collection. Tissue samples from Boğaziçi University were collected between 2008 and 2010, following the non-destructive method described by Worthington Wilmer and Barratt (1996), and stored in 80% ethanol in the field.

2.2.2. Genetic Analyses

Total DNA was extracted from tissue samples following the Roche High Pure PCR Template Preparation Kit (Mannheim, Germany) protocol. For the phylogenetic analyses, we amplified two commonly used mitochondrial markers, NADH dehydrogenase subunit 1 (ND1) and cytochrome b (Cytb). The ND1 amplifications were done with the primers ER65 and ER66 as described in Mayer and von Helversen (2001) and the partial Cytb gene was amplified with the primers Molcit-F (Ibáñez et al., 2006) and MVZ-16 (Smith and Patton, 1993) as outlined in Ibáñez et al. (2006). The PCR products were cleaned by QIAquick PCR Purification Kit and the sequencing reactions were commercially performed by Macrogen Inc. (Korea). The primers ER70 and ER175 (Mayer and von

Helversen, 2001) were used for ND1 and the primers Molcit-F and MVZ-16 for Cytb sequencing reactions. The sequences were edited with Sequencher 4.8 (Gene Codes Corp.) and aligned using Clustal X2 (Larkin et al., 2007). The sequences obtained have been deposited to GenBank (Table A.1).

2.2.3. Phylogenetic Analyses

Phylogenetic trees were constructed using a Bayesian MCMC algorithm as implemented in the software BEAST v.1.6.1 (Drummond and Rambaut, 2007). For each species, three independent MCMC analyses were run for 5×10^6 generations and sampled every 1000^{th}; the first 10% were discarded as burn-in. As different runs gave similar outputs, results were not pooled together. Best fitting substitution models for the analysed sequence sets were selected by jModelTest2 (Darriba et al., 2012; Guindon and Gascuel, 2003) according to the corrected Bayesian information criterion (Appendix B). In most of the cases, closely related species were included in the analysis, and therefore Yule Process was used for the tree prior with A UPGMA starting tree (Drummond and Rambaut, 2007). The trees were rooted with an appropriate outgroup for each species. The convergence and the effective sample sizes (ESS) of the estimates were evaluated by exploring the likelihood plots using TRACER v 1.5 (Rambaut and Drummond, 2007). In all cases, ESS values of all parameters were above 200 and independent runs gave similar estimates. The MCMC samples were summarized using the maximum clade credibility topology in TreeAnnotator v1.6.1 (Drummond and Rambaut, 2007), with the posterior probability limit set to 0.5 and median node heights summarized. Genealogical relationships among ND1 haplotypes were estimated by a statistical parsimony network (Templeton et al., 1992) with the software TCS v. 1.21 (Clement et al., 2000). For intraspecific and interspecific comparisons, uncorrected-p distances were calculated in MEGA5 (Tamura et al., 2011) for both ND1 and Cytb.

2.3. Results

2.3.1. Intraspecific Genetic Variability

To assess the intraspecific structure of the investigated 33 species, we analysed 228 ND1 sequences from the present study together with 418 relevant sequences from GenBank (Table A.2). Nineteen of the analysed species sampled in Europe and the Near East formed single monophyletic clades. In ND1, the mean intraspecific genetic divergence in these species was lower than 2% and the maximum differences among conspecific individuals typically did not exceed 3% (Table 2.1). In the remaining 12 species and the large *Myotis* complex (*Myotis myotis* and *M. blythii*), we were able to define intraspecific clades. These clade definitions were based on the clustering of individuals into monophyletic groups in the phylogenetic trees and their intraspecific divergence levels (Table 2.2 and Appendix B). Except two of these, *Pipistrellus pipistrellus* and the large *Myotis* complex, the mean genetic divergences on ND1 between the intraspecific clades were greater than 3%. Both in *P. pipistrellus* and the large *Myotis* complex, the ND1 divergence was ca. 2 %. However, given the geographically wide distribution of the identified lineages and the clear clustering of the individuals, we defined the two species as having multiple clades.

The species with more than one genetically identified clade were further analysed with Cytb marker (65 Cytb sequences from the present study and the 310 relevant GenBank sequences) (Table A.2). Based on Cytb and ND1 analyses, the number of intraspecific clades varied from two to four, and the clades differed considerably in their geographical distributions (Figure 2.1 and Appendix B). Eleven of the identified clades were found only in the Near East: Clade 2 and Clade 3 of *R. hipposideros*, Clade 2 of *M. bechsteinii*, Clade 2 of *M. capaccinii*, Clade 2 of *P. pipistrellus*, Clade 2 of *P. kuhlii*, Clade 4 of *P. auritus*, Clade 2 of *P. kolombatovici*, Clade 2 of *P. macrobullaris*, Clade 2 of *E. serotinus*, Clade 2 of *M. schreibersii*. Clade 2 of large *Myotis* complex and Clade 2 of *M. capaccinii*, which were present in the eastern distributional ranges, were also found in Europe.

2.3.2. Phylogeographical Patterns

To facilitate taxonomic interpretation of our results and to evaluate their conservation implications, we investigated the phylogeographic patterns of the species, based on the mtDNA divergence of the clades and their geographic distributions (allopatric or sympatric). Large *Myotis* complex and four species, *M. bechsteinii*, *M. capaccinii*, *P. kolombatovici*, and *M. schreibersii*, consisted of two genetically distinct clades. The two clades within large *Myotis* complex did not correspond to the morphologically distinct *M. myotis* and *M. blythii*, and the divergence between Clade 1 (Europe) and Clade 2 (Near East and Eastern Europe) was ca. 2% on ND1 and Cytb. Clade 2 of *M. bechsteinii* (north-eastern Turkey and Caucasus) differed from Clade 1 (Europe) by ca. 6% on ND1 and 7% on Cytb. In *M. capaccinii*, Clade 1 (Europe) differed from Clade 2 (Near East) by ca. 4% on ND1 and 5% on Cytb. In *P. kolombatovici*, Clade 1 (south-eastern Europe) differed from Clade 2 (south-western Anatolia) by ca. 5% on ND1. The genetic divergence in *M. schreibersii* between Clade 1 (Europe and the coastal regions of Anatolia; the nominal form) and Clade 2 (Near East; *M. s. pallidus*) was ca. 3% on ND1 and 4% on Cytb. Only in large *Myotis* complex and *M. capaccinii*, the identified lineages had sympatric distributions; in the former the clades overlapped in Central Europe and in the latter in the Balkans. Apparently, there are also two clades within *Taphozous nudiventris* (Appendix C). However, the small number of samples and lack of GenBank references did not allow further analysis of this species.

Six species, *R. hipposideros*, *H. savii*, *E. serotinus*, *P. pipistrellus*, *P. kuhlii*, and *P. macrobullaris*, consisted of three clades. The clades of *R. hipposideros* were basically allopatric and all of them differed by ca. 3-4% on ND1 and Cytb. The phylogenetic constructions of both markers suggest that Clade 2 (western Anatolia) and Clade 3 (eastern Anatolia) are more closely related to each other than to Clade 1 (Europe). In *H. savii*, the divergences between the clades were relatively high; Clade 2 (south-eastern Europe and Near East) differed from Clade 1 (south-western Europe) by ca. 7% on ND1 and 8% on Cytb and by ca. 9% on ND1 and 8% on Cytb from Clade 3 (North Africa and Iberia). However, the relationship between the lineages was unresolved.

Table 2.1. ND1 percent intraspecific divergences of 19 of the studied bats species, which did not exhibit a genetic discontinuity particular to Anatolian populations. Intraspecific divergences (d), standard errors (S.E.), ranges (minimum - maximum), and sample sizes (n).

Species	**d ± S.E.**	**Min - Max**	**n**
Rhinolophus blasii [a]	0.2 ± 0.1	0 - 0.4	12
Rhinolophus euryale [a]	0.4 ± 0.1	0 - 0.7	15
Rhinolophus ferrumequinum [a]	0.8 ± 0.2	0 - 1.6	14
Rhinolophus mehelyi [a, b]	0.1 ± 0.1	0 - 0.1	3
Myotis alcathoe	0.6 ± 0.2	0 - 3.0	33
Myotis aurascens	1.2 ± 0.4	0 - 1.8	11
Myotis daubentonii [c]	0.4 ± 0.1	0 - 1.4	44
Myotis emarginatus	0.6 ± 0.2	0 - 1.1	12
Myotis mystacinus	0.1 ± 0	0 - 3.0	40
Myotis schaubi	1.2 ± 0.3	0 - 2.1	5
Pipistrellus nathusii	0 ± 0	0 - 0.2	9
Pipistrellus pygmaeus	0.5 ± 0.1	0 - 1.5	15
Nyctalus lasiopterus	0.6 ± 0.2	0 - 1.1	7
Nyctalus leisleri	0.2 ± 0.1	0 - 0.7	19
Nyctalus noctula	0.4 ± 0.1	0 - 1.3	23
Eptesicus anatolicus	0.2 ± 0.1	0 - 0.8	20
Barbastella barbastellus	0.7 ± 0.2	0 - 1.2	8
Plecotus austriacus	1.1 ± 0.2	0 - 2.9	10
Tadarida teniotis	0.5 ± 0.3	0.5	2

[a] As *Rhinolophus* species had only a few ND1 sequences available in GenBank, we also analysed their Cytb region (Appendix A) and did not detect distinct lineages (data not shown). [b] In Cytb, one sample from Greece (GenBank Accession no FJ185208) differed by about 4% from the rest *R. mehelyi* samples. [c] Three shallow lineages differing between 2 to 2.8% were identified and the Anatolian sample clustered with the individuals from Scotland (Clade A as identified by Ngamprasertwong et al. [2008]).

Sequences of *E. serotinus* were analysed together with two closely related species, *E. bottae* and *E. anatolicus*. Phylogenetic constructions of both markers were consistent. Clade 2 (southern Anatolia) and Clade 3 (North Caucasus) clustered together, which subsequently clustered with *E. bottae*; Clade 1 (Europe and Near East) was located at the

basal node of this group. Clade 2 differed from Clade 1 by ca. 7% and from Clade 3 by ca. 5% on both markers. Similarly, the average divergence between the Clade 2 and the Clade 3 was ca. 5% on ND1 and Cytb. In *P. macrobullaris*, Clade 1 (Europe) differed both from Clade 2 (Anatolia) and from Clade 3 (Iran) by ca. 5% on ND1 and 3% on Cytb. On the other hand, the differences between Clade 2 and Clade 3 were less pronounced; ca. 3% on ND1 and 2% on Cytb and both clades were present in Iran.

Pipistrellus kuhlii sequences were analysed together with the sequences of *P. maderensis*. In both markers, Clade 3 (Western Europe) was located at the basal node. On the other hand, the relationship of Clade 2 (Eastern Europe, Anatolia and North Africa), Clade 3 (Near East), and *P. maderensis* remained unresolved. In ND1, *P. maderensis* was closely related to Clade 2, whereas in Cytb, it first clustered with Clade 1. Clade 2 differed from Clade 1 by ca. 5% on ND1 and Cytb and from Clade 3 by ca. 7% on both markers. The divergence between Clade 1 and Clade 3 was ca. 6% on ND1 and Cytb.

The divergence between Clade 1 (Europe and western Anatolia) and Clade 2 (eastern Anatolia) of *P. pipistrellus* was relatively low; by ca. 2% on both markers. Their divergence from Clade 3 (North Africa) was deeper, with ca. 3% on ND1 and 5% on Cytb. These clades had a contact zone in Central Anatolia (Figure 2.1).

Plecotus auritus consisted of four deeply diverged and allopatric clades. Clade 1 (Western Europe) diverged from Clade 3 (Caucasus and north-eastern Turkey) by ca. 4% on ND1 and 6% Cytb. Similarly, the average divergence between the Clade 2 (Eastern Europe) and the Clade 3 was ca. 5% on ND1 and Cytb. Both Clade 2 and Clade 3 were present in Turkey; however, they were geographically isolated.

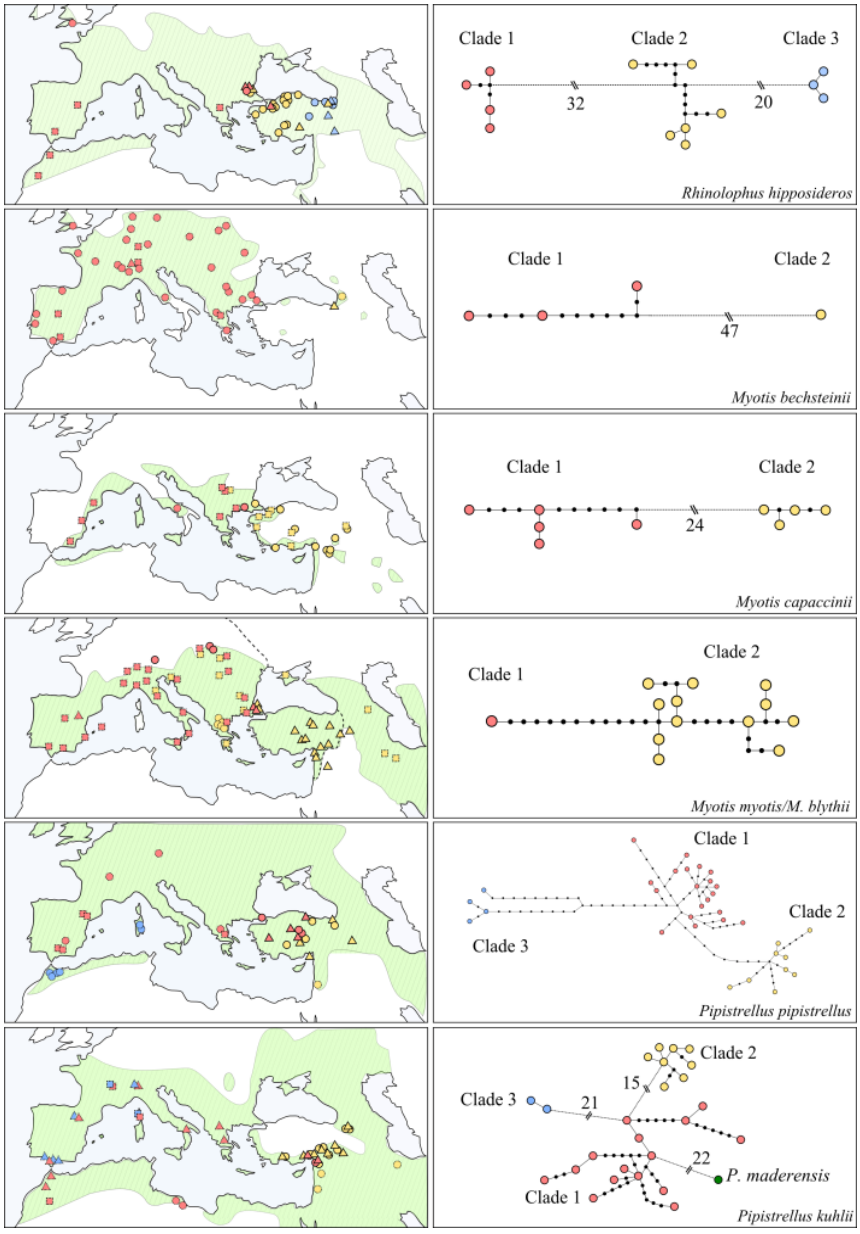

Figure 2.1. Distribution of the identified clades in 11 species and large *Myotis* complex and TCS networks of ND1 haplotypes. Shaded areas show the range of the species (dashed line in large *Myotis* complex map shows the eastern range of *M. myotis*). The distribution maps are taken from IUCN Red List of Threatened Species website; maps of *E. serotinus* and *P. kolombatovici* were updated based on the data from Dietz et al. (2009). Circles indicate ND1 sequences, squares Cytb, and triangles both of the markers. Numbers in TCS networks show base pair differences.

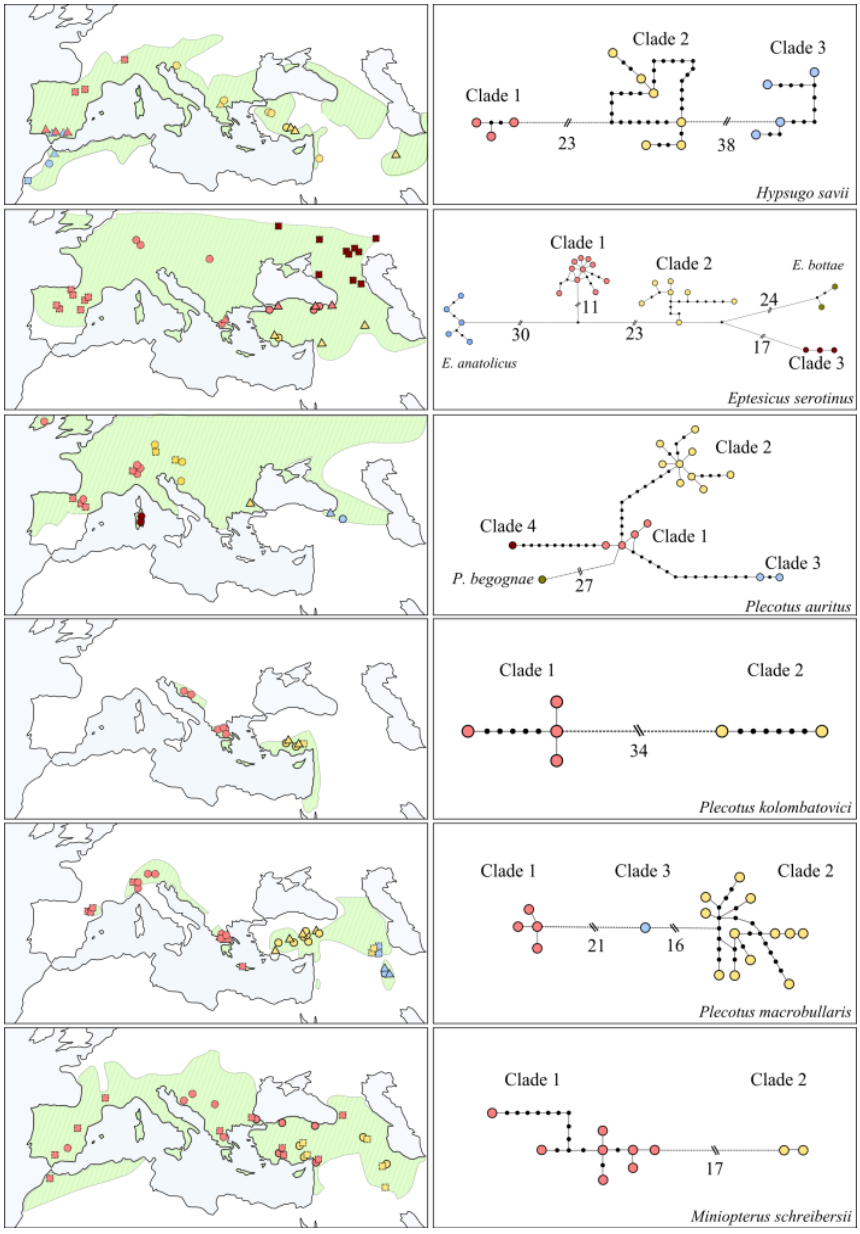

Figure 2.1. (Continued.)

Table 2.2. Genetic divergence of the lineages identified in 12 species and *Myotis myotis*/*M blythii* complex at Cytb and ND1 markers. Percent inter-specific and intraspecific divergences (d), standard errors (S.E.), and ranges (minimum - maximum) are shown. Clade identities are as shown in Figure 2.1.

Species	Gene	Between 1 – 2	Between 1 – 3	Between 2 – 3	Within 1	Within 2	Within 3
R. hipposideros [a]	Cytb	3.6 ± 0.6 (3.0 – 4.4)	4.1 ± 0.6 (3.6 – 4.4)	2.8 ± 0.5 (2.5 – 3.0)	1.2 ± 0.2 (0 – 1.8)	0.7 ± 0.2 (0 – 1.4)	0.3 ± 0.1 (0 – 0.4)
	ND1	4.1 ± 0.6 (3.8 – 4.4)	4.1 ± 0.6 (4.0 – 4.3)	2.6 ± 0.5 (2.5 – 2.9)	0.3 ± 0.1 (0 – 0.5)	0.4 ± 0.1 (1.0 – 1.4)	0.1 ± 0 (0 – 0.2)
M. bechsteinii	Cytb	6.8 ± 0.9 (6.6 – 7.0)	-	-	0.4 ± 0.2 (0 – 0.7)	NA	-
	ND1	5.8 ± 0.9 (4.4 – 7.0)	-	-	0.6 ± 0.1 (0 – 1.7)	0.5 ± 0.2 (0.2 – 1.1)	-
M. capaccinii	Cytb	5.3 ± 0.8 (4.6 – 5.8)	-	-	0.7 ± 0.4 (0.2 – 1.2)	0.4 ± 0.1 (0 – 0.8)	-
	ND1	3.9 ± 0.3 (3.5 – 4.5)	-	-	0.7 ± 0.2 (0.1 – 1.4)	0.2 ± 0.1 (0 – 0.5)	-
M. myotis/M. blythii	Cytb	2.1 ± 0.3 (1.0 – 3.4)	-	-	0.3 ± 0.1 (0 – 1.6)	1.0 ± 0.2 (0 – 1.9)	-
	ND1	2.2 ± 0.5 (1.7 – 2.8)	-	-	0 ± 0 (0 – 0.1)	0.8 ± 0.2 (0 –1.9)	-
P. pipistrellus [b]	Cytb	1.5 ± 0.4 (0.5 – 3.5)	4.7 ± 0.7 (3.7 – 6.0)	5.1 ± 0.7 (4.0 – 6.2)	0.6 ± 0.1 (0 – 0.1)	0.9 ± 0.2 (0 – 1.6)	2.3 ± 0.4 (0.1 – 4.7)
	ND1	2.0 ± 0.4 (1.5 – 2.7)	2.9 ± 0.4 (1.6 – 3.4)	3.2 ± 0.5 (2.5 – 3.9)	0.9 ± 0.2 (0 – 1.6)	0.6 ± 0.1 (0 – 0.1)	1.5 ± 0.3 (0 – 2.8)
P. kuhlii	Cytb	4.5 ± 0.7 (4.1 – 5.4)	5.6 ± 0.8 (4.9 – 6.7)	6.6 ± 0.8 (5.6 – 7.1)	1.0 ± 0.2 (0 – 2.4)	0.3 ± 0.1 (0 – 0.6)	0.3 ± 0.1 (0 – 0.6)
	ND1	5.4 ± 0.7 (4.3 – 6.5)	6.0 ± 0.9 (5.2 – 6.9)	6.9 ± 1.0 (6.6 – 7.6)	1.7 ± 0.2 (0 – 3.6)	0.2 ± 0.1 (0 – 0.9)	0.2 ± 0.2 (0.2)
H. savii [c]	Cytb	7.9 ± 0.9 (7.3 – 8.5)	7.0 ± 0.8 (5.8 – 8.2)	8.3 ± 0.8 (6.8 – 9.0)	0.3 ± 0.1 (0 – 0.8)	1.1 ± 0.3 (0 – 2.1)	1.3 ± 0.3 (0.1 – 2.3)
	ND1	7.3 ± 0.8 (6.0 – 10.4)	8.8 ± 0.9 (8.4 – 9.4)	9.2 ± 0.9 (7.6 – 11.2)	0.5 ± 0.2 (0.4 – 0.6)	1.3 ± 0.3 (0 – 3.9)	1.2 ± 0.2 (0.6 – 2.0)
P. auritus [d]	Cytb	3.0 ± 0.6 (2.6 – 3.6)	5.5 ± 0.9 (5.3 – 5.9)	4.7 ± 0.8 (4.4 – 5.0)	0.6 ± 0.2 (0.1 – 1.3)	0.3 ± 0.1 (0.1 – 0.4)	NA
	ND1	3.7 ± 0.6 (2.5 – 4.6)	4.4 ± 0.7 (4.0 – 4.9)	5.2 ± 0.8 (4.6 – 6.1)	0.5 ± 0.1 (0 – 1.2)	0.5 ± 0.2 (0 – 1.4)	0.3 ± 0.2 (0.3)
P. kolombatovici	Cytb	NA	-	-	NA	0 ± 0 (0)	-
	ND1	5.0 ± 0.7 (4.4 – 5.4)	-	-	0.4 ± 0.1 (0 – 0.9)	0.5 ± 0.2 (0 – 0.8)	-
P. macrobullaris	Cytb	2.8 ± 0.5 (2.2 – 3.8)	2.7 ± 0.5 (2.3 – 3.3)	2.2 ± 0.4 (2.7 – 1.6)	1.1 ± 0.3 (0.3 – 2.0)	1.3 ± 0.3 (0.1 – 2.3)	0.6 ± 0.2 (0 – 0.9)
	ND1	5.0 ± 0.8 (3.9 – 6.1)	4.7 ± 0.8 (4.4 – 5.6)	3.1 ± 0.7 (2.7 – 4.5)	0.2 ± 0.1 (0 – 0.7)	0.9 ± 0.2 (0 – 2.1)	0 ± 0 (0)

Table 2.2. (Continued.)

Species	Gene	Between 1 – 2	Between 1 – 3	Between 2 – 3	Within 1	Within 2	Within 3
E. serotinus	Cytb	6.9 ± 0.9 (6.5 – 7.7)	7.3 ± 0.9 (6.3 – 8.5)	5.1 ± 0.8 (4.3 – 5.7)	0.3 ± 0.1 (0 – 0.8)	0.7 ± 0.2 (0 – 1.9)	0.3 ± 0.1 (0 – 0.7)
	ND1	7.4 ± 0.9 (6.9 – 8.0)	7.5 ± 1.0 (7.1 – 8.0)	5.0 ± 0.8 (4.7 – 5.4)	0.2 ± 0.1 (0 – 0.9)	0.7 ± 0.2 (0 – 1.7)	0.1 ± 0.1 (0 – 0.3)
M. schreibersii	Cytb	4.4 ± 0.9 (3.9 – 5.2)	-	-	1.2 ± 0.3 (0 – 2.1)	0.3 ± 0.3 (0.3)	-
	ND1	3.4 ± 0.7 (3.0 – 5.0)	-	-	0.7 ± 0.2 (0 – 2.6)	0.1 ± 0.1 (0 – 0.2)	-
T. nudiventris	Cytb	3.8 ± 0.7 (3.8)	-	-	-	-	-
	ND1	3.2 ± 0.6 (3.2)	-	-	0 ± 0	-	-

NA: Sequences are not available for this comparison. [a] One sample from Greece (GenBank Accession no FJ185200) differed between 3.3% to 5.7% (in Cytb) from rest of the lineages and is not included here (see Appendix B). [b] Clade 3 has substructure; Morocco and Sardinian populations. [c] Clade 2 has substructure; Eastern Europe and Asia Minor populations. [d] The Sardinian population forms a distinct lineage (Clade 4 in Figure 2.1 and Appendix B). It differed from rest of the lineages between 3 to 4.5% in ND1.

2.4. Discussion

2.4.1. Phylogeographical Concordance

Phylogenetic analyses of ND1 and Cytb reveal congruent topologies for all, but three species: *P. kuhlii*, *H. savii*, and *P. auritus*. In the *P. kuhlii* tree, *P. maderensis* stayed close to Clade 2 on ND1, but near Clade 1 on Cytb. Nevertheless, in ND1, the support for the node separating *P. maderensis* and Clade 2 was low, hence leaving the split of these three clades as a trichotomy. The split of Clade 1, Clade 2, and Clade 3 in *P. auritus* was unresolved on Cytb, whereas the grouping of Clade 2 and Clade 3 had a low support in ND1. Finally, in *H. savii*, Clade 1 was closer to Clade 2 on ND1, but to Clade 3 on Cytb; the tree topologies being well supported for both of the genes. The observed differences might be caused by lineage-specific rate heterogeneity. Alternatively, the discrepancy might arise from the tendency of Bayesian analyses to overestimate, in some cases, the posterior probabilities (Yang and Rannala, 2005). Accordingly, some of the supports might be lower, which would result in an unresolved trichotomy.

Most of the phylogeographical structures identified in this study fit certain patterns observed in multiple taxa in and around Anatolia (Bilgin, 2011). *Myotis capaccinii*, *P. kolombatovici*, and *P. macrobullaris* fit Pattern I where the Anatolian and the Balkan populations are differentiated from each other on mtDNA and may show some overlap in their ranges (Figure 2.1). The species that fit Pattern II, where the differentiation is within Anatolia, are *P. pipistrellus*, *E. serotinus*, and *M. schreibersii*. *Rhinolophus hipposideros* shows three clades that represents an amalgamation of Patterns I and II. Finally, *Hypsugo savii* populations in the Balkans and Anatolia show no differentiation between the two regions that presents another special case under the categorization of Bilgin (2011).

The similarities of the observed phylogeographic patterns among different taxa and the exceptionally high mitochondrial genetic diversity of bats in Turkey can be linked to its specific location amid several glacial refugia. These are situated in the Balkans (including Thrace and North Western Anatolia), in the lowlands of Western Georgia (Hewitt, 1999; Krebs et al., 2004; Connor et al., 2007), and along the coastal zones of the Mediterranean

region of Southern Anatolia (Médail and Diadema, 2009). Indeed, the Balkans refugium is within the current distributional range of many clades found in Europe and Western Turkey. The present distributions of the eastern clades of *R. hipposideros* (Clade 2), *M. capaccinii*, *P. kuhlii* (Clade 2), *P. kolombatovici*, *P. macrobullaris*, and *E. serotinus* (Clade 2) partially overlap with the locations of the South Anatolian refugium. Finally, the current distribution of the eastern clades of *M. bechsteinii*, *M. schreibersii*, *P. pipistrellus*, *R. hipposideros* (Clade 3), and *P. auritus* include the location of the Caucasian refugium.

2.4.2. Conservation Implications

We identify that eleven species exhibit divergences that are deeper than 3% on ND1, which is considerably higher than the intraspecific divergences reported for most of the European bats (Mayer and von Helversen, 2001; Mayer et al., 2007). In four of these, *M. bechsteinii*, *P. kuhlii*, *H. savii*, and *E. serotinus*, the observed divergences were even more distinct; higher than 5% on ND1 and Cytb, which is a typical divergence level found between sister bat species (Ruedi and McCracken, 2009). These high levels of genetic differentiation found in several variants indicate that some of the identified lineages might actually represent biologically distinct taxa. For instance, based on the additional support from the nuclear DNA and morphological data, the Anatolian clades of *M. schreibersii* are proposed as two distinct species (Bilgin et al., 2012; Furman, Postawa, et al., 2010).

The finding that some of the species present in Europe and Near East consists of several genetically discrete clades, which might be evaluated as separate taxa, can have a substantial effect on their conservation statuses. Particularly for *R. hipposideros*, *M. capaccinii*, *P. kuhlii*, *P. macrobullaris*, *P. kolombatovici*, and *M. schreibersii*, whose eastern clades occupy a large part of the species ranges, splitting them into two or more smaller units would require a re-evaluation of their specific conservation statuses. Nevertheless, we have to stress that our study did not intend to clarify the taxonomic classification of bats in Anatolia. Instead, we aimed to identify taxa consisting of discrete genetic units and to point out to the regions harbouring them. To resolve taxonomic affiliations of Anatolian bats, additional and more detailed follow-up studies are urgently needed; each of them focusing on both nuclear DNA and phenotypic traits particularly in

the contact zones of the identified intraspecific lineages. Furthermore, investigating the ecological niches of genetically distinct populations can provide information on their evolution, as well as possible divergences in their adaptations.

About one third of the investigated species consist of at least two distinct intraspecific clades, many of which are confined exclusively to the Near East. Based on the phylogeographic analyses, eastern clades of *M. bechsteinii*, *M. capaccinii*, *P. pipistrellus*, *P. kuhlii*, *H. savii*, *E. serotinus*, *P. kolombatovici*, *M. schreibersii*, *R. hipposideros*, *P. macrobullaris*, and *P. auritus* could be classified as evolutionarily significant units (ESU; Moritz, 1994b). However, these assessments require support from nuclear DNA. For instance, the clades identified in *M. capaccinii* do not show differentiation in nuclear microsatellites and would not qualify as ESUs (Bilgin et al., 2008). On the other hand, the nuclear differences observed between the mtDNA clades within *M. schreibersii* (Bilgin, 2012; Furman, Postawa, et al., 2010) and *P. pipistrellus* (Hulva et al., 2010) satisfies the criteria for their ESU designation.

Based on the distributions of the genetically distinct units, we suggest that protection of populations in three regions, the Balkans, the Caucasus, and Southern Anatolia, should have high priority for preservation of intraspecies genetic variability. Previous studies investigating population genetics of different bat species showed that the populations in the Balkans have relatively high levels of intraspecific diversity, suggesting that Europe was re-colonized from here, after the last glacial maximum (*M. schreibersii*; [Furman, Öztunç, Postawa et al., 2010] and *M. bechsteinii*; [Kerth et al., 2008]). Considering that most of the lineages identified in Europe are present in the Balkans and the populations in this peninsula served as sources for the genetic variability in the western ranges, their protection is crucial for preserving the current European genetic diversity. The populations in the Caucasus and Southern Anatolia, on the other hand, harbour genetically distinct lineages, which are not present in Europe. The eastern clades of many species are present in both of these regions and Clade 2 of *E. serotinus* and Clade 2 of *P. kolombatovici* are found in Southern Anatolia, and Clade 2 of *M. bechsteinii* and Clade 3 of *P. auritus* only in the Caucasus. Because most of these eastern lineages might represent distinct ESUs, their protection is crucial not only for regional conservation efforts but also for increasing the

overall survival chances of species. Accordingly, the genetic diversity of the eastern populations should be incorporated into both local and large-scale management plans for more effective protection strategies.

Using molecular markers enabled us to evaluate the distributions of cryptic species, which are difficult to recognize based solely on morphological features. In this regard, our findings do not support the currently recognized distributions of two pairs of sibling species: *M. aurascens* - *M. mystacinus* and *M. nattereri* - *M. schaubi* (Appendix D). Out of 10 samples from *M. aurascens* - *M. mystacinus* pair, only one individual (from the Turkish-Armenian border) genetically belonged to the former. Also all samples initially identified as *M. nattereri* genetically represented *M. schaubi*. It should be noted that, although it has not been reported explicitly, a comprehensive analysis of *M. nattereri* complex (Puechmaille et al., 2011) with one sequence from GenBank (Benda et al., 2007; GenBank Accession no EU086527) verifies that *M. schaubi* is also present in Cyprus. These results show that unlike previously suggested (Benda and Karataş, 2005) the distribution of *M. aurascens* in the Near East is likely to be confined to the Caucasian region. The presence of *M. nattereri* in Anatolia is also questionable. On the other hand, our results suggest that the distributions of *M. schaubi* and *M. mystacinus* seem to include the Mediterranean coast of Turkey, covering larger areas than previously anticipated (Sharifi and Tsytsulina, 2008; Benda and Karataş, 2005). It has to be noted that the changes in the recognized distributions of these species will also have implications on their estimated abundances and accordingly, their conservation statuses. Our results indicate that *M. aurascens* is very rare and *M. nattereri* is most probably not present in Anatolia. However, we acknowledge that these results are based on very limited sampling and further research is necessary to have a better understanding of their distribution in Turkey.

3. RESPONSE OF KUHL'S PIPISTRELLE TO CLIMATE CHANGE

This chapter is being prepared for publication with a working title "Differential Responses of Genetical Lineages to Climate Change: Evidence from Past and Current Range Expansion of Kuhl's Pipistrelle." This study was done in collaboration with Andrzej Furman from Institute of Environmental Sciences, Boğaziçi University, Panagiotis Georgiakakis from Natural History Museum of Crete, Petr Benda from Natural History Museum of Prague, Frieder Mayer from Natural History Museum of Berlin, and Christian Dietz.

3.1. Introduction

Genetically distinct lineages that evolved in long term isolation are likely to acquire different ecological adaptations (Moritz, 1994a; Crandall et al., 2000). Apparently, such differences not only alter their evolutionary histories, but also affect their responses to changing environmental conditions. On the other hand, estimating adaptive distinctiveness of geographically segregated populations and evaluating its possible implications are challenging (Peterson, 2011). First of all, it is difficult to assess if such differences arise from a real niche divergence or simply because the available niches are different. For example, the Anatolian lineages that we describe in the previous section are likely to exhibit differences in their occupied niches then their European conspecifics. However, depending on their niche plasticity, they might survive in each other's habitats as well. Apparently, testing such hypotheses is practically difficult, especially for wild populations.

Integrating phylogeographical inferences into ecological modeling practices provide insights about niche divergences of genetic lineages (Alvarado-Serrano and Knowles, 2014). Ecological Niche Models (ENM) aim to explain geographical distribution of species in correlation to their occupied environmental spaces. These models construct a statistical association between distribution data and environmental variables, and accordingly, estimate suitability probabilities for habitats. Niche similarity of lineages can be assessed

by comparing these suitability scores that are generated for each lineage separately (Warren et al., 2008; Broennimann et al., 2012).

Projecting ENMs into the past for each lineage separately and comparing them with phylogeographical inferences can provide further information about their niche identities (e.g. Jezkova et al., 2009; Hornsby and Matocq, 2012; Fitze et al., 2011). Phylogeographical practices analyse current distribution of genetic variability to infer the distributional ranges and the past demographics of ancestral populations. Assuming that niches of genetical lineages are conserved throughout the time, past projections of their ENMs should give similar inferences to those obtained from phylogeographical analyses (Alvarado-Serrano and Knowles, 2014).

Identifying differences in environmental niches which are also conserved for long time periods provide a strong evidence for distinct ecological adaptations. Intraspecific groups with such distinct niches are likely to respond differently to changing environmental conditions and apparently require separate conservation strategies. In these regards, the genetic variability of Anatolian bats needs further attention. Considering that almost one third of the bats species in Turkey are represented by more than one phylogeographic lineage, understanding their adaptive capabilities and assessing their implications are crucial for better management plans.

In order to evaluate the possible niche divergences and their implications, we decided to analyse one of these species complexes in greater detail. Considering the levels of within and in between genetic diversities, the variability of habitats they occupy, and the current population trends, we selected Kuhl's Pipistrelle, *Pipistrellus kuhlii*.

This species is widely distributed throughout the Mediterranean area and also occurs in the Middle East extending eastwards through to Southeast Asia. In the last few decades, the northern limit of its range increased drastically; from ca. 45° N in 1985 to 48° N (Austria) in 1994; to 51° N (Ukraine) in 2001; and more recently to almost 57° N (Russia) (Aulagnier et al., 2014; Robinson et al., 2005). It has been also documented in areas, where it was not recorded previously (Barti, 2010; Popczyk et al., 2008; Sachanowicz et

al., 2006; Bogdanowicz, 2004). We also found this bat for the first time in the Central Black Sea coast of Turkey, which is approximately 500 km away from the known records.

The expansion of this species, especially in the Eastern Europe, is considered to be related to climate change, yet this has not been supported by any analysis (Robinson et al., 2005). Some other researchers propose that it might be linked to its adaptation to inhabit human settlements (Sachanowicz et al., 2006). Again there is no documented evidence for this explanation, and considering history of human presence in these regions, it is unlikely that increased anthropogenic activity can alone explain this current range expansion.

An interesting aspect of this current spread is that it might be limited to one of the genetic lineages. Molecular studies show that *P. kuhlii* is composed of deeply diverged clades (Ibáñez et al., 2006; García-Mudarra et al., 2009; Mayer et al., 2007). One of these lineages, which was identified only in three localities in Syria, Turkey, and Israel (Mayer et al., 2007), was recently found in Ukraine and Poland (Veith et al., 2011). In the previous chapter, we showed that this lineage is very common in Anatolia and also present in the Caucasus. Considering that these areas overlap with the ranges of recent expansion, it is possible that only one of clades might be spreading.

In order to evaluate the recent expansion of the *P. kuhlii* complex in association to its phylogeographic patterns, we use an integrated approach using both molecular and ENM methods. In the first part of the study, we run phylogeographical analyses to investigate the current genetic structure of the *P. kuhlii* complex. Using mtDNA and nuclear markers, we assess the ancestral relationship of the identified clades and infer their past population demographics. In the second part of the study, we use ENMs to infer the distribution patterns of the clades by constructing lineage specific and combined models. The possible niche differentiations are assessed by qualitatively comparing the model predictions with the phylogeographical inferences and by quantitatively measuring the overlap of the models' projections. Finally, we project the models to the future to evaluate the possible responses of the *P. kuhlii* complex.

3.2. Material and Methods

3.2.1. Genetic Data Collection

For the genetic analysis, we used a total of 298 samples collected from 128 locations, spanning almost the entire known range of the species complex (Figure 3.1). Sampling also included individuals from four closely related species: *P. deserti*, *P. maderensis*, and *P. hesperidus*. A partial fragment of the Cytb gene was used for the phylogenetic and the phylogeographical analysis, and the sequences were obtained as described in the previous chapter. For the mtDNA analysis, we added 53 related sequences from GenBank, which represented 103 individuals.

For the microsatellite analysis, we used a subset of 136 samples. Eight microsatellite loci were amplified, which were previously developed for *P. pipistrellus* (Pip2, Pip3, and Pip4 [Racey et al., 2007]) or other vespertilionid bat species (A2-Mluc, A24-Mluc, and G6-Mluc [Jan et al., 2012]; P217 [Mayer et al., 2000]; EF4 [Vonhof et al., 2002]). Fragment analysis was done by Macrogen Inc. Korea and the allele sizes were scored with GeneMarker 1.8. One locus, which was monomorphic (G6-Mluc), and two other (Pip2 and Pip3), which had very few alleles, were not used in the further analysis. The rest of the loci were tested for Hardy–Weinberg equilibrium and for the presence of null alleles with MICRO-CHECKER 2.2 (Van Oosterhoutet al., 2004). None of them had null alleles or large allelic dropout.

3.2.2. Phylogenetic Reconstruction and Molecular Dating

A Bayesian MCMC algorithm was used to construct the phylogenetic tree, using the software BEAST v.2.1.3 (Drummond and Rambaut, 2007). HKY was selected as the substitution model, based on the corrected Bayesian information criterion scores calculated in jModelTest2 (Darriba et al., 2012; Guindon and Gascuel, 2003). As the dataset included closely related taxa and the *P. kuhlii* lineages were highly diverged, we selected Yule process for the tree prior and used a UPGMA starting tree (Drummond and Rambaut, 2007). For dating the divergences, a divergence rate of 4% per million years was used,

which was suggested for Pipistrelle bats (Hulva et al., 2004). Three independent MCMC analyses were run for 50×10^6 generations and sampled every 1000^{th}, and their first 10% were discarded as burn-in. The convergence and the effective sample sizes (ESS) of the estimates were evaluated by exploring the likelihood plots using TRACER v 1.5 (Rambaut and Drummond, 2007). In all runs, ESS of all parameters had values above 200 and independent runs gave similar estimates. The independent runs were combined in LogCombiner v.2.1.3 and the MCMC samples were summarized using the maximum clade credibility topology in TreeAnnotator v.2.1.2 (Drummond and Rambaut, 2007), with the posterior probability limit set to 0.5 and median node heights summarized. For intraspecific and interspecific comparisons, uncorrected-p distances were calculated in MEGA6 (Tamura et al., 2011).

3.2.3. Population Structure and Demographic Analyses

Haplotype and nucleotide diversities, for each clade were calculated in DnaSP v.5.10.01 (Librado and Rozas, 2009). The genealogical relationships within the clades were investigated by analysing the statistical parsimony networks (Templeton et al., 1992) with the software TCS v. 1.21 (Clement et al., 2000).

In order to check, if the identified clades carry any signatures of population expansion, we ran neutrality tests, Tajima's D and Fu's Fs, as implemented in Arlequin 3.5.1.3 (Excoffier and Lischer, 2010), and their significance was assessed by 10,000 coalescent simulations. In addition to these, we ran mismatch distribution analysis to test population expansion scenarios. These analysis estimate population expansion parameters, such as the age of the expansion (τ) and the mutation parameters for before (θ_0) and after an expansion (θ_1). The θ parameter is proportional to the effective population size (Ne) and accordingly, it is expected to increase in case of a population expansion. In order to test the significance of such an increase, we ran 10,000 bootstrap replicates to reconstruct confidence intervals (CI) and cases, which had non-overlapping 99% CI for θ_0 and θ_1, are considered to have a significant signature of expansion. There are two possible expansion models available in the software: demographic expansion and spatial expansion. In order to check which model fits to our dataset, we plotted the observed mismatched distributions

with the simulated runs and Harpending's Raggedness index (Rag.) and sum of squared deviations (SSD) were used to evaluate their fit. Again, these runs were done in the Arlequin software and 10,000 bootstrap replicates were run to test the probability of the calculated index and also to construct 95% CI for the expected mismatch distributions.

Finally, we also used a Bayesian MCMC algorithm to infer the population size changes through the time. BEAST v.2.1.3. was used to construct Bayesian skyline prior plots, where a divergence rate of 4% per million years was used. The MCMC runs were 20 million chains long and the skylines were plotted in Tracer v1.5.

3.2.4. Population Structure Based on Microsatellites

Two different algorithms were used to evaluate the population genetic structure based on the microsatellite data. In the first approach, we used Structure v. 2.3.4 (Pritchard et al., 2000) to cluster the samples into 'K' possible populations based on their allele frequencies. As the initial simulations with no a priori information did not reveal any structure (except clustering samples from Europe), we used the LocPrior model, which was suggested for recovering structure when the level divergences were low (see results section for details of used groupings). Ten replicates were run for K values from 1 to 8 (250,000 burn-in followed by 350,000 chains under admixture and correlated allele frequency models) and their likelihood were evaluated in Structure Harvester (Earl and Von Holdt, 2012) by using the criterion proposed by Evanno et al. (2005). The assignment scores of individuals in replicate runs were averaged in CLUMPP (Jakobsson and Rosenberg, 2007) using the Large K Greedy algorithm, and visualized with the software DISTRUCT (Rosenberg, 2004).

As a second approach, we used a structuring algorithm that makes use of the spatial information as implemented in the R Package GENELAND v. 4.0.4 (Guillot at al., 2005). Independent allele frequency model was selected and the MCMC was run for 100,000 iterations, saving each 100^{th}. Similar to Structure runs, the likelihood of simulations was evaluated up to 8 populations and the estimated population memberships were mapped for

the K value with the maximum a posteriori estimate. Finally, inbreeding coefficient (F_{IS}) and the pairwise fixation indices (F_{ST}) were calculated for the estimated clusters.

3.2.5. Ecological Niche Modeling

Ecological niche models (ENM) were constructed using the Maxent software (Phillips et al., 2006), based on 19 bioclimatic variables (Appendix E), which were obtained from WorldClim website (http://www.worldclim.org). Considering the mobility of bats, 5 arc-minutes (~ 10 km2 spatial resolutions) was selected for the data resolution. The constructed models were projected into the past, the Last Glacial Maximum (LGM; ~ 21,000 years before present), the Last Interglacial period (LIG; ~ 140,000 – 150,000 years before present), and also into the future, 2020 and 2040. For the past projections, we used the projections of CCSM and MIRCO General Circulation Models (GCMs). The future models were run for the A2 scenario of the Intergovernmental Panel on Climate Change with two different projection data sets, IPSL and CSRIO mk2, obtained from the CCAFS-climate data portal (http://www.ccafs-climate.org/).

For each model, 20 replicates were run and 20% of the locations were randomly selected to test the accuracy of the models. The estimates of these run were then averaged for constructing niche availability maps and the performances of the models were evaluated based on the Area Under the Curve (AUC) of the receiver operator characteristics as implemented in Maxent.

In order to evaluate the possible niche differentiation of the identified clades, the models were run for the clade only and the combined data sets. We used two approaches to assess possible differences: First, the model predictions were qualitatively compared with the phylogeographical inferences and second, identity and background tests were used to quantitatively measure the overlap of the model inferences. In the second approach, the similarities of the identified niches were compared by calculating two overlap metrics, Schoener's *D* and Hellinger's *I* (Warren et al., 2008). Both of these metrics compare the habitat suitability scores of each grid calculated for two different models by normalising them. Therefore, their results vary from 0, which suggests that there is no niche overlap

between the compared data sets, to 1, which suggest identical niches. Later, the significance of these calculated overlap values were assessed by comparing them to the distribution of these metrics obtained from 100 replicates, where the occurrence points were randomly assigned to one of the groups. Finally, in order to evaluate if the identified differences are caused by a niche divergence among the analysed groups or because they occupy niches that are inherently different, we ran background tests. In the background tests, the models obtained from one particular data set was run for the background points of the other one. Again the distributions of overlap metrics were constructed by running 100 replicates to test the significance of background tests. All these tests were run in ENMTools v.1.4.3 (Warren et al., 2008).

For the presence data, we used the coordinates of genetically identified samples, except for Clade western. For this clade, the localities of *P. kuhlii* samples from Spain, France, and Portugal that were obtained from Global Diversity Information Facility (http://www.gbif.org) were used. In order to eliminate geographically clustered localities, a spatial auto-correlation analysis was run (as implemented in ArcGIS v10) for each data set and the locations were removed till the sets had a random distribution.

3.3. Results

3.3.1. Phylogeny of *Pipistrellus kuhlii sensu lato*

Phylogenetic reconstruction of Cytb sequences revealed five highly diverged clades (Table 3.1 and Figure 3.1). Two of these corresponded to *P. hesperidus* and *P. maderensis* and the other three lineages were within the *P. kuhlii* complex. First clade within this group was formed by samples from Western Europe (from now on referred as Clade western). Second clade was composed of samples from South-Eastern Europe, North Africa, Canary Islands, and parts of Cyprus and Turkey (from now on referred as Clade kuhlii). Finally, samples from Middle East and part of Eastern Europe formed the third clade (from now on referred as Clade lepidus). Specimens, which were identified as *P. deserti* based on their morphology, did not form a monophyletic clade but grouped with the Clade kuhlii samples.

The deepest divergence within the identified clades was the split of *P. hesperidus* samples from rest of the group. Its divergence was dated to around 3.5 million years ago (mya) and it differed by up to 18% from the rest of the clades. High levels of within group differences suggested that this clade might harbour further cryptic genetic diversity; samples from Ethiopia diverged up to 8% from the samples from South Africa and Madagascar (this split is marked with a star in Figure 3.1). As this clade was highly diverged from the rest, we excluded it in the further analysis.

Within the rest of the group, Clade western was located at the basal node and it split from the rest around 1.5 mya. The remaining samples split into three clades approximately 1 mya, forming Clade lepidus, Clade kuhlii, and *P. maderensis* clades. In the phylogenetic tree, the split of Clade lepidus preceded the split of Clade kuhlii and *P. maderensis* samples. Nevertheless, the 95% high posterior probability ranges of these divergence times had overlapping ranges, suggesting that their split might have happened during the same time period.

Table 3.1. Mean percent of genetic distance between (lower diagonal) and within the identified (diagonal; in bold) clades.

Clade	western	kuhlii	lepidus	*P. maderensis*	*P. hesperidus*
western	**0.001**				
kuhlii	0.060	**0.012**			
lepidus	0.067	0.049	**0.004**		
P. maderensis	0.063	0.044	0.051	**0.016**	
P. hesperidus	0.160	0.174	0.175	0.170	**0.03**

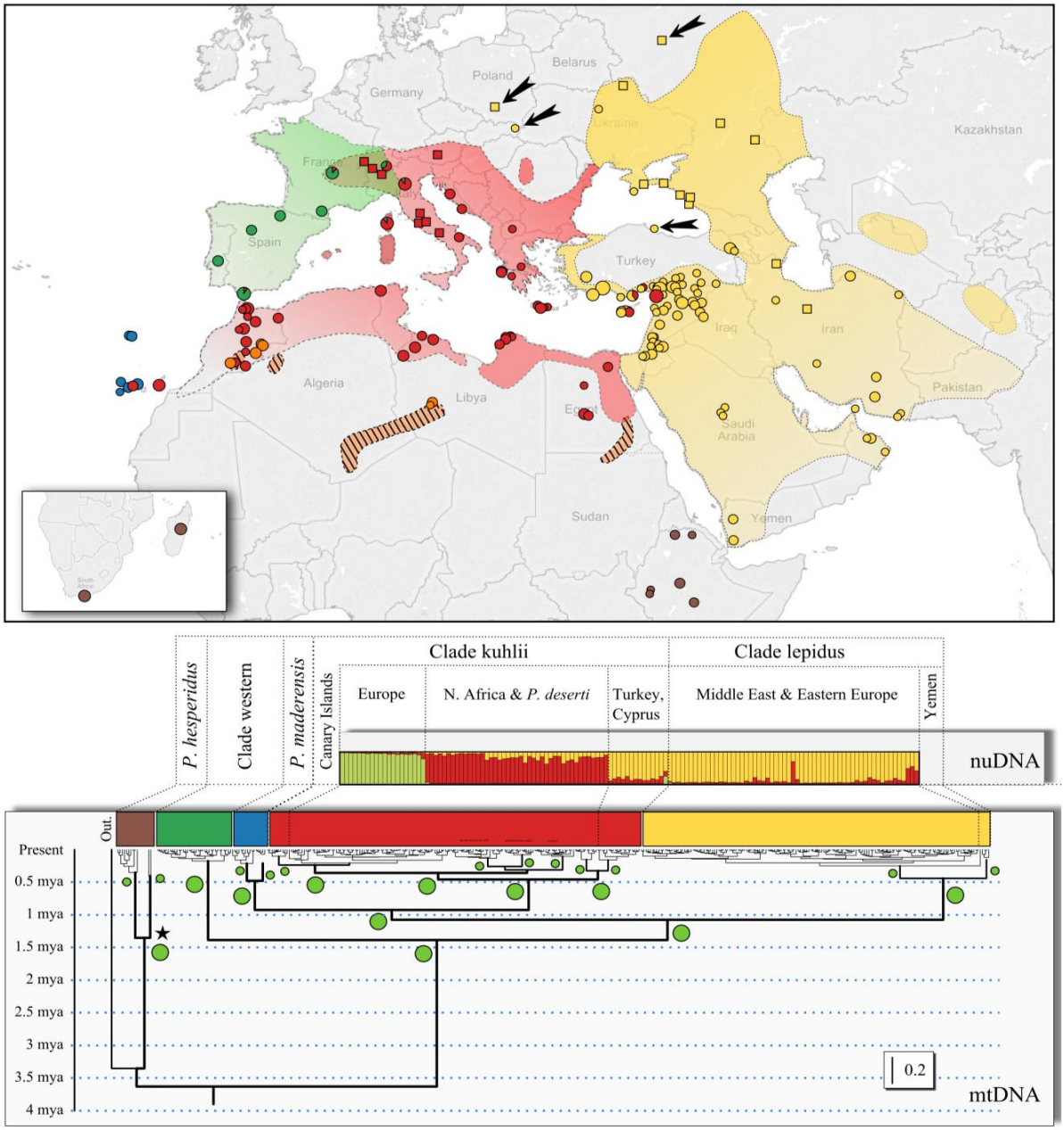

Figure 3.1. Distribution and the phylogenetic reconstruction of the identified clades. Orange circles and orange shaded areas in the map denote *P. deserti*, and the insert shows samples from Madagascar and South Africa. In the tree, nodes that had posterior probability higher than 0.7 are marked with green circles. The nuDNA panel shows Structure results for K = 3. Note that not all the samples used for mtDNA analysis were run for microsatellite analysis. Arrows shows samples that are found outside the known distribution range.

In terms of level of divergences, the highest differentiations within the *P. kuhlii* complex were among Clade western and the others; on average they differed 6.5% (Table 3.1). The other clades also had high level of divergences; with a minimum of 4.4% between Clade kuhlii and *P. maderensis* and a maximum of 5.1% between *P. maderensis* and Clade lepidus. On the other hand, genetic variability within the clades were relatively shallow; Clade western and Clade lepidus had less than 0.5%, and Clade kuhlii and *P. maderensis* had slightly more than 1% mean within group differences.

3.3.2. Within Clade Structures

3.3.2.1. Clade kuhlii. Clade kuhlii had the highest haplotype and nucleotide diversities among the identified clades and the age of this clade was the oldest (Table 3.2). Both the phylogenetic and the network analysis recovered sub-clades which showed geographical structuring (Figure 3.2). Among these, the clade formed by the populations from Northern Morocco (olive green circles in Figure 3.2) and Europe (red circles) had the largest distribution. Surprisingly, despite being few thousand kilometres apart, some samples from Morocco and Greece shared the same haplotype. This sub-clade was basically located at the centre of the haplotype network and the other groups were connected to it with few base differences. Genetically, the closest neighbor of this central group was formed by the samples from Canary Islands (dark blue circles). In the phylogenetic tree, these two sub-clades clustered together and were connected to three other sub-clades, which also formed a group at a higher level. The first one of these three was formed by samples from Southern Morocco. This group included samples identified as *P. kuhlii* (yellow circles) and *P. deserti* (purple circles). Second sub-clade was formed of samples from North-Western Libya and Algeria (green circles) and the third one was mainly dominated by samples from the Sahara (orange circles), including the ones identified as *P. deserti* (turquoise circles). These five sub-clades grouped together in the tree and were connected to the samples from Eastern Mediterranean, which also separated into two subgroups: Turkey and Cyprus (pink circles), and Egypt and western Libya (brown circles). All of the identified sub-clades and their parent nodes were highly supported, except for the first sub-clade (from Northern Morocco and Europe).

Table 3.2. Genetic diversity statistics and the age of the identified clades: Number of analysed samples (n) with length of the sequences in parentheses; nucleotide (π) and haplotype (Hd) diversities with their standard deviations; mean time to most recent common ancestor (TMRCA) and 95% HPD intervals.

Statistic	Clade western	Clade kuhlii	Clade lepidus
n	35 (515 bp)	175 (515 bp)	160 (757 bp)
π	0.00067 (0.00023)	0.01166 (0.00063)	0.00380 (0.00045)
Hd	0.318 (0.102)	0.886 (0.018)	0.871 (0.016)
TMRCA	93,116	490,460	481,600
	(35,896 – 169,262)	(354,740 – 649,030)	(306,800 – 671,200)

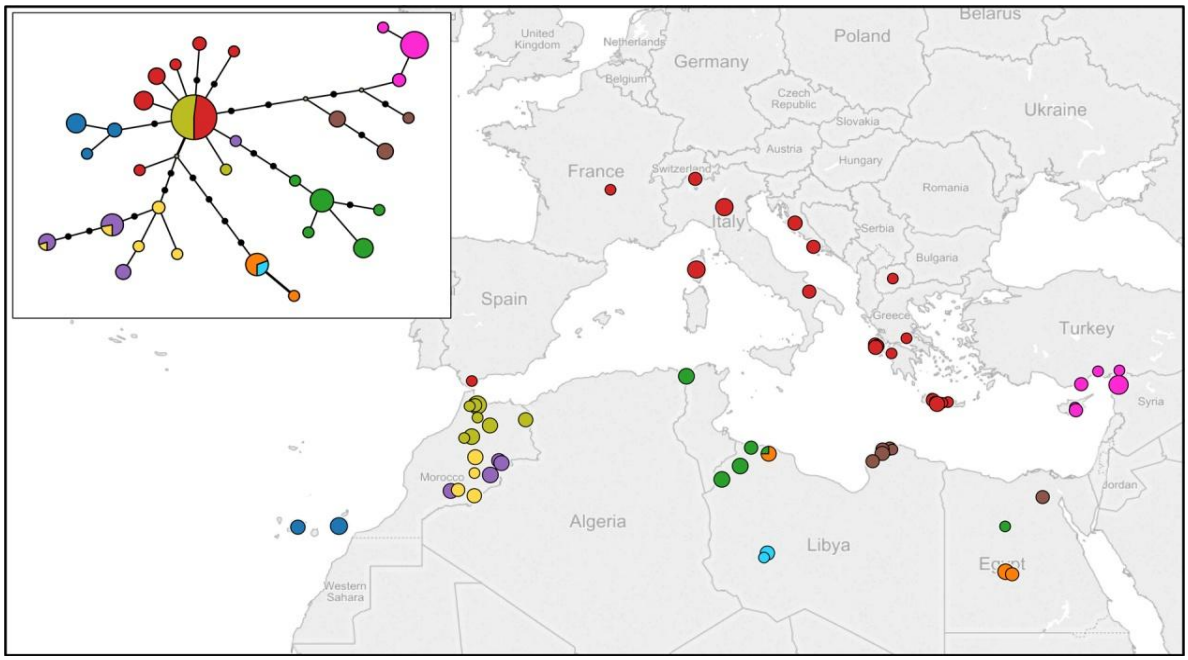

Figure 3.2. Haplotype network of Clade kuhlii samples and the distribution of the identified sub-clades. Samples identified as *P. deserti* from Morocco are marked with purple and from Libya are with turquoise. Inset shows the parsimony network of the identified haplotypes.

3.3.2.2. Clade lepidus. Although the analysed samples covered a wide distribution range, most of the identified haplotypes in Clade lepidus were closely related (Figure 3.3). Only samples from Yemen and one GenBank sequence from Saudi Arabia were different from the main group, which differed from the rest up to 2.6%. Similar to Clade kuhlii, the distribution of the identified haplotypes were moderately structured. For instance, one of the most common haplotype was found almost exclusively in Western Mediterranean coast of Turkey and Cyprus (light green circles) and another one was dominantly in the South-Eastern Turkey and Syria (yellow circles). Other haplotypes, which were connected to these common and central haplotypes, were found in the Mediterranean coastal zone. Only one of them was distributed in a very wide range, covering the Northern Iran, Armenia, the Northern Turkey, and Ukraine (red circles).

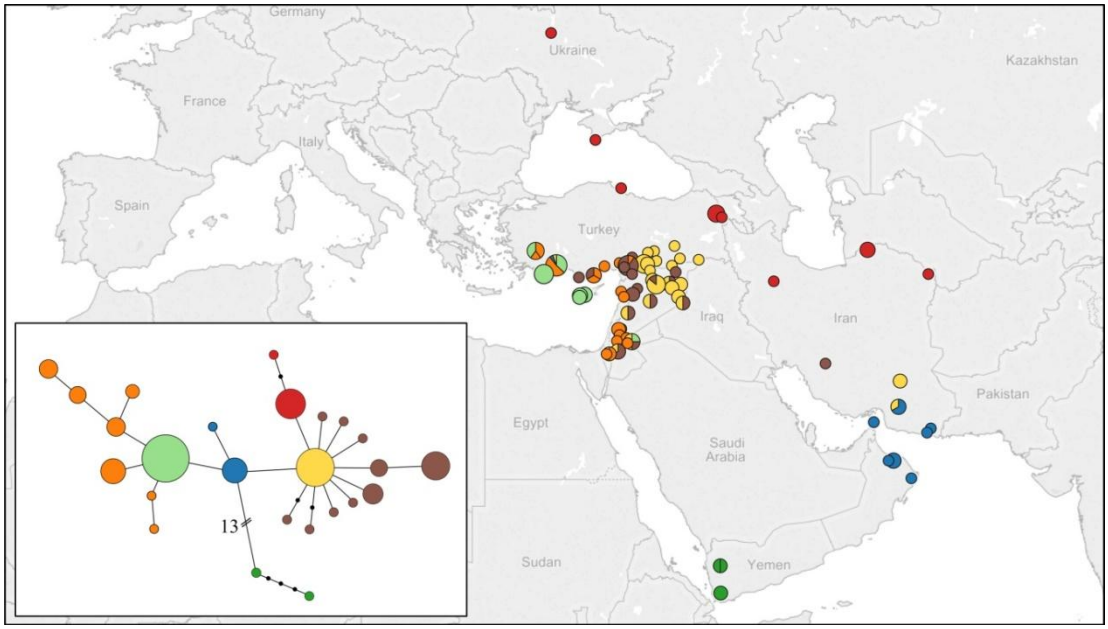

Figure 3.3. Haplotype network of Clade lepidus samples and the distribution of the identified sub-clades. Inset shows the parsimony network of the identified haplotypes.

3.3.2.3. Clade western. Clade western had the lowest haplotype and nucleotide diversities (Table 3.3). All of the identified haplotypes were only few bases different from the central one, which was found throughout the clade's distribution range (yellow circles in Figure 3.4). This clade was the youngest of the identified lineages, with a mean most common recent ancestor age around 90,000 years.

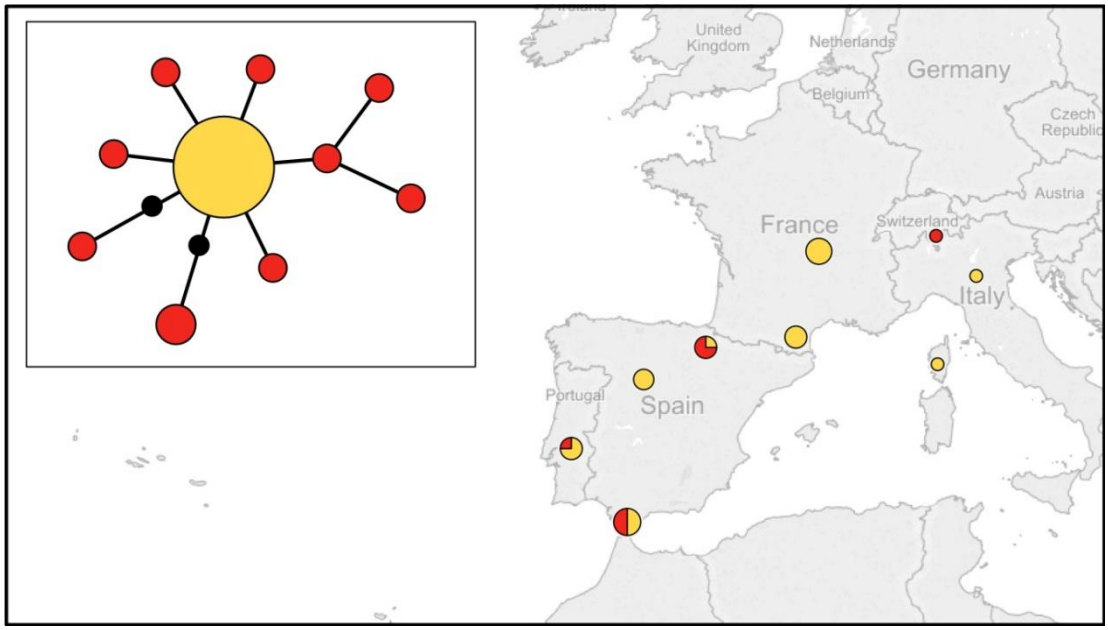

Figure 3.4. Haplotype network of Clade western samples and the distribution of the identified sub-clades. Inset shows the parsimony network of the identified haplotypes.

3.3.3. Past Population Demographics

In neutrality tests, Clade western and Clade lepidus had statistically significant negative values for Tajima's *D* and Fu's *Fs*, indicating a population expansion (Table 3.3). Although Clade kuhlii also had negative scores, they were not significant. In mismatch analysis, the mutation parameters had non-overlapping 99% confidence intervals for all the cases and in both, the demographic and the spatial expansion models, the observed mismatch distributions did not significantly differ from the expected distributions (Table 3.3).

Bayesian skyline (BSL) plots recovered similar inferences with neutrality tests and their expansion time estimates were comparable with the results of the mismatch distribution analysis (Figure 3.5 and Table 3.4). In the BSL plots, Clade western and Clade lepidus showed a population expansion trend, which roughly started 50,000 years ago. In mismatch analyses, the Clade western's population expansion was dated to start around 17,000 BP and the Clade lepidus' one around 70,000 years ago. On the other hand, BSL

plot of Clade kuhlii did not identify a population expansion in accordance with the neutrality tests and indeed, detected a decline in the last few 10,000 years.

Table 3.3. Neutrality tests and statistics of mismatch distribution analysis: *D*, Tajima's D; *Fs*, Fu's Fs; *Rag*, Harpending's raggedness index; SSD, sum of squared deviations. The latter two were run for demographic (*dem*) and spatial (*spa*) expansion models.

Statistic	Clade western	Clade kuhlii	Clade lepidus
D	-2.103 (0.002)	-0.700 (0.272)	-1.7665 (0.010)
Fs	-7.041 (< 0.001)	-4.754 (0.138)	-9.2697 (0.008)
Rag_{dem}	0.224 (0.688)	0.023 (0.283)	0.0212 (0.837)
Rag_{spa}	0.224 (0.553)	0.023 (0.809)	0.0212 (0.863)
SSD_{dem}	0.005 (0.307)	0.013 (0.279)	0.0009 (0.791)
SSD_{spa}	0.001 (0.307)	0.010 (0.703)	0.0008 (0.826)

Table 3.4. Estimated expansion times (in years; with their 95% CI) based on mutational times (Tau) calculated in mismatch distribution analyses for pure demographic and spatial expansion models.

		Clade western	Clade kuhlii	Clade lepidus
Tau				
	Dem.	0.326 (0.004 – 0.840)	7.935 (2.921 – 12.215)	2.455 (0.998 – 4.152)
	Spa.	0.393 (0.081 – 1.305)	5.253 (2.550 – 12.059)	2.021 (0.799 – 3.167)
Exp. time				
	Dem.	15,825 (194 – 40,776)	385,194 (141,796 – 592,961)	81,076 (32,959 – 137,120)
	Spa.	19,077 (3,932 – 63,349)	255,000 (123,786 – 585,388)	66,743 (26,387 – 104,590)

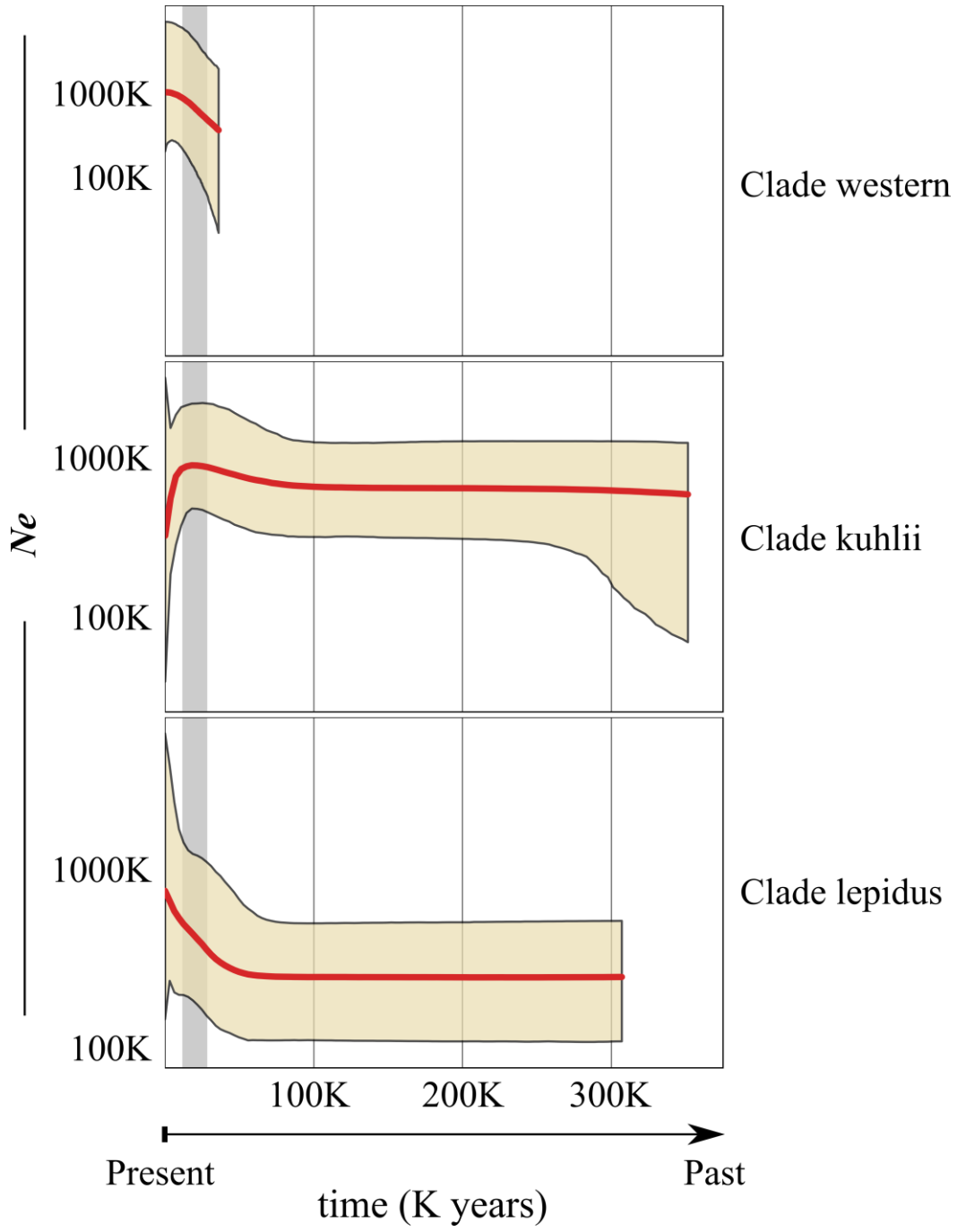

Figure 3.5. Bayesian skyline reconstructions of the identified clades. Plots show the effective population size (Ne) through time (in thousand years). Red line indicates the median and values and beige shaded areas show 95% HPD intervals. Grey shaded bar shows approximate period of the LGM (~19,000 – 26,500 years before present).

3.3.4. Population Structure Based on Microsatellite Markers

For the microsatellite analyses, we selected a subset of samples that were used for the mtDNA analysis. As microsatellite structuring analyses depend on the allele frequencies, their results can be influenced by sampling design, especially when study area covers a large geographical range. In such cases, using many samples from same or close by localities can affect the results by sticking to local population structures, whereas using single samples from distant isolated locations can change population inferences, as they might not belong to any of them. In order to minimise such possible biases, only samples belonging to Clade kuhlii and Clade lepidus were used and the number samples from close by vicinities were reduced.

We used two different methods to infer population structure based on microsatellite markers. In the Structure analysis, the initial runs with no a priori information did not recover any structure. Therefore, we used a LocPrior model, where we assigned the individuals to five groups based on their sampling location, mtDNA clade membership, and taxonomical identification. The LocPrior model is known not to find structure when none is present and to ignore the membership information when the ancestry of individuals is uncorrelated with their sampling locations (Hubisz, et al., 2009). These groups were: 1) Clade kuhlii samples from Europe, 2) Clade kuhlii samples from North Africa, 3) Clade kuhlii samples from Turkey and Cyprus, 4) samples identified as *P. deserti* based on their morphology, and 5) Clade lepidus samples.

Structure and GENELAND analyses identified different number of likely populations: the former suggested that there were most likely two clusters, whereas the latter suggested three clusters. On the other hand, the assignments of the individuals to the identified clusters were quite similar for the same K values. At K = 2, both analyses first separated European Clade kuhlii samples, which grouped together with the North African populations in mtDNA analysis. At K = 3, the other identified two clusters overlap with the mtDNA clades, except Clade kuhlii samples from Turkey and Cyprus which grouped with the Clade lepidus samples (Figure 3.6 and Figure 3.7). A minute difference between the two of the analyses was that, in GENELAND inference, samples from Egypt clustered

with the populations from Middle East, whereas in the Structure runs, they grouped with the populations from North Africa.

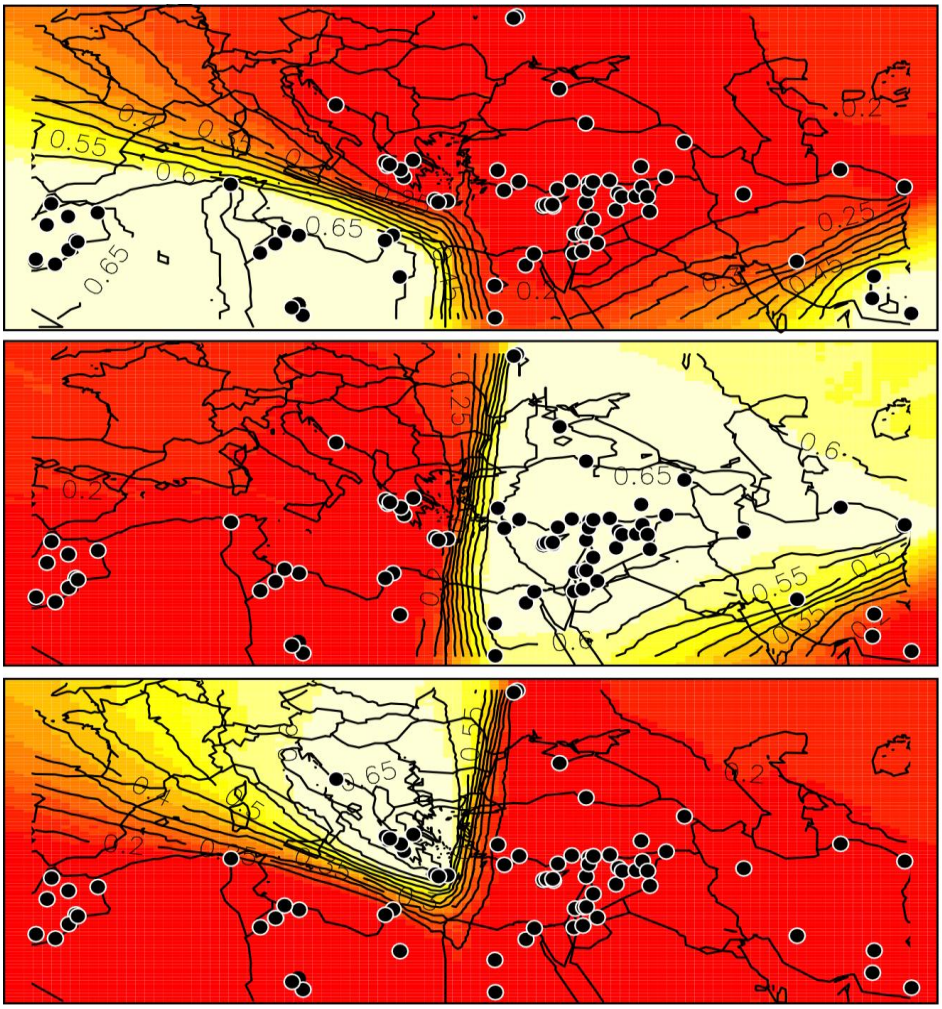

Figure 3.6. Posterior probability maps for the three clusters identified in the Geneland analysis. Lighter colours indicate higher membership probability of the analysed individuals to that particular cluster.

Higher K values did not indicate any further meaningful clusters that were concordant either with the spatial distribution of the populations or with their taxonomical identifications. Nevertheless, similar to mtDNA results, samples identified as *P. deserti*, did not form a monophyletic group. Only in the Structure analysis, at K = 5, some of them formed a distinct group, yet their membership probabilities were relatively low (Figure 3.7).

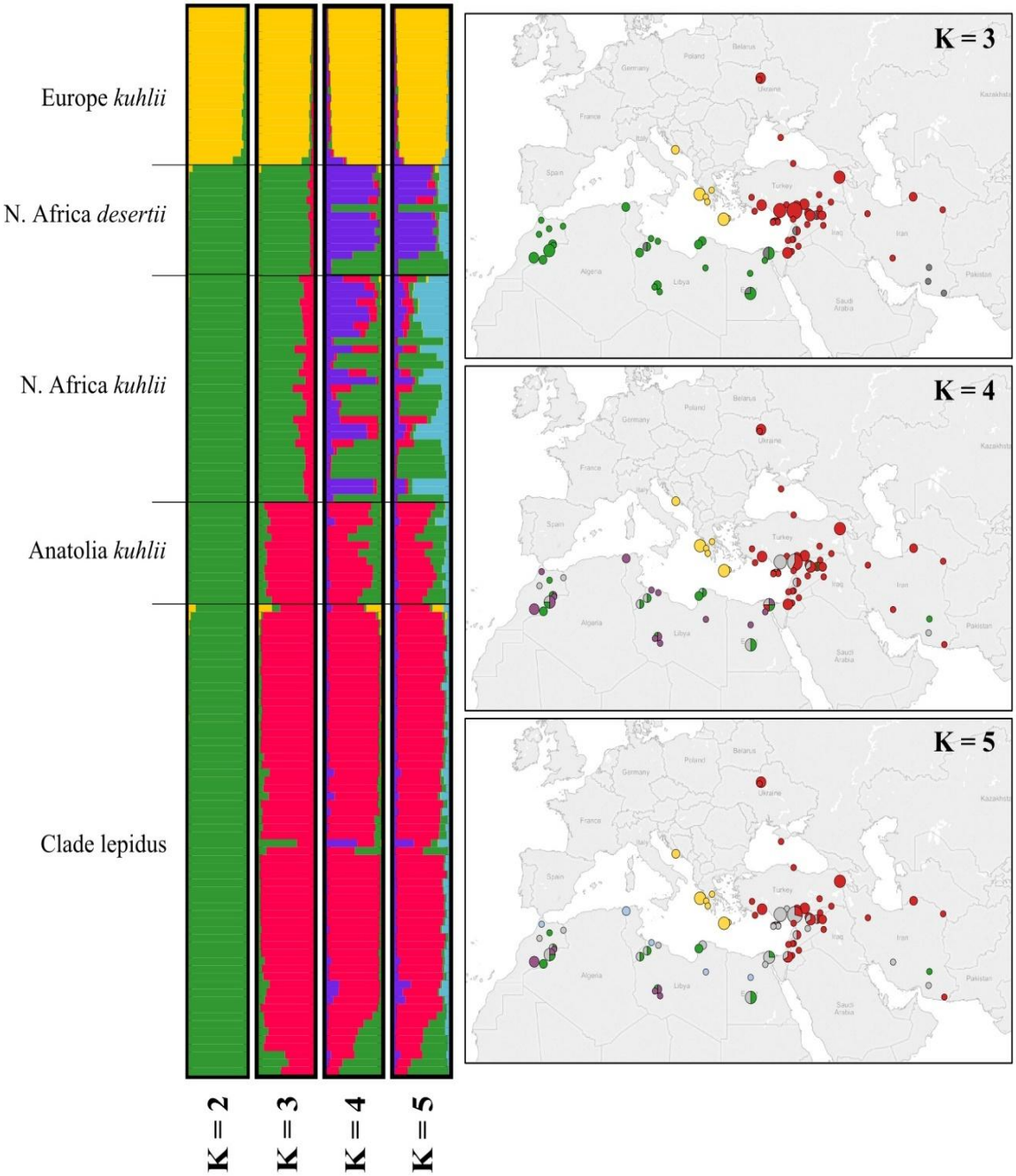

Figure 3.7. Inference of the Structure analysis. Horizontal bars illustrate the inferred population membership of the individuals up to 5 clusters. The groups used as the a priori information are shown on the left of these panels. Maps show the geographical distribution of the assigned individuals for K = 3 up to K = 5. Individuals that had lower than 0.7 membership probabilities are marked with grey.

Based on the GENELAND inference of three clusters, the highest inbreeding coefficient (F_{IS}) was found for the European group (0.26). The other clusters had relatively lower F_{IS} values with 0.14 for the North African and 0.13 for the Middle Eastern group. Pairwise fixation indices (F_{ST}) showed that the genetic differentiation between the North African and Middle Eastern clusters was low (0.04), whereas the European cluster differed moderately from both them; 0.13 and 0.11, respectively.

3.3.5. Ecological Niche Modeling

All the constructed ecological niche models were highly predictive (Table 3.5) and they captured the current distribution ranges of their respective data sets (Figure 3.8, Figure 3.9, Figure 3.10, and Figure 3.11). In the 'clade only' models, the predictions of particular clades exceeded their current ranges. Nonetheless, most of these did not exceed the species' range but overlapped with the distributions of the other clades. For instance, in the Clade kuhlii model, the predicted areas also covered the ranges that were occupied by the Clade western populations and in the Clade lepidus model, the predicted areas highly overlapped with the ranges that were inhabited by the Clade kuhlii populations. On the other hand, both, the 'clade only' and the combined data set models had an underestimation in predicting the eastern distributional ranges; the areas that were occupied with Clade lepidus. Although the Clade lepidus model had relatively better prediction, still it could not fully capture the North-Eastern distributional ranges.

Each ENM had three to five (out of 19) parameters, which had more than 10% variable contribution and on average, their overall contributions were more than 70% (Table 3.5). These informative variable sets were distinct for each model, yet some of the parameters were present in more than one model. For instance, in the Clade western and the Clade kuhlii models, four variables had contributions of more than 10%. In the former one, isothermality (Bio_3) and temperature seasonality (Bio_4) had the highest contributions, followed by precipitation of coldest quarter (Bio_19). First two of these also had high contribution in the Clade kuhlii model, following temperature of coldest quarter (Bio_11). In the Clade lepidus model, three variables, precipitation of coldest quarter

(Bio_19), precipitation of warmest quarter (Bio_18), and minimum temperature of coldest month (Bio_6), had contributions over 10%.

Table 3.5. Average Area Under Curve (AUC) scores of the replicate runs and the variables that had more than 10% contribution.

	Model			
	Clade western	Clade kuhlii	Clade lepidus	Combined dataset
AUC	0.978	0.938	0.874	0.873
Variable Contributions	Bio_3 (23.4%)	Bio_11 (28.1%)	Bio_19 (32.5%)	Bio_8 (25.3%)
	Bio_4 (23.2%)	Bio_4 (24.5%)	Bio_18 (22.8%)	Bio_11 (12.4%)
	Bio_19 (16.8%)	Bio_3 (12.6%)	Bio_6 (17.8%)	Bio_1 (12.3%)
	Bio_17 (15.8%)	Bio_8 (11.9%)		Bio_4 (10.7%)
				Bio_6 (10.5%)

The projections of the ‘clade only’ and the combined dataset models had remarkable differences. In the past predictions, the combined dataset model showed a general contraction and expansion pattern around the Mediterranean, whereas, the predictions of the ‘clade only’ models had distinctive responses for each clade. For instance, most of the areas that are currently occupied by Clade western were identified as suitable in the combined data set model (both for the LIG and the LGM period), while the ‘clade only’ model suggested that these areas were mainly uninhabitable, except a few tiny pockets in the coastal zones.

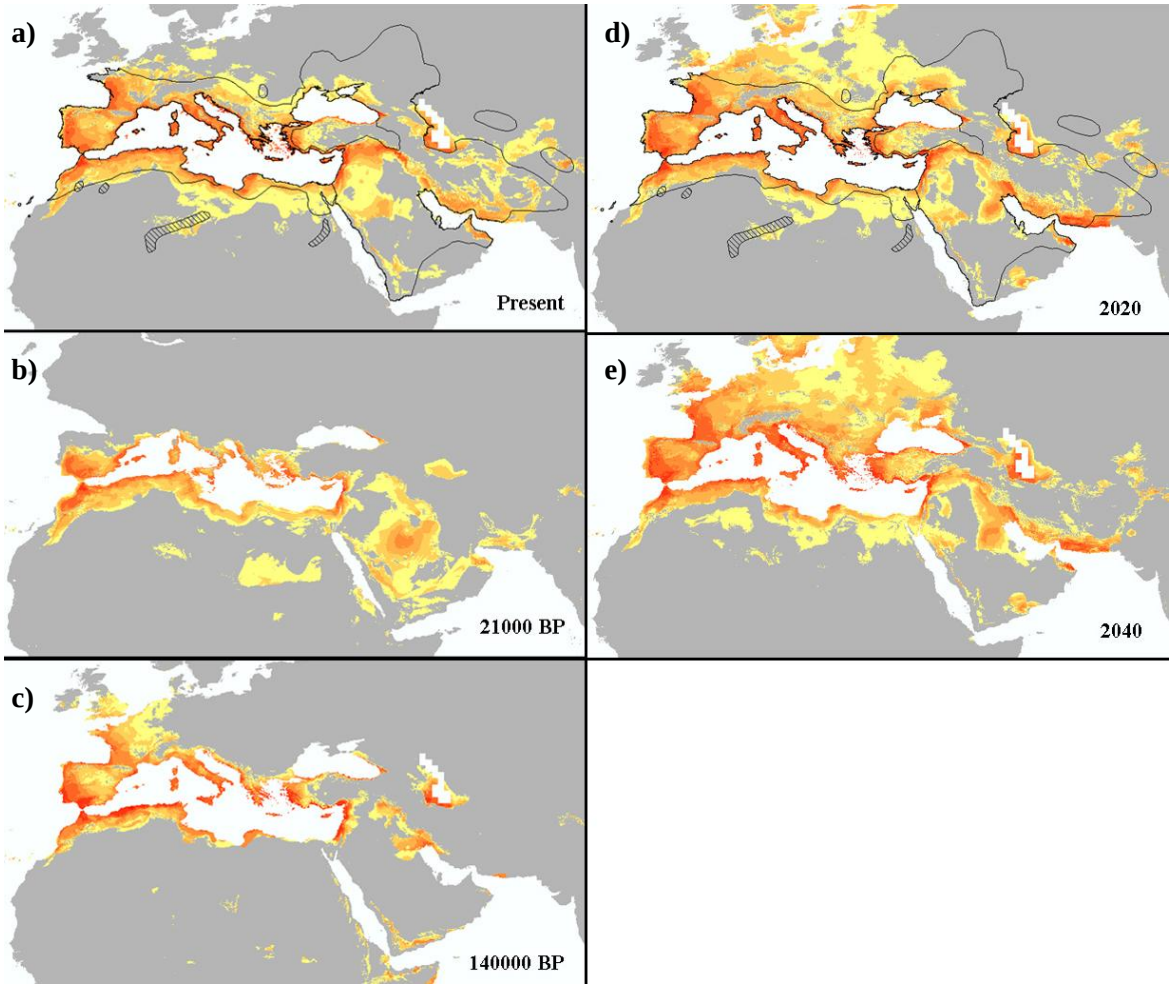

Figure 3.8. ENMs of the combined data set: a) Present, b) LGM, c) LIG, d) 2020, and e) 2040. The contour in present and 2020 maps show the current distribution range of *P. kuhlii* and the dashed areas show the range of *P. deserti*. Areas that had habitat suitability score lower than the presence threshold are shown with grey. Warmer colours indicate higher habitat suitability.

Similar differences were found for the future projections. The combined data set model again showed a general trend, a northward spread in Europe throughout the species range. The extent of this spread, however, highly differed in the 'clade only' models; notably, for Clade lepidus. In the 2020 projections of the Clade lepidus model, the predicted niches almost completely captured the currently recognized distribution of Clade lepidus. On the other hand, in all the models, both the IPSL and the CSRIO mk2 projections revealed similar inferences (Appendix F).

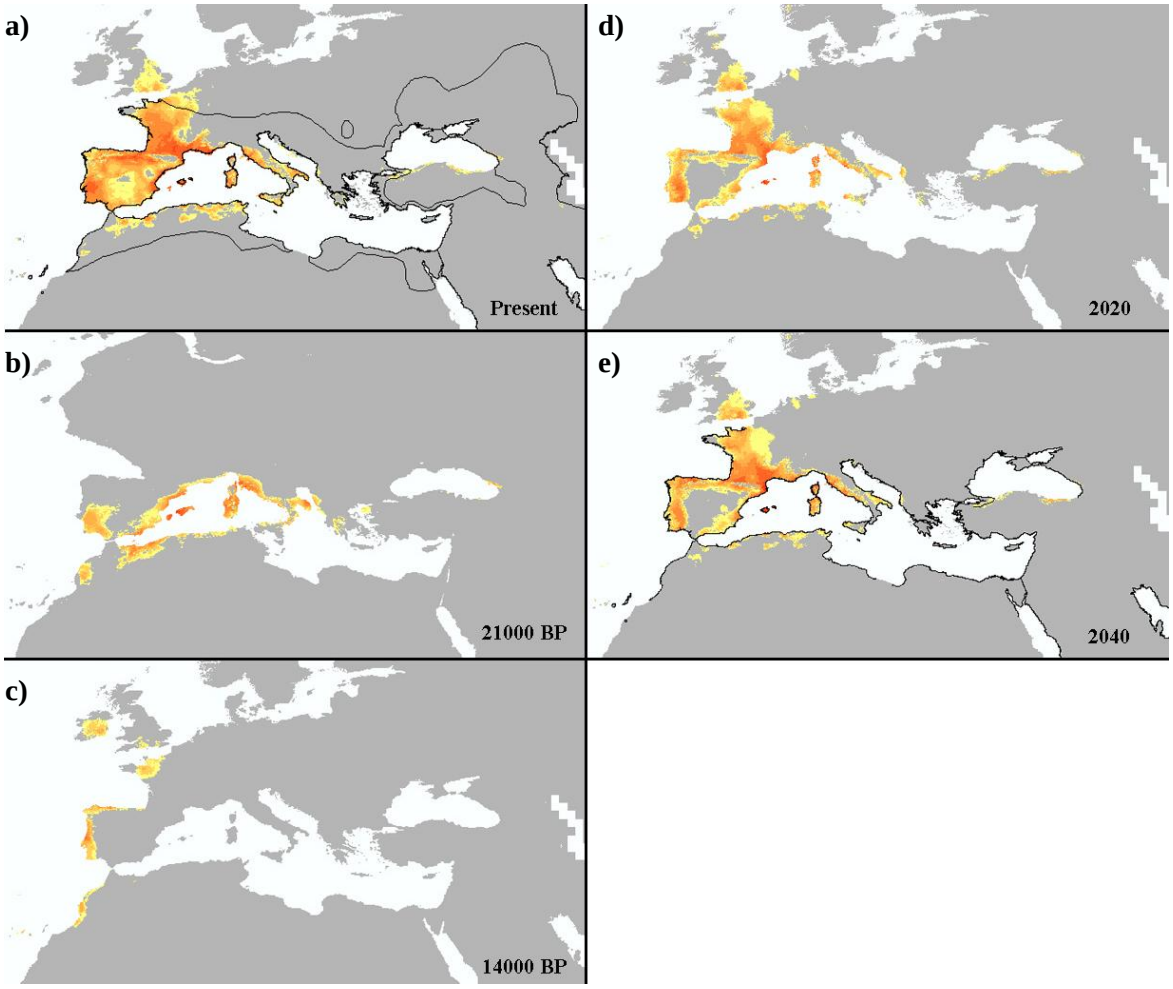

Figure 3.9. ENMs of Clade western

For both of the similarity indices (Hellinger's *I* and Schoener's *D*), the comparison of the Clade kuhlii and the Clade lepidus models had the highest values, whereas the Clade western and the Clade lepidus models had the lowest (Table 3.6). On the other hand, when these similarity scores were compared to a null hypothesis of niche equivalency, they were significantly smaller; rejecting that the environmental niches of the clades were similar (Figure 3.12 and Figure G.1).

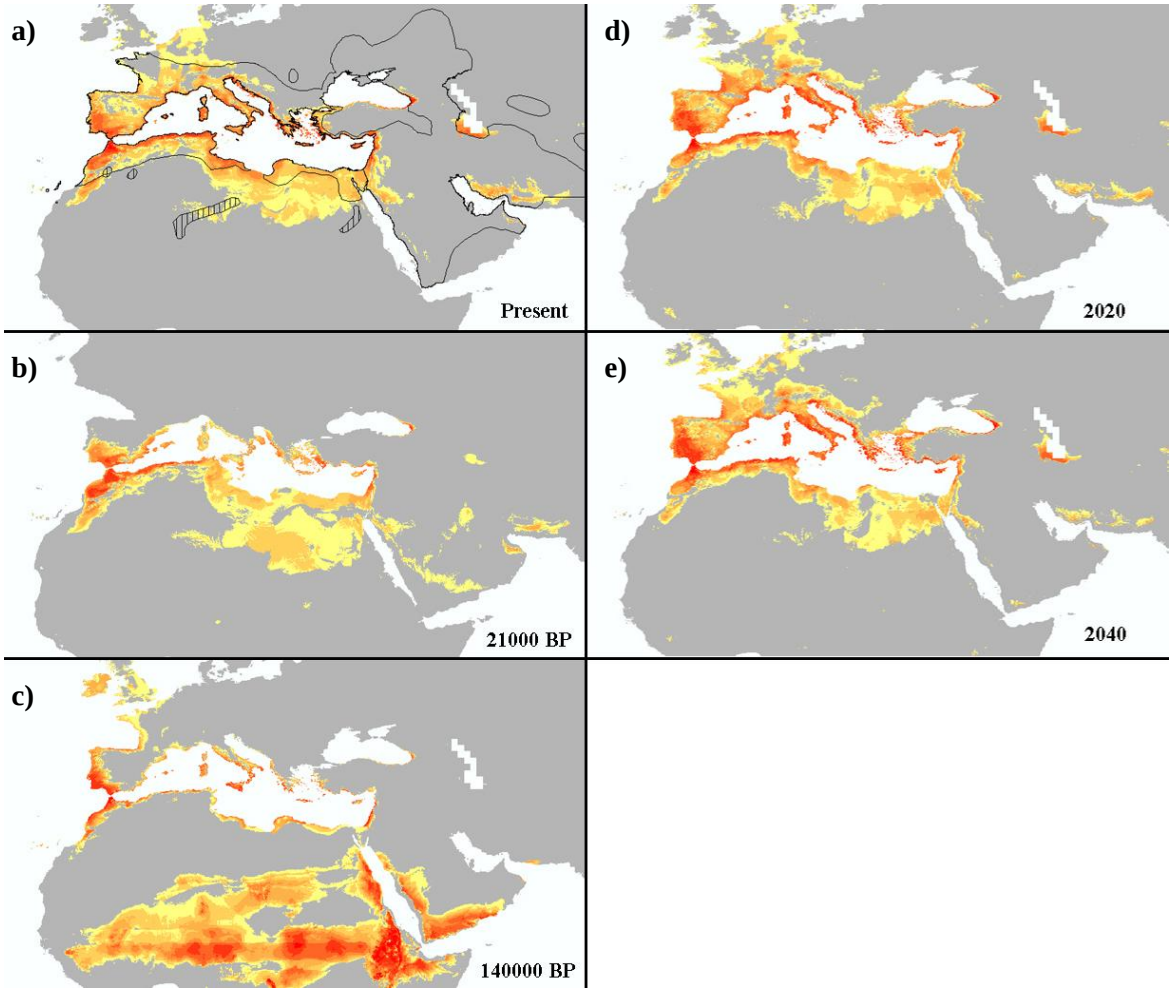

Figure 3.10. ENMs of the Clade kuhlii

Table 3.6. Similarity scores of the pairwise comparisons of the clade only models. Hellinger's *I* values are shown in the upper and Schoener's *D* in the lower diagonal.

Model	Clade western	Clade kuhlii	Clade lepidus
Clade western	-	0.68	0.52
Clade kuhlii	0.39	-	0.76
Clade lepidus	0.25	0.50	-

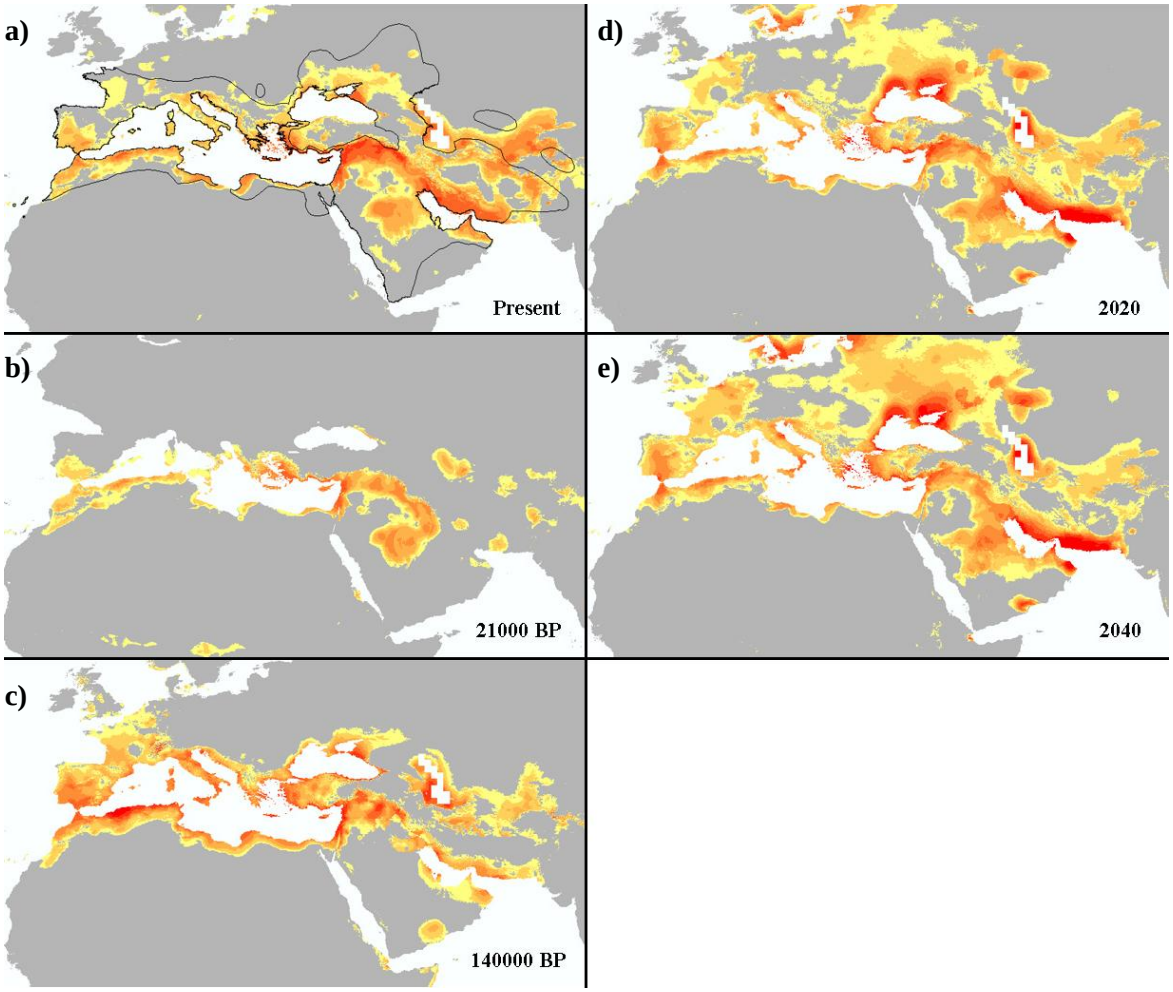

Figure 3.11. ENMs of the Clade lepidus

Background tests showed that Clade western and Clade kuhlii used a subset of Clade lepidus' habitat. When we ran the Clade lepidus model in the backgrounds of Clade kuhlii and Clade western, the distributions of the observed similarity scores were overlapping with the calculated niche overlap values (Figure G.2 and Figure G.3). On the other hand, when we ran the Clade kuhlii and the Clade western models in the background of Clade lepidus, the distributions of their observed similarity scores were significantly smaller than the calculated niche overlap values. In Clade kuhlii and Clade western comparisons, the distributions of the similarity indices were overlapping, suggesting that both clades occupied similar niches (Figure G.4). However, both of the observed similarity distributions were significantly different than the calculated niche overlap value.

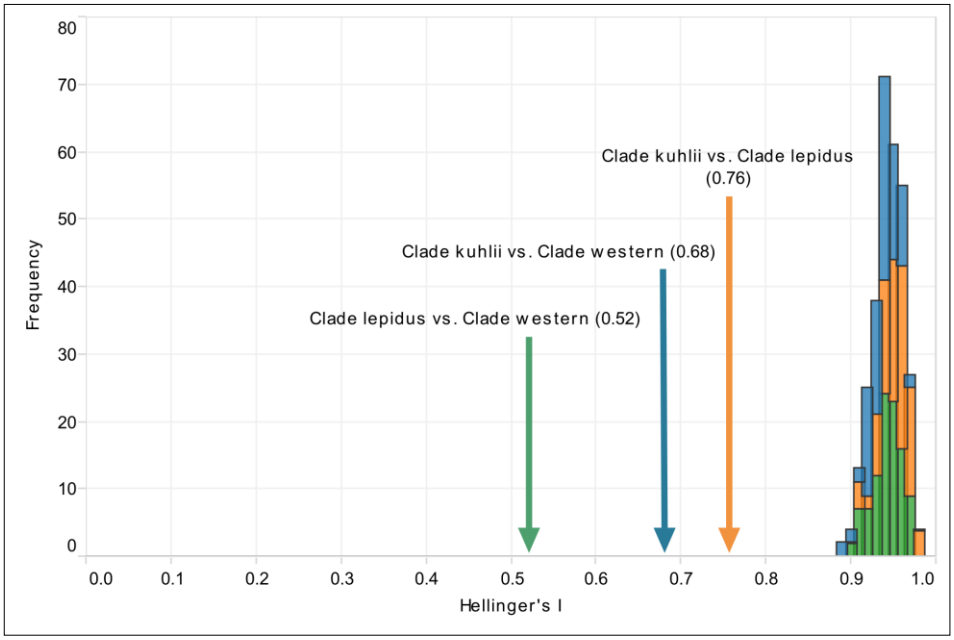

Figure 3.12. Niche overlap for values for Hellinger's *I* compared to the null distributions. Clade lepidus vs. Clade kuhlii comparisons are shown with green bars, Clade lepidus vs. Clade western with blue, and Clade western vs. Clade kuhlii with orange. The arrows show the similarity scores.

3.4. Discussion

3.4.1. Phylogeography of the *Pipistrellus kuhlii* Complex: Deep Divergences

Phylogenetic analyses suggest that the identified clades within the *P. kuhlii* complex evolved from a common ancestral population, which was present during the Pleistocene. The ages of the clades and the distribution of the genetic diversity within them indicate that this ancestral population was distributed in the Southern Mediterranean Basin and the Middle East. In the late Pleistocene, consecutive vicariance and dispersal events split this ancestral population into four main groups, shaping the current genetic structure.

The first split is dated back to 1.5 mya, when the ancestors of Clade western separated from the rest of the group. Probably this population originated from the ancestral North African populations, which dispersed to the Iberian Peninsula. During that era, the Straits of Gibraltar was already open and there had not been any major geophysical events

that could lead to a vicariance event between the European and the African populations. Therefore, we interpret this split to be caused by a dispersal event; the first episode of colonisation of Europe by the *P. kuhlii* complex.

Colonization of Europe from North Africa is a common dispersal pattern observed for many other taxa. Various studies showed that Europe was colonized by the North African population through the Straits of Gibraltar at different time periods (Husemann et al., 2014). However, bats species, which show similar genetic discontinuities on both sides of the Straits of Gibraltar, exhibit an opposite pattern, where European populations colonised North Africa (*Hypsugo savii*, *Myotis mystacinus*, *M. nattereri* [García-Mudarra et al., 2009], and *P. pipistrellus* [Hulva et al., 2004]).

The other three clades separated roughly at the same time period, approximately 1.0 mya. Among these, the split of *P. maderensis* show similar patterns to the separation of Clade western, suggesting that they also originated in North Africa. Currently, this clade is present in the Canary and the Madeira archipelagos and populations from these archipelagos have moderate levels of divergences (around 2.8%, [Jesus et al., 2013]). This isolation by distance pattern suggests that their ancestors first moved to the close by islands in the Canaries and around 500 kya these colonisers expanded to Madeira. We also found Clade kuhlii haplotypes in the Canary Islands, which were genetically similar to the Moroccan populations. This suggests there was a secondary expansion to the Canaries relatively recently.

The other two clades, Clade kuhlii and Clade lepidus, have diverse genetic variability and their ages (approximately 500 ky) are relatively old compared to their divergence time. These patterns coupled with their wide distributional ranges suggest that they were separated by a vicariance event. The ancestors of these two clades were most likely distributed in the Southern and the Eastern Mediterranean coast extending to the Persian Gulf through the Mesopotamia. The diverged haplotypes from Yemen suggest that they might be also present around the Red Sea. Around 1.0 mya, with the increase of aridification in the subtropical Africa (deMenocal, 2004), this ancestral population was split into two disjunct groups, separated roughly by the ‘Arabian-Syrian’ desert belt. In the

West, ancestors of Clade kuhlii were confined mainly to the coastal zones in the North Africa, and in the East, ancestors of Clade lepidus were confined to the Mesopotamia.

3.4.2. Past Population Demographics of the Clades

Genetic structures within the clades allowed us to make further inferences about their past population demographics. Apparently, all the clades are affected by the climatic fluctuations during the glacial periods and experienced many phases of population expansion and retractions. Especially after the LGM, all of them expanded their population sizes, as well as their distribution ranges.

3.4.2.1. Clade western. Clade western, for instance, has substantially low within genetic diversity; despite its early split. This lack of genetic variability suggests that the ancestors of this clade went through a bottleneck after they colonised the Iberian Peninsula. Nevertheless, expansion time estimates indicate a relatively recent population increase, which dates back after the LGM.

3.4.2.2. Clade lepidus. A similar expansion pattern is found for Clade lepidus. This clade has high haplotype diversity, yet within group differences are relatively shallow. Except for few diverged haplotypes found in Yemen and Saudi Arabia, the remaining group was closely related, without showing any genetic discontinuity. On the other hand, even these shallow differences exhibit geographical structuring. These patterns indicate that most of the Clade lepidus populations expanded from a single source in a relatively short period of time, compared to the clade's age. Both the mismatch analyses and the BSL plots confirm these findings, with an expansion time estimate dating back to around 50 kya (the age of the clade is approximately 480 ky). Furthermore, samples from Northern Iran, the Caucasus, and Eastern Europe shared the same haplotype, suggesting that this vast area is colonised even more recently. Nevertheless, *P. kuhlii* have been recorded in these regions only for the last few decades (Aulagnier et al., 2014; Robinson et al., 2005).

In the Eastern Mediterranean coast of Turkey and Cyprus, we found the Clade lepidus and the Clade kuhlii populations in sympatry. These populations clustered together in

nuclear DNA, indicating the presence of gene flow between them. This cytonuclear discordance suggests that Clade lepidus colonised the Mediterranean coast relatively recently. Probably, some of the Clade kuhlii populations survived the LGM in isolated areas along the Mediterranean coast of the Middle East, however, in contrast to the Clade lepidus populations, they did not expand in the following periods. With the arrival of the Clade lepidus populations, gene flow started between these two clades. The residents maintained their maternal genetic signature as they were philopatric, but lost the nuclear type because of the high asymmetric gene flow from the arriving Clade lepidus populations.

3.4.2.3. Clade kuhlii. Based on the current distribution of Clade kuhlii, the European populations would be expected to be genetically similar to those from Anatolia. However, they are more similar to the populations from the North Western Africa; some even share the same haplotypes. On the other hand, microsatellite analysis shows that no evidence for gene flow across the Mediterranean, indicating that the populations from Europe and Africa are isolated. These phylogeographical patterns result from founder effects of a colonisation event followed by a long-term isolation (Rossiter et al., 2007). High genetic variability of the North African populations, both in mtDNA and nuclear markers, suggests that the colonisers originated from the North Africa and apparently, after that the first dispersal, the gene flow between the continents ceased.

As the Iberian Peninsula was already occupied by the Clade western populations, it is likely that this second colonisation happened through the Strait of Sicily. Nonetheless, we do not find any trace of this European haplogroup in the Iberian Peninsula. The shallow genetic variability of the European samples suggests an expansion during the LGM, when the distance between Sicily and Tunisia was significantly shorter (Lambeck et al., 2004). Considering that the Apennine Peninsula was a refugium for many other species (Hewitt, 1999), including bats (Ruedi et al., 2008), it is likely that *P. kuhlii* also found suitable habitats in this area even during the last ice age. After the LGM, with the retreat of the ice-sheets, they expanded to the North, colonising the current European distribution range of Clade kuhlii. Meanwhile, the Mediterranean sea level increased and closed the passage through the Strait of Sicily, hindering the prospective gene flow.

The available fossils records are also in agreement with this scenario: in most of the Europe, *P. kuhlii* is missing (Spitzenberger, 2001) and all the existing records from southern areas are dated to after the LGM (Bogdanowicz, 2004).

To the best of our knowledge, such dispersal pattern is reported for first time for bats. Nevertheless, there are only few cases documented for other taxa (Husemann et al., 2014) and most of these date back to earlier than the Pleistocene period (Giovannotti et al., 2007; Habel et al., 2010, 2009; Stöck et al., 2008).

Genetic diversity of the populations from North Africa suggests that this area was occupied by the *P. kuhlii* complex probably throughout the species' evolutionary history. Phylogeographical analyses of these populations indicate that they were also affected by the climatic fluctuations during the Late Pleistocene. Most of the sub-clades within the Clade kuhlii are found along the patchy areas along the Mediterranean coastal zone. These populations from Morocco, Libya, and Egypt show isolation by distance pattern, indicating the presence of a continuous distribution in the past. It is likely that these populations were isolated during the arid periods when the Sahara extended, and expanded back during the humid periods when the desert retreated.

On the other hand, some of the sub-clades occupy very arid areas in the Sahara Desert and its vicinity. Although the current climatic conditions of these areas are drastically different then the coastal zones, palaeo-hydrological studies suggest that these regions had more humid periods during the Late Pleistocene and early Holocene (~11 to 8 ka) and even in the LIG (~ 125 ka) (Drake et al., 2010). During these humid periods, the Sahara contained a series of linked lakes, rivers, and inland deltas, which facilitated the dispersal of many animal species. It is possible that *P. kuhlii* also used these pathways to disperse through the Sahara.

These desert populations, on the other hand, are genetically more similar to the Moroccan populations than to their coastal neighbours. For instance, the samples from the Egyptian Sahara and the Nile Delta differ from each other by more than 2%, whereas the difference between them and the Moroccan populations are both around 1%. Nonetheless,

some of these desert populations share the same haplotypes, despite being separated by more than 1,500 km apart. These results suggest that the North African populations followed different dispersal paths during the wetter periods; some expanded through the coastal zones and some dispersed through the humid corridor in the South of retracted Sahara.

Various North African species has shown to exhibit high genetic variability, most of which are also composed of multiple endemic lineages (Husemann et al., 2014; Madec et al., 2003; Kapli et al., 2008; Fritz et al., 2009). On the other hand, the majority of these studies focus on the North Western coast and even fewer cover whole North Africa (Barrientos et al., 2014); even rarer are those that also include the Sahara. In most of these cases, the divergence estimates for the identified clades date back to the Pliocene or even earlier periods. Considering that the most of the studied organisms had low mobility, they are more likely to exhibit deep genetic divergences than the ones we found.

3.4.3. Taxonomical Implications

The identified clades within the *P. kuhlii* complex exhibit high levels of diversification in Cytb sequences, which are considerably higher than the intraspecific divergences reported for most of the European bats (Mayer and von Helversen, 2001; Mayer et al., 2007; Ruedi and McCracken, 2009). These deep divergences indicate that the *P. kuhlii* complex might be composed of cryptic species. Nevertheless, previous studies also questioned the presence of possible cryptic species, yet none of the proposed taxa have been agreed upon (Ibáñez et al., 2006; Mayer et al., 2007; García-Mudarra et al., 2009).

Ibáñez et al. (2006) used one nuclear (RAG2) and two mitochondrial (Cytb and ND1) markers to compare the Iberian and the Eastern European populations. Their results showed that the deep divergence in mtDNA was not reflected in the nuclear gene; accordingly, the authors suggested that Clade western should be regarded as a subspecies of *P. kuhlii*. In our study, we have few individuals from Clade western and all of them are from a single colony. Therefore, we are unable to comment on the gene flow between the

Clade western and the Clade kuhlii populations. The distribution of the compiled Cytb data set shows that Clade western and Clade kuhlii have a contact zone in the Eastern France. In order to clarify the taxonomic relations of these clades, we suggest the populations in this contact area be investigated by using the nuclear markers.

Clade lepidus was previously suggested to represent a distinct species by Mayer et al. (2007). However, their study had very few samples and their inference was based on solely the mtDNA markers. We show that this lineage is very common in the Middle East, even extending to Eastern Europe and also have a sympatric distribution with Clade kuhlii in some parts of Turkey and Cyprus. Microsatellite analyses group the North African Clade kuhlii and the Middle Eastern Clade lepidus populations into two distinct clusters, suggesting that they represent different gene pools. On the other hand, the Clade kuhlii samples from Turkey and Cyprus clustered within Clade lepidus, indicating the presence of gene flow in the contact zone. These results suggest that Clade kuhlii and Clade lepidus are not reproductively isolated, and can breed with each other. Accordingly, we suggest that Clade lepidus represents an ecotype of Clade kuhlii and it should be regarded as an Evolutionarily Significant Unit (ESU).

On the other hand, samples identified as *P. deserti* do not exhibit any genetic diversification from the North African *P. kuhlii* populations; neither on mtDNA nor on microsatellites. These findings raise a question about the taxonomic classification of *P. deserti* as a distinct species. This species' identification is based on its smaller size and lighter fur coloration and they do not differ from the rest of the complex in nonmetric traits (e.g. shapes of skull and teeth) (Benda et al., 2004). Apparently, the *P kuhlii* complex has high phenotypic plasticity and these differences are likely to be affected by environmental conditions. For instance, in Egypt, the forearm length of the largest measured individual is approximately 20% larger than the smallest one (Dietz, 2005); and in Italy, the cranial sizes of the populations increased just after 1950 as a response to changing prey availability (Tomassini et al., 2014). Morphological differences of *P. deserti* can also represent its adaptation to environmental conditions. This form is often found in desert areas in the central Sahara and its smaller size and light colour might be an adaptation to

these arid environments. Accordingly, we suggest that the populations regarded as *P. deserti* represents another ecotype of *P. kuhlii*.

3.4.4. Predicting the Effects of Climate Change

Comparing phylogeographic inferences with past projections indicate that the 'clade only' models are more informative. The combined model shows a general expansion and retraction pattern, however, it cannot capture the individual responses of the clades. For example, phylogeographic analyses reveal that Clade western went through a bottleneck after colonising the Iberian Peninsula. The combined dataset model, however, suggests that this area was suitable both in the LGM and the LIG periods, whereas, the Clade western model identifies the range retraction; showing that except for a few tiny pockets in the coastal areas, most of the Iberia was unsuitable.

Differential past responses of the clades suggest that they might have acquired different adaptations. Nevertheless, identity estimates indicate that each clade has its own environmental space and the background tests show some of these differences are not related to the available habitats. For example, the distributional ranges of Clade western and Clade kuhlii provide similar environmental conditions and therefore, their niche differences might indicate an adaptive divergence. On the other hand, Clade lepidus occupy similar habitats with the other clades but they are also present in areas with distinct environmental conditions. Apparently, they can survive in the ranges of Clade kuhlii and Clade western, but we do not know if the others can survive throughout the range of Clade lepidus.

The future projections of the 'clade only' and the combined dataset models show differences as well. Again the combined model predicts a general pattern, an expansion to north; but cannot capture the Clade lepidus' recent dispersal. However, the 'clade only' model recognizes this and predicts a range expansion around the Black Sea region and to the further north. The Clade kuhlii and the Clade western models also identify a northern expansion. Nevertheless, in the last few years, *P. kuhlii* have been recorded both to the north of the Alps in Germany and Southern England (Robinson et al., 2005). The latter

model also identifies a range contraction in Iberia; a pattern which was also found for the grey long-eared bat, *Plecotus austriacus* (Razgour et al., 2013).

The impact of climate change on European bats was previously evaluated by Rebelo et al. (2010). They compared the future projections of 28 species in Europe and showed that their distributions will change in relation to their biogeographic patterns. Species associated with colder climates are likely to decline and the Mediterranean and temperate taxa seem to be more tolerant of increasing temperatures. On the other hand, this study did not distinguish the intraspecific groups. Our results and a recent study, which incorporated phylogenetic information to ENMs, show considerable differences between the predicted ranges, as well as the responses, between 'clade only' and combined models (D'Amen et al., 2012). Considering that most of the bat species in Europe are composed of genetically distinct lineages (Mayer and von Helversen, 2001; Ibáñez et al., 2006; García-Mudarra et al., 2009; Mayer et al., 2007), their responses might be more complicated than predicted.

4. CONCLUSION

4.1. The Importance Anatolian Bats for European Genetic Diversity

Taking into consideration that Turkey has one of the richest bat fauna in the Mediterranean region and its biodiversity is under threat from the current developmental practices (Şekercioğlu et al., 2011), an urgent protection program for Anatolian populations is of the uttermost importance. Genetically diverse populations identified in Turkey highlights the region's significance even further, suggesting that their conservation should be focus of not only regional biodiversity management plans, but also should be included large-scale planning. Because ranges of many Turkish bat species extend to Europe, protecting bats in Turkey should be a part of the European bat conservation program as well.

Molecular methods have been very instrumental in discovery of cryptic diversity in species from a conservation perspective. Focusing in a generally threatened order, Chiroptera, in Turkey showed the presence of genetically distinct populations in at least 12 species. The source of this cryptic diversity is probably related to the fact that Turkey is surrounded by major refugial areas, such as the Balkans and the Caucasus. The perspective outlined here has implications for other non-volant species: seeing genetic breaks in volant species like bats suggests that even greater levels of genetic differentiation can be expected for non-volant species and these might comprise a greater number of ESUs within their ranges, requiring greater protection than if these species are considered as a single unit of evolution.

4.2. Differential Responses of Genetical Lineages to Climate Change

Using phylogeographical inferences in association with ecological niche modelling techniques allow us to infer the response of the *P. kuhlii* lineages to the climate change. Both of the methodologies reveal similar population expansion scenarios, both for the past and the future. The consistency of the current and the past expansion patterns of each clade

suggests that they conserved their niches throughout the time. On the other hand, the differential responses among the clades indicate that their niches are diverged. These divergences are likely to arise because they were long time isolated in distinct ecological habitats. Considering that various species exhibit similar genetic discontinuities in Anatolia (Bilgin, 2011), they might be also diverged in their ecological adaptations.

Although the importance of conservation of genetic diversity has been long recognized, it has been neglected in conservation policy implementations (Laikre et al., 2010). Our results show that genetical lineages are likely to respond differently to current climate change and the models that do not distinguish subspecific units might fail to identify potential risks. Nowadays, numerous studies investigate the possible effects of current climate change; however, only very few of them utilize phylogenetic information (D'Amen et al., 2012). Evidently, whenever available, phylogenetic information should be included in the conservation planning practices and the previous assessments of genetically diverged species complexes should be revised.

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APPENDIX A: SAMPLE COLLECTION

Table A.1. List of specimens. Sample codes, localities (province and country (Ctry.): ARM, Armenia; AZ, Azerbaijan; GEO, Georgia; GR, Greece; IR, Iran, SY, Syria; TR: Turkey), and haplotypes codes for ND1 and Cytb, fragment lengths (bp), and GenBank accession numbers of the analysed haplotypes.

Species	**Sample code**	**Province**	**Ctry.**	**ND1 haplotype**	**ND1 GenBank**	**ND L.**	**Cytb haplotype**	**Cytb GenBank**	**Cytb L.**
Rhinolophus blasii	Rhibla_2002/092	Mersin	TR	Rhibla_TR1_ND1	KF218528	957	-	-	-
	Rhibla_50	Balıkesir	TR	Rhibla_TR1_ND1	KF218528	957	-	-	-
	Rhibla_2002/091	Mersin	TR	Rhibla_TR1_ND1	KF218528	957	-	-	-
	Rhibla_TR36	Hatay	TR	Rhibla_TR1_ND1	KF218528	957	-	-	-
	Rhibla_2002/159	Zonguldak	TR	Rhibla_TR2_ND1	KF218531	957	-	-	-
	Rhibla_TR23	Mersin	TR	Rhibla_TR3_ND1	KF218529	957	-	-	-
	Rhibla_TR34	Hatay	TR	Rhibla_TR4_ND1	KF218530	957	-	-	-
	Rhibla_TR100	Antalya	TR	Rhibla_TR5_ND1	KF218533	957	-	-	-
	Rhibla_K3	Kırklareli	TR	Rhibla_TR6_ND1	KF218532	957	Rhibla_TR1_cytb	KF218411	909
	Rhibla_K4	Kırklareli	TR	Rhibla_TR7_ND1	KF218534	957	Rhibla_TR1_cytb	KF218411	909
	Rhibla_K2	Burdur	TR	Rhibla_TR8_ND1	KF218526	957	-	-	-
	Rhibla_G84	-	GR	Rhibla_GR1_ND1	KF218527	957	-	-	-
R. euryale	Rhieur_Arm_5_ 1	-	ARM	Rhieur_GEO1_ND1	KF218540	957	-	-	-
	Rhieur_Geo_3_24	-	GEO	Rhieur_GEO1_ND1	KF218540	957	Rhieur_GEO1_cytb	KF218412	780
	Rhieur_2002/008	Mersin	TR	Rhieur_TR1_ND1	KF218537	957	-	-	-
	Rhieur_2002/006	Mersin	TR	Rhieur_TR1_ND1	KF218537	957	-	-	-
	Rhieur_2002/009	Mersin	TR	Rhieur_TR1_ND1	KF218537	957	-	-	-
	Rhieur_2002/007	Mersin	TR	Rhieur_TR1_ND1	KF218537	957	-	-	-

Table A.1. (Continued.)

Species	Sample code	Province	Ctry.	ND1 haplotype	ND1 GenBank	ND L.	Cytb haplotype	Cytb GenBank	Cytb L.
R. euryale	Rhieur_2004/157	Hatay	TR	Rhieur_TR2_ND1	KF218536	957	-	-	-
	Rhieur_TR35	Hatay	TR	Rhieur_TR2_ND1	KF218536	957	-	-	-
	Rhieur_TR42	Hatay	TR	Rhieur_TR2_ND1	KF218536	957	-	-	-
	Rhieur_2001/155	Zonguldak	TR	Rhieur_TR3_ND1	KF218538	957	-	-	-
	Rhieur_2001/154	Zonguldak	TR	Rhieur_TR3_ND1	KF218538	957	-	-	-
	Rhieur_257	Kocaeli	TR	Rhieur_TR4_ND1	KF218539	957	-	-	-
	Rhieur_2004/257	Kocaeli	TR	Rhieur_TR4_ND1	KF218539	957	-	-	-
	Rhieur_326	Sinop	TR	Rhieur_TR5_ND1	KF218535	957	-	-	-
	Rhieur_2001/141	Denizli	TR	Rhieur_TR6_ND1	KF218541	957	-	-	-
	Rhieur_5_10	Aydın	TR	-	-	-	Rhieur_TR1_cytb	KF218413	780
	Rhieur_83	Zonguldak	TR	-	-	-	Rhieur_TR1_cytb	KF218413	780
	Rhieur_10	Eskişehir	TR	-	-	-	Rhieur_TR2_cytb	KF218414	780
R. ferrumequinum	Rhifer_2004/114	Gaziantep	TR	Rhifer_TR1_ND1	KF218542	957	Rhifer_TR1_cytb	KF218415	
	Rhifer_2003/060	Balıkesir	TR	Rhifer_TR1_ND1	KF218542	957	-	-	-
	Rhifer_2004/310	Karabük	TR	Rhifer_TR1_ND1	KF218542	957	-	-	-
	Rhifer_2004/299	Zonguldak	TR	Rhifer_TR1_ND1	KF218542	957	-	-	-
	Rhifer_2003/032	Kars	TR	Rhifer_TR2_ND1	KF218544	957	Rhifer_TR6_cytb	KF218418	803
	Rhifer_105	Eskişehir	TR	Rhifer_TR3_ND1	KF218550	957	Rhifer_TR4_cytb	KF218417	803

Table A.1. (Continued.)

Species	Sample code	Province	Ctry.	ND1 haplotype	ND1 GenBank	ND L.	Cytb haplotype	Cytb GenBank	Cytb L.
R. ferrumequinum	Rhifer_119	Tekirdağ	TR	Rhifer_TR4_ND1	KF218551	957	Rhifer_TR1_cytb	KF218415	803
	Rhifer_2002/157	Mardin	TR	Rhifer_TR5_ND1	KF218548	957	Rhifer_TR2_cytb	KF218419	803
	Rhifer_2002/174	Niğde	TR	Rhifer_TR6_ND1	KF218549	957	Rhifer_TR5_cytb	KF218416	803
	Rhifer_2002/068	Van	TR	Rhifer_TR7_ND1	KF218546	957	-	-	-
	Rhifer_2003/076	Çanakkale	TR	Rhifer_TR8_ND1	KF218543	957	-	-	-
	Rhifer_2004/158	Hatay	TR	Rhifer_TR9_ND1	KF218545	957	Rhifer_TR3_cytb	KF218420	803
	Rhifer_2004/170	Şanlıurfa	TR	Rhifer_TR10_ND1	KF218547	957	-	-	-
R. hipposideros	Rhihip_2003/061	Balıkesir	TR	Rhihip_TR1_ND1	KF218563	957	-	-	-
	Rhihip_2004/294	Zonguldak	TR	Rhihip_TR1_ND1	KF218563	957	-	-	-
	Rhihip_2004/301	Zonguldak	TR	Rhihip_TR1_ND1	KF218563	957	-	-	-
	Rhihip_2004/325	Sinop	TR	Rhihip_TR1_ND1	KF218563	957	-	-	-
	Rhihip_2002/127	Bursa	TR	Rhihip_TR1_ND1	KF218563	957	-	-	-
	Rhihip_A1	Kocaeli	TR	Rhihip_TR1_ND1	KF218563	957	Rhihip_TR7_cytb	KF218423	800
	Rhihip_A4_Çal	Trabzon	TR	Rhihip_TR1_ND1	KF218563	957	Rhihip_TR2_cytb	KF218422	800
	Rhihip_A2_Porsuk	Kocaeli	TR	Rhihip_TR1_ND1	KF218563	957	-	-	-
	Rhihip_A4_Porsuk	Kocaeli	TR	Rhihip_TR1_ND1	KF218563	957	-	-	-
	Rhihip_210	Kırklareli	TR	Rhihip_TR1_ND1	KF218563	957	-	-	-
	Rhihip_214	Kırklareli	TR	Rhihip_TR1_ND1	KF218563	957	Rhihip_TR2_cytb	KF218422	800
	Rhihip_T1	Isparta	TR	Rhihip_TR2_ND1	KF218561	957	-	-	-

Table A.1. (Continued.)

Species	Sample code	Province	Ctry.	ND1 haplotype	ND1 GenBank	ND L.	Cytb haplotype	Cytb GenBank	Cytb L.
R. hipposideros	Rhihip_100	Isparta	TR	Rhihip_TR2_ND1	KF218561	957	-	-	-
	Rhihip_211	Kırklareli	TR	Rhihip_TR3_ND1	KF218555	957	Rhihip_TR5_cytb	KF218424	800
	Rhihip_213	Kırklareli	TR	Rhihip_TR3_ND1	KF218555	957	-	-	-
	Rhihip_2003/025	Erzincan	TR	Rhihip_TR4_ND1	KF218558	957	Rhihip_TR1_cytb	KF218426	800
	Rhihip_2002/150	Şanlıurfa	TR	Rhihip_TR4_ND1	KF218558	957	Rhihip_TR3_cytb	KF218427	800
	Rhihip_2004/350	Trabzon	TR	Rhihip_TR4_ND1	KF218558	957	-	-	-
	Rhihip_A5_Çal	Trabzon	TR	Rhihip_TR4_ND1	KF218558	957	Rhihip_TR1_cytb	KF218426	800
	Rhihip_A6_Çal	Trabzon	TR	Rhihip_TR4_ND1	KF218558	957	-	-	-
	Rhihip_A3_Çal	Trabzon	TR	Rhihip_TR4_ND1	KF218558	957	-	-	-
	Rhihip_88	Eskişehir	TR	Rhihip_TR5_ND1	KF218554	957	Rhihip_TR6_cytb	KF218425	800
	Rhihip_212	Kırklareli	TR	Rhihip_TR6_ND1	KF218553	957	-	-	-
	Rhihip_2001/162	Karabük	TR	Rhihip_TR7_ND1	KF218562	957	-	-	-
	Rhihip_2002/175	Niğde	TR	Rhihip_TR8_ND1	KF218552	957	Rhihip_TR4_cytb	KF218421	800
	Rhihip_2003/048	Ordu	TR	Rhihip_TR9_ND1	KF218557	957	-	-	-
	Rhihip_2003/051	Sivas	TR	Rhihip_TR10_ND1	KF218556	957	-	-	-
	Rhihip_2003/103	Zonguldak	TR	Rhihip_TR11_ND1	KF218560	957	-	-	-
	Rhihip_2003/136	Antalya	TR	Rhihip_TR12_ND1	KF218559	957	-	-	-
R. mehelyi	Rhimeh_6_3	İzmir	TR	Rhimeh_TR1_ND1	KF218565	957	Rhimeh_TR1_cytb	KF218428	812
	Rhimeh_G47	-	GR	Rhimeh_TR1_ND1	KF218565	957	Rhimeh_TR1_cytb	KF218428	812

Table A.1. (Continued.)

Species	Sample code	Province	Ctry.	ND1 haplotype	ND1 GenBank	ND L.	Cytb haplotype	Cytb GenBank	Cytb L.
R. mehelyi	Rhimeh_G92	-	GR	Rhimeh_GR1_ND1	KF218564	957	-	-	-
Myotis alcathoe	Myoalc_702	Kırklareli	TR	Myoalc_TR1_ND1	KF218451	957	-	-	-
	Myoalc_703	Kırklareli	TR	Myoalc_TR1_ND1	KF218451	957	-	-	-
M. aurascens	Myoaur_100927	Iğdır	TR	Myoaur_TR1_ND1	KF218453	832	-	-	-
	Myoaur_2006/074	-	IR	Myoaur_IR1_ND1	KF218452	650	-	-	-
M. bechsteinii	Myobec_2001/142	Artvin	TR	Myobec_TR1_ND1	KF218454	800	Myobec_TR1_cytb	KF218378	784
M. blythii	Myola_2002/062	Van	TR	Myola_TR1_ND1	KF218474	637	Myola_TR5_cytb	KF218389	785
	Myola_2002/058	Bitlis	TR	Myola_TR1_ND1	KF218474	637	Myola_TR2_cytb	KF218388	785
	Myola_2002/163	Gaziantep	TR	Myola_TR1_ND1	KF218474	637	Myola_TR2_cytb	KF218388	785
	Myola_2002/146	Şanlıurfa	TR	Myola_TR1_ND1	KF218474	637	Myola_TR2_cytb	KF218388	785
	Myola_2002/071	Erzincan	TR	Myola_TR5_ND1	KF218469	637	Myola_TR6_cytb	KF218381	785
M. blythii	Myola_316	Hatay	TR	Myola_TR6_ND1	KF218471	637	Myola_TR9_cytb	KF218382	785
	Myola_519	Nevşehir	TR	Myola_TR7_ND1	KF218468	637	Myola_TR1_cytb	KF218379	785
	Myola_SY26	-	SY	Myola_SY1_ND1	KF218467	637	Myola_TR1_cytb	KF218379	785
	Myola_24	Tokat	TR	-	-	-	Myola_TR7_cytb	KF218385	785
	Myola_26	Tokat	TR	-	-	-	Myola_TR8_cytb	KF218387	785
	Myola_82	Kırşehir	TR	-	-	-	Myola_TR11_cytb	KF218386	785
M. capaccinii	Myocap_03/108	Nevşehir	TR	Myocap_TR1_ND1	KF218456	957	-	-	-
	Myocap_107048	Antalya	TR	Myocap_TR1_ND1	KF218456	957	-	-	-

Table A.1. (Continued.)

Species	Sample code	Province	Ctry.	ND1 haplotype	ND1 GenBank	ND L.	Cytb haplotype	Cytb GenBank	Cytb L.
M. capaccinii	Myocap_TR88	Hatay	TR	Myocap_TR2_ND1	KF218457	957	-	-	-
	Myocap_100901	Karabük	TR	Myocap_TR2_ND1	KF218457	957	-	-	-
	Myocap_1010002	Kırklareli	TR	Myocap_TR2_ND1	KF218457	957	-	-	-
	Myocap_2002/155	Şanlıurfa	TR	Myocap_TR3_ND1	KF218458	957	-	-	-
	Myocap_2001/218	Bitlis	TR	Myocap_TR4_ND1	KF218455	671	-	-	-
	Myocap_SY25	Idlib	SY	Myocap_TR1_ND1	KF218456	957	-	-	-
	Myocap_SY14	Idlib	SY	Myocap_TR1_ND1	KF218456	957	-	-	-
	Myocap_SY15	Idlib	SY	Myocap_TR1_ND1	KF218456	957	-	-	-
	Myocap_G40	Greece	GR	Myocap_GR1_ND1	KF218459	957	-	-	-
M. daubentonii	Myodau_2002/115	Bolu	TR	Myodau_TR1_ND1	KF218460	590	-	-	-
M. emarginatus	Myoema_264	Kırklareli	TR	Myoema_TR1_ND1	KF218461	663	-	-	-
	Myoema_223	Hatay	TR	Myoema_TR2_ND1	KF218464	957	-	-	-
	Myoema_224	Hatay	TR	Myoema_TR3_ND1	KF218462	663	-	-	-
	Myoema_K1	Kırklareli	TR	Myoema_TR4_ND1	KF218466	957	-	-	-
	Myoema_2002/099	Mersin	TR	Myoema_TR5_ND1	KF218465	957	-	-	-
M. emarginatus	Myoema_2004/300	Bartın	TR	Myoema_TR6_ND1	KF218463	957	-	-	-
M. myotis	Myola_508	Nevşehir	TR	Myola_TR1_ND1	KF218474	637	Myola_TR10_cytb	KF218384	785
	Myola_110	İstanbul	TR	Myola_TR2_ND1	KF218472	637	Myola_TR3_cytb	KF218383	785
	Myola_07010	Kırklareli	TR	Myola_TR2_ND1	KF218472	637	Myola_TR3_cytb	KF218383	785

Table A.1. (Continued.)

Species	Sample code	Province	Ctry.	ND1 haplotype	ND1 GenBank	ND L.	Cytb haplotype	Cytb GenBank	Cytb L.
M. myotis	Myola_905	İstanbul	TR	Myola_TR3_ND1	KF218473	637	Myola_TR1_cytb	KF218379	785
	Myola_319	Hatay	TR	Myola_TR3_ND1	KF218473	637	Myola_TR1_cytb	KF218379	785
	Myola_1006	İstanbul	TR	Myola_TR4_ND1	KF218470	637	Myola_TR4_cytb	KF218380	785
M. mystacinus	Myomys_107001	Karaman	TR	Myomys_TR1_ND1	KF218480	957	-	-	-
	Myomys_2002/011	Ankara	TR	Myomys_TR2_ND1	KF218478	957	-	-	-
	Myomys_2002/023	Yozgat	TR	Myomys_TR3_ND1	KF218481	957	-	-	-
	Myomys_2003/006	Niğde	TR	Myomys_TR4_ND1	KF218476	655	-	-	-
	Myomys_2003/059	Uşak	TR	Myomys_TR5_ND1	KF218479	957	-	-	-
	Myomys_2010/040_4	Antalya	TR	Myomys_TR6_ND1	KF218475	610	-	-	-
	Myomys_603	Nevşehir	TR	Myomys_TR7_ND1	KF218482	957	-	-	-
	Myomys_TR106	Antalya	TR	Myomys_TR8_ND1	KF218477	655	-	-	-
M. schaubi	Myosch_TR89	Hatay	TR	Myosch_TR1_ND1	KF218483	667	-	-	-
	Myosch_TR90	Hatay	TR	Myosch_TR1_ND1	KF218483	667	-	-	-
	Myosch_2008/129	Mersin	TR	Myosch_TR2_ND1	KF218484	667	-	-	-
Pipistrellus kuhlii	Pipkuh_2001/214	Diyarbakır	TR	Pipkuh_TR1_ND1	KF218496	647	-	-	-
	Pipkuh_2002/034	Mersin	TR	Pipkuh_TR1_ND1	KF218496	647	Pipkuh_TR2_cytb	KF218392	803
	Pipkuh_2002/056	Şanlıurfa	TR	Pipkuh_TR1_ND1	KF218496	647	Pipkuh_TR1_cytb	KF218390	803
	Pipkuh_2002/134	Adıyaman	TR	Pipkuh_TR1_ND1	KF218496	647	Pipkuh_TR1_cytb	KF218390	803

Table A.1. (Continued.)

Species	Sample code	Province	Ctry.	ND1 haplotype	ND1 GenBank	ND L.	Cytb haplotype	Cytb GenBank	Cytb L.
Pipistrellus kuhlii	Pipkuh_2002/135	Adıyaman	TR	Pipkuh_TR1_ND1	KF218496	647	-	-	-
	Pipkuh_2002/139	Adıyaman	TR	Pipkuh_TR1_ND1	KF218496	647	-	-	-
	Pipkuh_2002/159	Mardin	TR	Pipkuh_TR1_ND1	KF218496	647	Pipkuh_TR1_cytb	KF218390	803
	Pipkuh_2002/161	Gaziantep	TR	Pipkuh_TR1_ND1	KF218496	647	-	-	-
	Pipkuh_2003/033	Iğdır	TR	Pipkuh_TR1_ND1	KF218496	647	Pipkuh_TR5_cytb	KF218394	803
	Pipkuh_Z3	Iğdır	TR	Pipkuh_TR1_ND1	KF218496	647	-	-	-
	Pipkuh_Z4	Iğdır	TR	Pipkuh_TR1_ND1	KF218496	647	-	-	-
	Pipkuh_2002/176	Adana	TR	Pipkuh_TR2_ND1	KF218492	647	Pipkuh_TR2_cytb	KF218392	803
	Pipkuh_2003/008	Adana	TR	Pipkuh_TR2_ND1	KF218492	647	-	-	-
	Pipkuh_TR108	Antalya	TR	Pipkuh_TR2_ND1	KF218492	647	-	-	-
	Pipkuh_2004/001	Hatay	TR	Pipkuh_TR3_ND1	KF218491	647	Pipkuh_TR3_cytb	KF218395	806
	Pipkuh_2004/003	Hatay	TR	Pipkuh_TR3_ND1	KF218491	647	-	-	-
	Pipkuh_2003/018	Kahramanmaraş	TR	Pipkuh_TR4_ND1	KF218493	647	Pipkuh_TR4_cytb	KF218391	803
	Pipkuh_TR109	Antalya	TR	Pipkuh_TR5_ND1	KF218495	647	Pipkuh_TR6_cytb	KF218393	803
	Pipkuh_TR60	Hatay	TR	Pipkuh_TR6_ND1	KF218494	647	-	-	-
	Pipkuh_1999/098	Mersin	TR	Pipkuh_TR7_ND1	KF218490	647	Pipkuh_TR3_cytb	KF218395	806
P. nathusii	Pipnat_2003/063	Balıkesir	TR	Pipnat_TR1_ND1	KF218499	957	-	-	-
	Pipnat_2003/064	Balıkesir	TR	Pipnat_TR2_ND1	KF218498	957	-	-	-
	Pipnat_2006/052	Afyonkarahisar	TR	Pipnat_TR1_ND1	KF218499	957	-	-	-

Table A.1. (Continued.)

Species	Sample code	Province	Ctry.	ND1 haplotype	ND1 GenBank	ND L.	Cytb haplotype	Cytb GenBank	Cytb L.
P. nathusii	Pipnat_2006/051	Afyonkarahisar	TR	Pipnat_TR3_ND1	KF218497	957	-	-	-
P. pipistrellus	Pippip_1999/078	Konya	TR	Pippip_TR1_ND1	KF218509	900	Pippip_TR1_cytb	KF218402	803
	Pippip_TR03	Nevşehir	TR	Pippip_TR1_ND1	KF218509	900	-	-	-
	Pippip_402	Nevşehir	TR	Pippip_TR2_ND1	KF218511	900	-	-	-
	Pippip_601	Nevşehir	TR	Pippip_TR2_ND1	KF218511	900	Pippip_TR8_cytb	KF218399	803
P. pipistrellus	Pippip_2002/070	Van	TR	Pippip_TR3_ND1	KF218506	900	Pippip_TR2_cytb	KF218400	803
	Pippip_2003/055	Samsun	TR	Pippip_TR4_ND1	KF218503	900	Pippip_TR7_cytb	KF218397	803
	Pippip_2003/057	Samsun	TR	Pippip_TR5_ND1	KF218507	900	-	-	-
	Pippip_2003/058	Uşak	TR	Pippip_TR6_ND1	KF218512	900	Pippip_TR5_cytb	KF218396	803
	Pippip_2003/138	Kırıkkale	TR	Pippip_TR7_ND1	KF218504	900	Pippip_TR6_cytb	KF218398	803
	Pippip_403	Nevşehir	TR	Pippip_TR8_ND1	KF218510	900	-	-	-
	Pippip_602	Nevşehir	TR	Pippip_TR9_ND1	KF218508	900	-	-	-
	Pippip_7_Ppi1	Trabzon	TR	Pippip_TR10_ND1	KF218513	900	Pippip_TR3_cytb	KF218401	803
	Pippip_8_Ppi2	Trabzon	TR	Pippip_TR11_ND1	KF218505	900	-	-	-
	Pippip_TR52	Hatay	TR	Pippip_TR12_ND1	KF218502	736	Pippip_TR4_cytb	KF218403	803
	Pippip_Z6	İstanbul	TR	Pippip_TR13_ND1	KF218501	624	-	-	-
P. pygmaeus	Pippyg_2003/062	Balıkesir	TR	Pippyg_TR1_ND1	-	800	-	-	-
Hypsugo savii	Hypsav_2003/013	Konya	TR	Hypsav_TR1_ND1	KF218444	900	Hypsav_TR2_cytb	KF218377	809
	Hypsav_2008/127	Karaman	TR	Hypsav_TR2_ND1	KF218443	900	Hypsav_TR1_cytb	KF218376	809

Table A.1. (Continued.)

Species	Sample code	Province	Ctry.	ND1 haplotype	ND1 GenBank	ND L.	Cytb haplotype	Cytb GenBank	Cytb L.
H. savii	Hypsav_2006/066	-	IR	Hypsav_IR1_ND1	KF218442	900	Hypsav_IR1_cytb	KF218375	809
	Hypsav_2010/041	Antalya	TR	-	-	-	Hypsav_TR1_cytb	KF218376	809
Nyctalus lasiopterus	Nyclas_2010/027	Antalya	TR	Nyclas_TR1_ND1	KF218485	800	-	-	-
	Nyclas_2010/028_6	Antalya	TR	Nyclas_TR1_ND1	KF218485	800	-	-	-
N. leisleri	Nyclei_705	Kırklareli	TR	Nyclei_TR1_ND1	KF218486	630	-	-	-
	Nyclei_1_Nle	Trabzon	TR	Nyclei_TR1_ND1	KF218486	630	-	-	-
	Nyclei_2_Nle1	Trabzon	TR	Nyclei_TR1_ND1	KF218486	630	-	-	-
	Nyclei_3_Nle2	Trabzon	TR	Nyclei_TR1_ND1	KF218486	630	-	-	-
	Nyclei_4_Nle4	Trabzon	TR	Nyclei_TR1_ND1	KF218486	630	-	-	-
N. leisleri	Nyclei_2010/030_4	Antalya	TR	Nyclei_TR2_ND1	KF218487	630	-	-	-
N. noctula	Nycnoc_2004/287	Kırklareli	TR	Nycnoc_TR1_ND1	KF218489	900	-	-	-
	Nycnoc_2008/133	Osmaniye	TR	Nycnoc_TR2_ND1	KF218488	900	-	-	-
Eptesicus anatolicus	Eptana_1999/095	Mersin	TR	Eptana_TR1_ND1	KF218435	668	-	-	-
	Eptana_2002/101	Mersin	TR	Eptana_TR1_ND1	KF218435	668	Eptana_TR1_cytb	KF218367	755
	Eptana_2010/042_9	Antalya	TR	Eptana_TR1_ND1	KF218435	668	-	-	-
	Eptana_2010/043	Antalya	TR	Eptana_TR1_ND1	KF218435	668	Eptana_TR1_cytb	KF218367	755
	Eptana_2008/128	Mersin	TR	Eptana_TR2_ND1	KF218434	600	-	-	-
	Eptana_2008/139	Gaziantep	TR	Eptana_TR3_ND1	KF218436	668	Eptana_TR2_cytb	KF218368	755
E. serotinus	Eptser_2001/100	Giresun	TR	Eptser_TR1_ND1	KF218441	665	-	-	-

Table A.1. (Continued.)

Species	Sample code	Province	Ctry.	ND1 haplotype	ND1 GenBank	ND L.	Cytb haplotype	Cytb GenBank	Cytb L.
E. serotinus	Eptser_2003/042	Giresun	TR	Eptser_TR1_ND1	KF218441	665	Eptser_TR5_cytb	KF218370	755
	Eptser_2003/044	Giresun	TR	Eptser_TR1_ND1	KF218441	665	-	-	-
	Eptser_2001/158	Zonguldak	TR	Eptser_TR2_ND1	KF218440	665	Eptser_TR3_cytb	KF218373	743
	Eptser_Ese1	Trabzon	TR	Eptser_TR2_ND1	KF218440	665	Eptser_TR4_cytb	KF218369	755
	Eptser_2002/066	Van	TR	Eptser_TR3_ND1	KF218439	665	Eptser_TR1_cytb	KF218371	755
	Eptser_2010/032	Antalya	TR	Eptser_TR4_ND1	KF218438	665	Eptser_TR2_cytb	KF218374	732
	Eptser_2010/039_3	Antalya	TR	Eptser_TR4_ND1	KF218438	665	-	-	-
	Eptser_SY45	-	SY	Eptser_SY1_ND1	KF218437	665	Eptser_SY1_cytb	KF218372	747
Barbastella barbastellus	Barbar_404	Nevşehir	TR	Barbar_TR1_ND1	KF218431	900	-	-	-
	Barbar_410	Nevşehir	TR	Barbar_TR1_ND1	KF218431	900	-	-	-
	Barbar_2001/117	Rize	TR	Barbar_TR2_ND1	KF218432	900	-	-	-
	Barbar_2001/199	Kırklareli	TR	Barbar_TR3_ND1	KF218433	900	-	-	-
Plecotus auritus	Pleaur_1010003	Kırklareli	TR	Pleaur_TR1_ND1	KF218515	900	Pleaur_TR1_cytb	KF218404	680
	Pleaur_2001/134	Rize	TR	Pleaur_TR2_ND1	KF218516	900	Pleaur_TR2_cytb	KF218405	680
	Pleaur_2003/037	Kars	TR	Pleaur_TR3_ND1	KF218514	623	-	-	-
P. austriacus	Pleaus_2003/075	Çanakkale	TR	Pleaus_TR1_ND1	KF218571	670	-	-	-
	Pleaus_2003/077	Çanakkale	TR	Pleaus_TR1_ND1	KF218571	670	-	-	-
P. kolombatovici	Plekol_1999/087	Karaman	TR	Plekol_TR1_ND1	KF218569	900	-	-	-
	Plekol_1999/085	Karaman	TR	Plekol_TR1_ND1	KF218569	900	-	-	-

Table A.1. (Continued.)

Species	Sample code	Province	Ctry.	ND1 haplotype	ND1 GenBank	ND L.	Cytb haplotype	Cytb GenBank	Cytb L.
P. kolombatovici	Plekol_2002/083	Konya	TR	Plekol_TR2_ND1	KF218570	900	-	-	-
	Plekol_2002/082	Konya	TR	Plekol_TR2_ND1	KF218570	900	-	-	-
P. macrobullaris	Plemac_2001/069	Kayseri	TR	Plemac_TR1_ND1	KF218519	660	-	-	-
	Plemac_2001/070	Kayseri	TR	Plemac_TR1_ND1	KF218519	660	-	-	-
	Plemac_2001/050	Kayseri	TR	Plemac_TR2_ND1	KF218520	660	Plemac_TR1_cytb	KF218407	803
	Plemac_2001/151	Kayseri	TR	Plemac_TR2_ND1	KF218520	660	-	-	-
	Plemac_2002/040	Nevşehir	TR	Plemac_TR3_ND1	KF218517	660	-	-	-
	Plemac_2003/012	Konya	TR	Plemac_TR3_ND1	KF218517	660	Plemac_TR2_cytb	KF218409	803
	Plemac_2003/010	Konya	TR	Plemac_TR4_ND1	KF218525	660	-	-	-
	Plemac_2003/053	Sivas	TR	Plemac_TR5_ND1	KF218524	660	-	-	-
	Plemac_2003/054	Sivas	TR	Plemac_TR6_ND1	KF218521	660	Plemac_TR3_cytb	KF218408	803
	Plemac_2010/035	Antalya	TR	Plemac_TR7_ND1	KF218523	660	-	-	-
	Plemac_2010/092	Konya	TR	Plemac_TR8_ND1	KF218522	660	Plemac_TR4_cytb	KF218410	803
	Plemac_2006/073	Zencan	IR	Plemac_IR1_ND1	KF218518	660	Plemac_IR1_cytb	KF218406	803
	Plemac_2006/072	Zencan	IR	Plemac_IR1_ND1	KF218518	660	-	-	-
	Plemac_2006/069	Zencan	IR	Plemac_IR1_ND1	KF218518	660	Plemac_IR1_cytb	KF218406	803
Miniopterus schreibersii	Minsch_024	Mersin	TR	Minsch_TR1_ND1	KF218449	575	-	-	-
	Minsch_098	Niğde	TR	Minsch_TR1_ND1	KF218449	575	-	-	-
	Minsch_111	İstanbul	TR	Minsch_TR2_ND1	KF218447	575	-	-	-

Table A.1. (Continued.)

Species	Sample code	Province	Ctry.	ND1 haplotype	ND1 GenBank	ND L.	Cytb haplotype	Cytb GenBank	Cytb L.
M. schreibersii	Minsch_242	Ordu	TR	Minsch_TR2_ND1	KF218447	575	-	-	-
	Minsch_074	Zonguldak	TR	Minsch_TR3_ND1	KF218445	575	-	-	-
	Minsch_088	Hatay	TR	Minsch_TR4_ND1	KF218448	575	-	-	-
	Minsch_103	Isparta	TR	Minsch_TR5_ND1	KF218446	575	-	-	-
	Minsch_130	-	AZ	Minsch_TR6_ND1	KF218450	575	-	-	-
	Minsch_154	-	IR	Minsch_TR1_ND1	KF218449	575	-	-	-
Tadarida teniotis	Tadten_2002/089	Aksaray	TR	Tadten_TR1_ND1	KF218566	603	-	-	-
Taphozous nudiventris	Tapnuv_2002/168	Gaziantep	TR	Tapnuv_TR1_ND1	KF218568	663	-	-	-
	Tapnuv_2002/169	Gaziantep	TR	Tapnuv_TR1_ND1	KF218568	663	Tapnuv_TR1_cytb	KF218430	798
	Tapnuv_SY50	-	SY	Tapnuv_SY1_ND1	KF218567	663	Tapnuv_SY1_cytb	KF218429	798

Table A.2. Sample sizes of the sequences obtained in this study and acquired from GenBank

Species	This study		GenBank		GenBank references [a]		
	ND1	Cytb	ND1	Cytb	ND1	Cytb	Other regions
Rhinolophus blasii	12	2	-	2	-	*1, 2*	GR (1)
R. euryale	15	4	-	3	-	*1, 3, 4, 5*	AM (1), GEO (1)
R. ferrumequinum	13	7	1	8	*6*	*1, 3, 4, 5*	-
R. hipposideros	29	9	1	11	*7*	*1, 3, 4, 5*	-
R. mehelyi	3	2	-	5	-	*1, 2, 3, 4, 5*	GR (2)
Myotis alcathoe	2	-	31	-	*6, 8, 9, 10, 11, 12*	-	-
M. aurascens	2	-	9	-	*10, 13*	-	IR (1)
M. bechsteinii	1	1	29	4	*8, 10, 14, 15*	*3, 14*	-
M. capaccinii	11	-	4	10	*8, 10*	*3, 14, 16*	GR (1), SY (3)
M. daubentonii	1	-	43	-	*8, 10, 14, 17, 18*	-	-
M. emarginatus	6	-	6	-	*8, 9, 10*	-	-
M. myotis/M. blythii	14	17	13	50	*3, 8, 10, 14*	*3, 14, 19, 20, 21*	SY (1)
M. mystacinus	8	-	32	-	*5, 8, 9, 10, 22, 23, 24*	-	-
M. schaubi	3	-	2	-	*10, 14*	-	-
Pipistrellus kuhlii	20	10	24	29	*3, 5, 8, 10*	*3, 5, 20, 29, 46, 47*	-
P. maderensis [b]	-	-	5	10	*10*	*29*	-
P. nathusii	4	-	5	-	*8, 10*	-	-
P. pipistrellus	15	8	31	65	*3, 8, 14*	*3, 5, 25, 26, 27, 28*	-
P. pygmaeus	1	-	14	-	*8*	-	-
Hypsugo savii	3	4	14	21	*3, 5, 8, 10*	*3, 5, 20, 29*	IR (1)
Nyctalus lasiopterus	2	-	5	-	*8*	-	-
N. leisleri	6	-	13	-	*8, 10, 14, 30*	-	-
N. noctula	2	-	21	-	*8, 9, 10, 31*	-	-
Eptesicus anatolicus	6	3	14	12	*3, 8, 32*	*3, 33*	-
E. bottae [b]	-	-	6	6	*34*	*35*	SYR (1)
E. isabellinus [b]	-	-	1	1	*10*	*36*	-
E. serotinus	9	6	39	54	*3, 8, 10, 14, 37*	*3, 14, 38, 39*	SYR (1)
Barbastella barbastellus	4	-	4	-	*8, 10*	-	-
Plecotus auritus	3	2	19	9	*8, 10, 40, 41*	*42*	-
P. austriacus	2	-	8	-	*8, 10, 40, 41*	-	-
P. begognae [b]	-	-	1	5	*40*	*42, 43*	-
P. kolombatovici	4	-	5	2	*8, 10*	*42, 44*	-
P. macrobullaris	14	6	10	9	*10, 40, 41*	*42*	IR (3)
Miniopterus schreibersii	9	-	7	13	*8, 10, 14*	*5, 14, 45*	AR (1), IR (1)
Tadarida teniotis	1	-	1	-	*10*	-	-
Taphozous nudiventris	3	2	-	-	-	-	SYR (1)

[a] References: *1*, Zhou et al. 2009; *2*, Stoffberg et al. 2010; *3*, Ibáñez et al. 2006; *4*, Li et al. 2006; *5*, García-Mudarra et al. 2009; *6*, Kössl et al. 1999; *7*, Li et al. 2007; *8*, Mayer and von Helversen 2001; *9*, von Helversen et al. 2001; *10*, Mayer et al. 2007; *11*, unpublished GenBank sequences with accession numbers GU182397 - GU182403; *12*, unpublished GenBank sequences with accession numbers HM042915 - HM042915; *13*, unpublished GenBank sequences with accession numbers AY699856 - AY699862; *14*, Ruedi and Mayer 2001; *15*, Kerth et al. 2008; *16*, Bilgin et al. 2008; *17*, Ngamprasertwong et al. 2008; *18*, unpublished GenBank sequences with accession numbers HQ657328 - HQ657356; *19*, Castella et al. 2000; *20*, Stadelmann et al. 2007; *21*, Bogdanowicz et al. 2009; *22*, Agirre-Mendi et al. 2004; *23*, Boston et al. 2011; *24*, unpublished GenBank sequences with accession numbers AY699863 and AY699865; *25*, Benda et al. 2004; *26*, Hulva et al. 2004; *27*, Hulva et al. 2007; 28, Stadelmann et al. 2004; *29*, Pestano, Brown, Suárez, and Fajardo 2003; *30*, Salgueiro et al. 2007; *31*, Petit et al. 1999; *32*, unpublished GenBank sequences with accession numbers EU786926 - EU786936; *33*, unpublished GenBank sequences with accession numbers EU786802 - EU786812; *34*, unpublished GenBank sequences with accession numbers EU786940 - EU786945; *35*, unpublished GenBank sequences with accession numbers EU786816 - EU786821; *36*, unpublished GenBank sequences with accession numbers EU786829; *37*, unpublished GenBank sequences with accession numbers EU786966 - EU786972 and EU786975 - EU786999; *38*, unpublished GenBank sequences with accession numbers EU786842 - EU786848, EU786851 - EU786863, and EU786870 - EU786875; *39*, Artyushin et al. 2009; *40*, Kiefer et al. 2002; *41*, Garin et al. 2003; *42*, Juste et al. 2004; *43*, Pestano, Brown, Suárez, Benzal, et al. 2003; *44*, unpublished GenBank sequence with accession number EU086528; *45*, Furman, Öztunç, and Çoraman 2010; *46*, Evin et al. 2010; *47*, unpublished GenBank sequence with accession number AJ426661. [b] Closely related species, which is not present in Turkey but was included in the analysis or used as an outgroup

APPENDIX B: PHYLOGENETIC TREES

Phylogenetic constructions of ND1 (left) and Cytb (right) sequences of eleven species and large *Myotis* complex, in which genetically distinct lineages are identified. Posterior probabilities of major clades are shown. Clade numbers are as in Figure 2.1 in the main text. Selected evolutionary models are provided in the figure captions: HKY, Hasegawa-Kishino-Yano' 1985 model; F81, Felsenstein' 1981 model; G, gamma shape parameter; I, invariant sites.

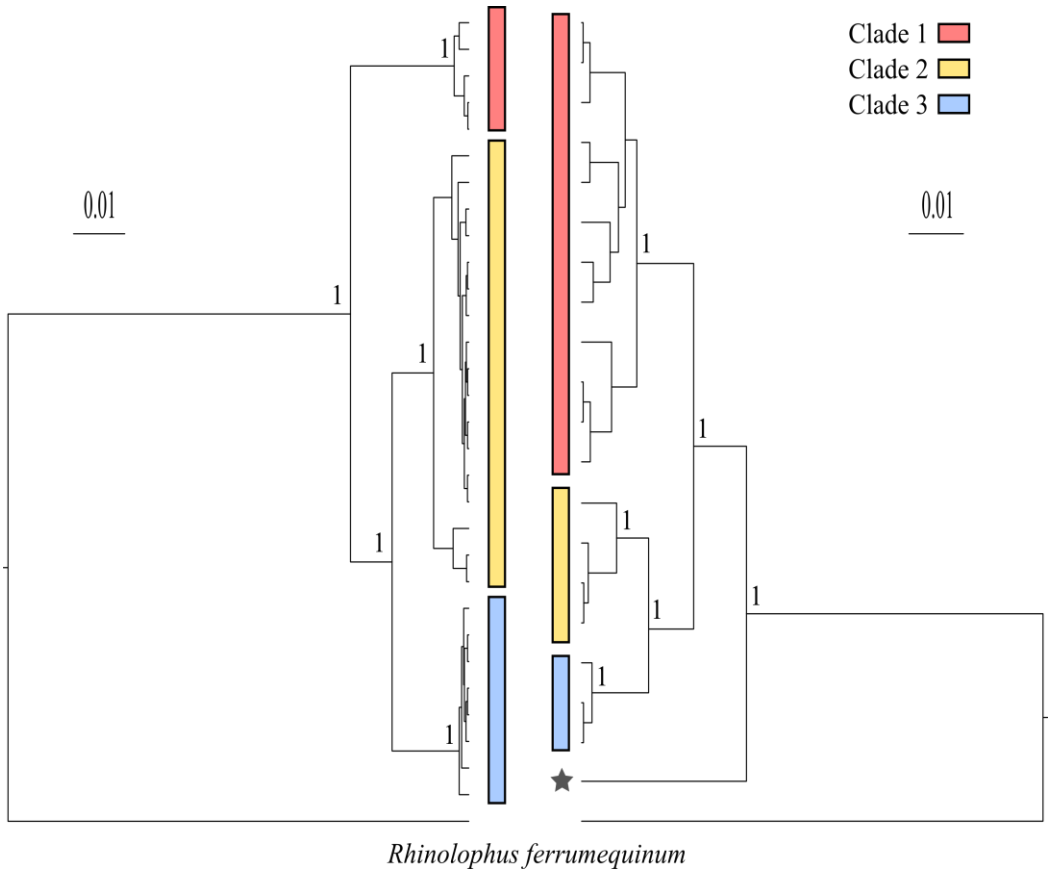

Figure B.1. *Rhinolophus hipposideros*; star denotes a single GenBank sequence from Greece (accession number FJ185200); ND1: HKY; Cytb: HKY+G.

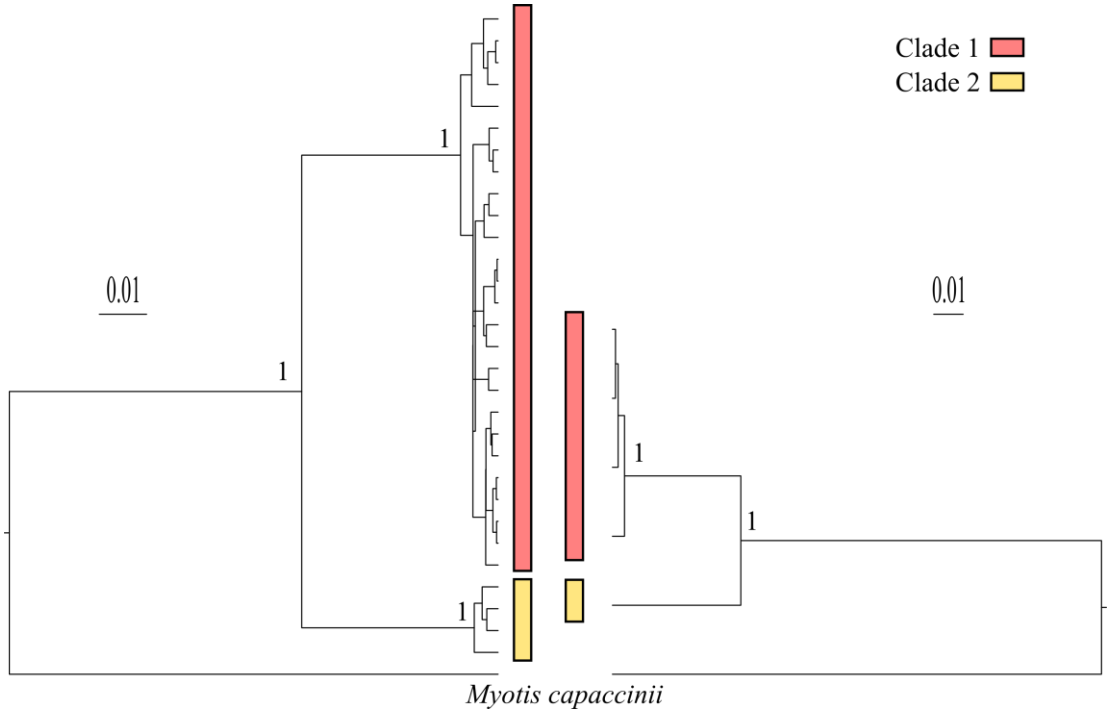

Figure B.2. *Myotis bechsteinii*; ND1: HKY; Cytb: HKY.

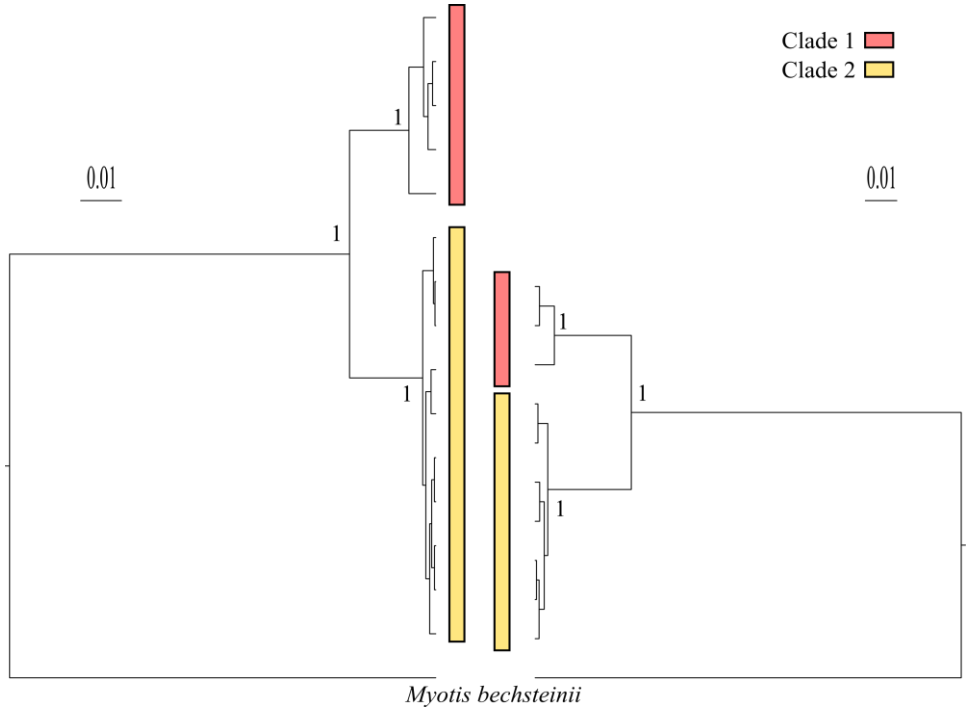

Figure B.3. *Myotis capaccinii*; ND1: HKY; Cytb: HKY.

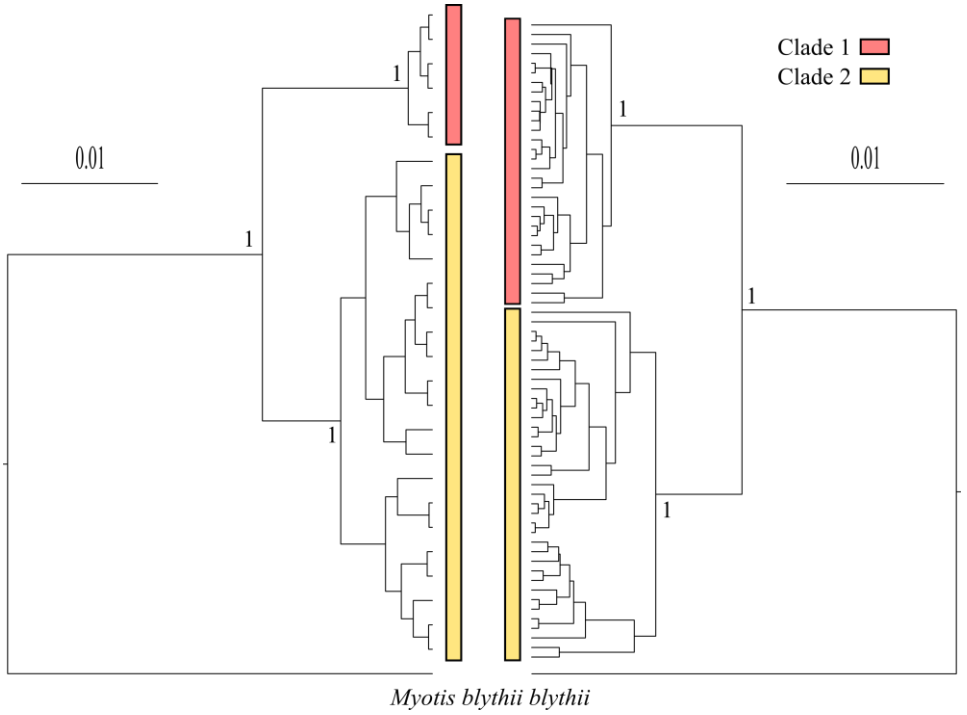

Figure B.4. *Myotis myotis/M. blythii*; ND1: HKY+G; Cytb: HKY.

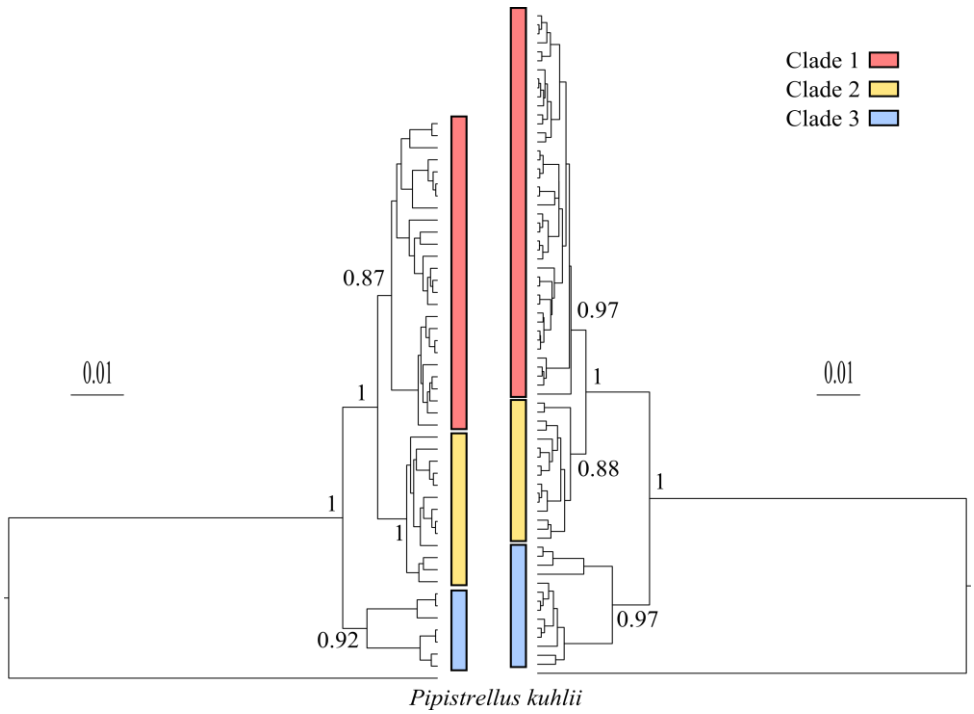

Figure B.5. *Pipistrellus pipistrellus*; ND1: HKY+I; Cytb: HKY.

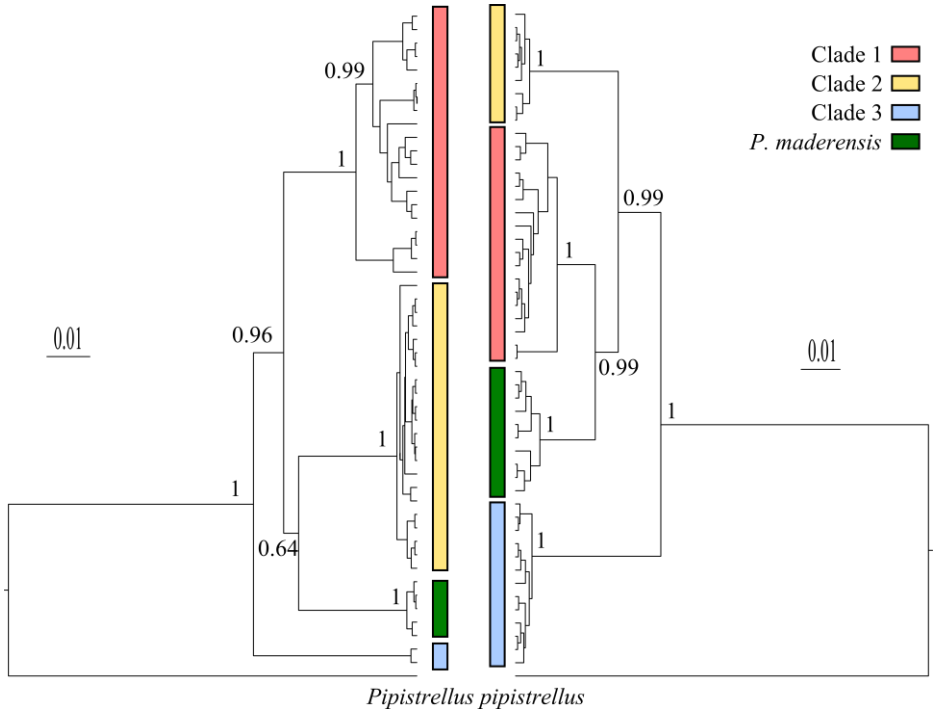

Figure B.6. *Pipistrellus kuhlii*; ND1: HKY; Cytb: HKY+I.

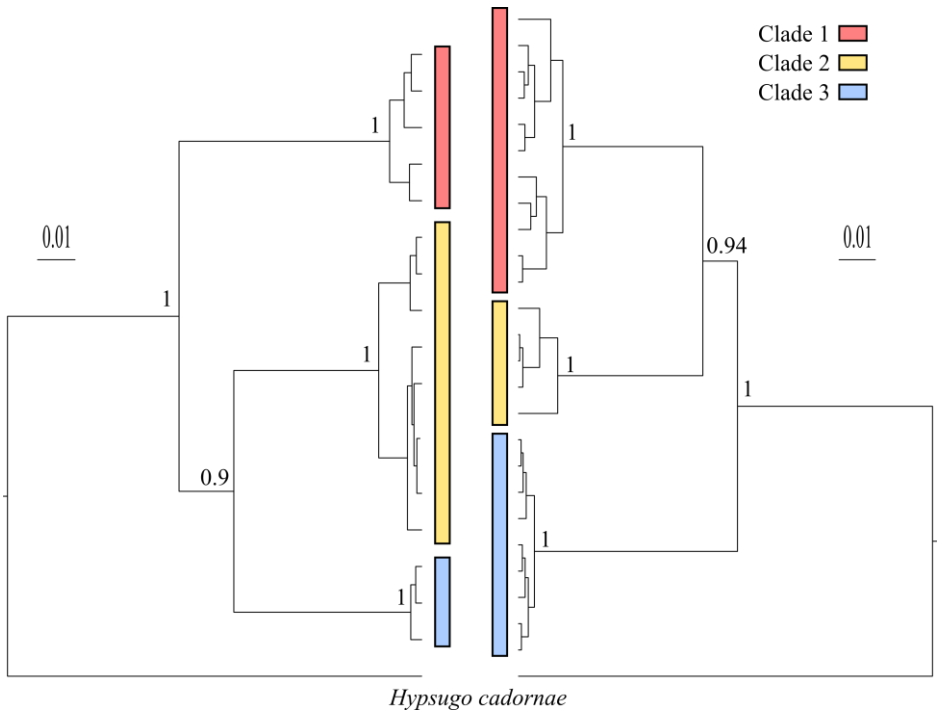

Figure B.7. *Hypsugo savii*; ND1: HKY+G; Cytb: HKY+I.

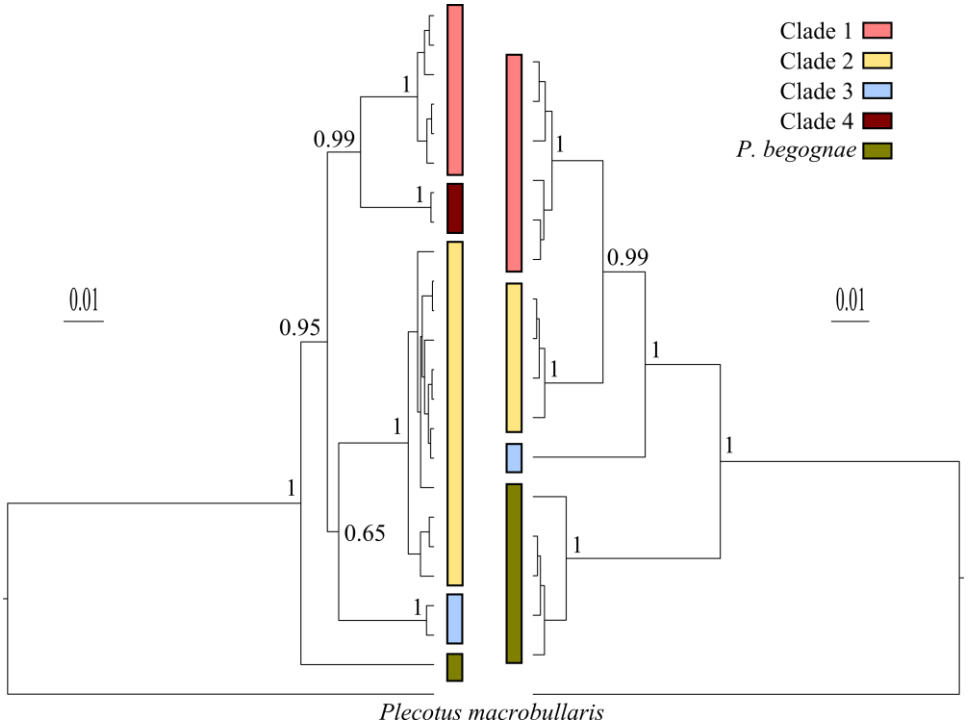

Figure B.8. *Plecotus auritus*; ND1: HKY+G; Cytb: HKY+G.

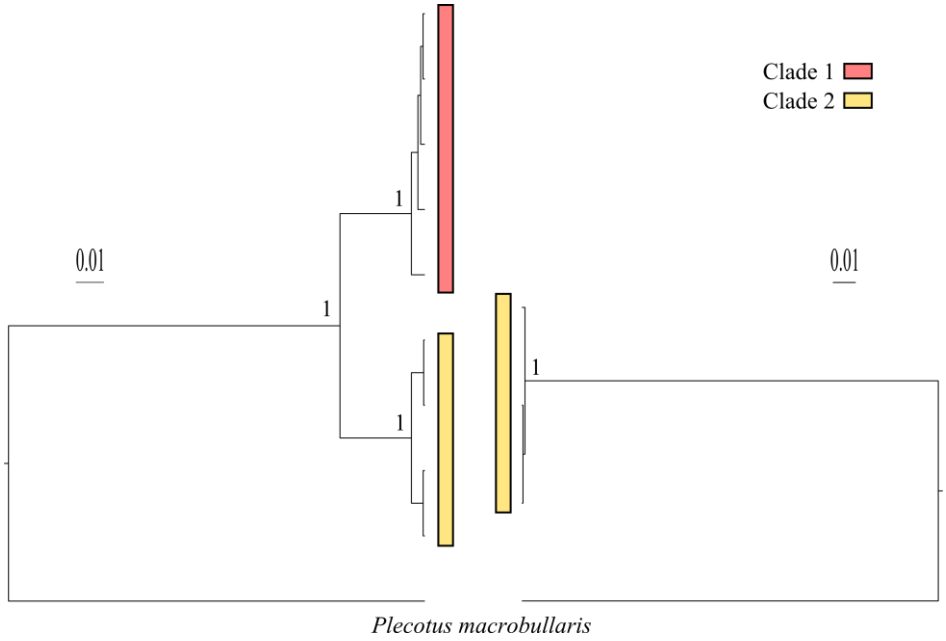

Figure B.9. *Plecotus kolombatovici*; no Cytb sequences are available in GenBank for European populations; ND1: HKY; Cytb: F81.

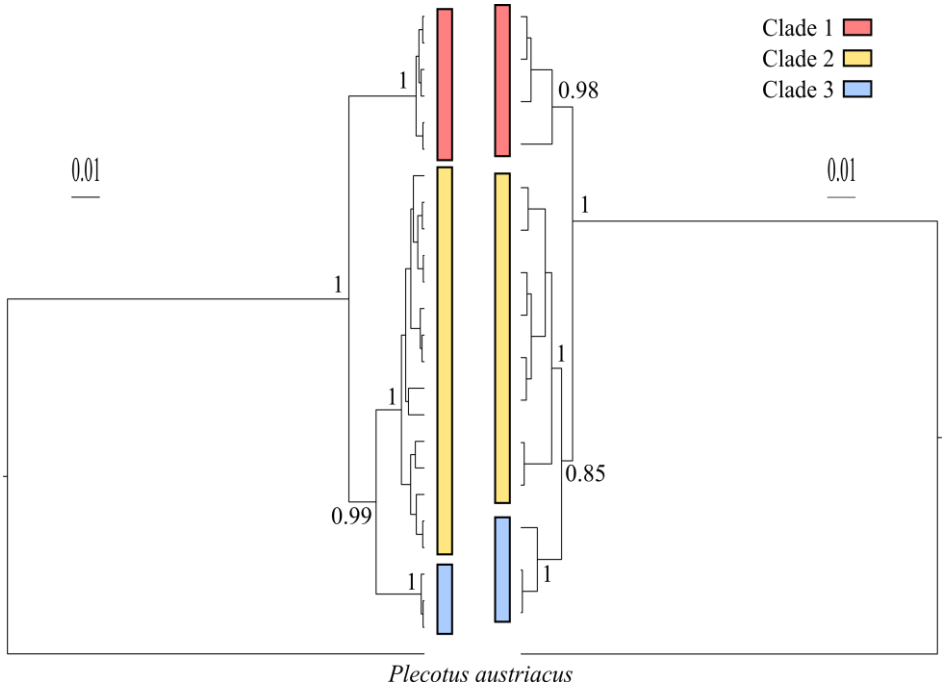

Figure B.10. *Plecotus macrobullaris*; ND1: HKY; Cytb: HKY.

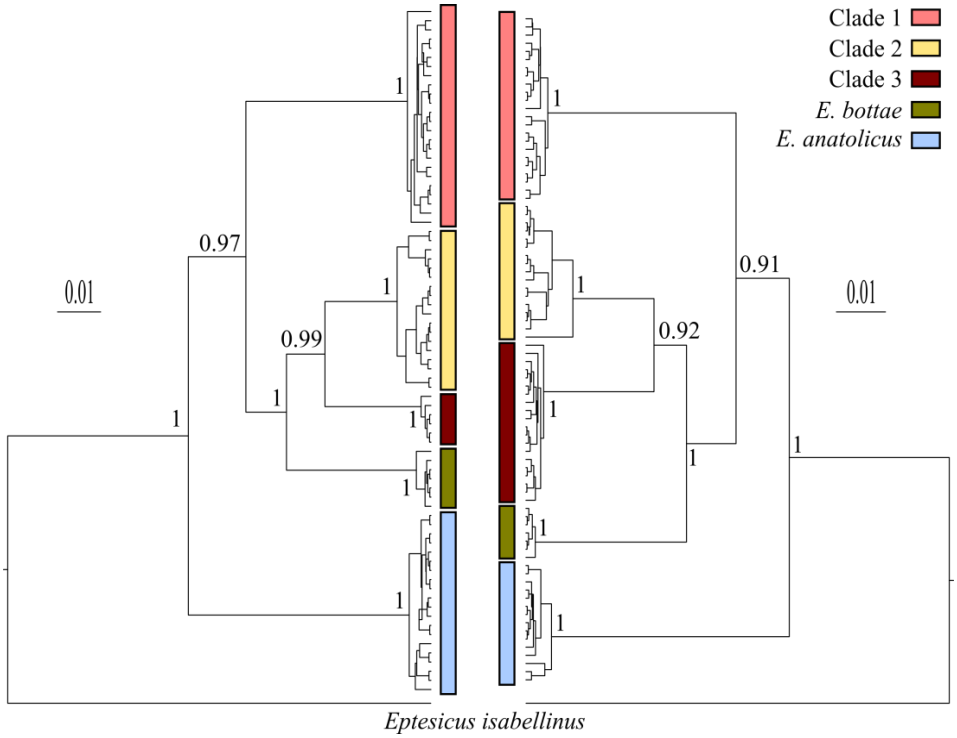

Figure B.11. *Eptesicus serotinus*; ND1: HKY+I; Cytb: HKY+G.

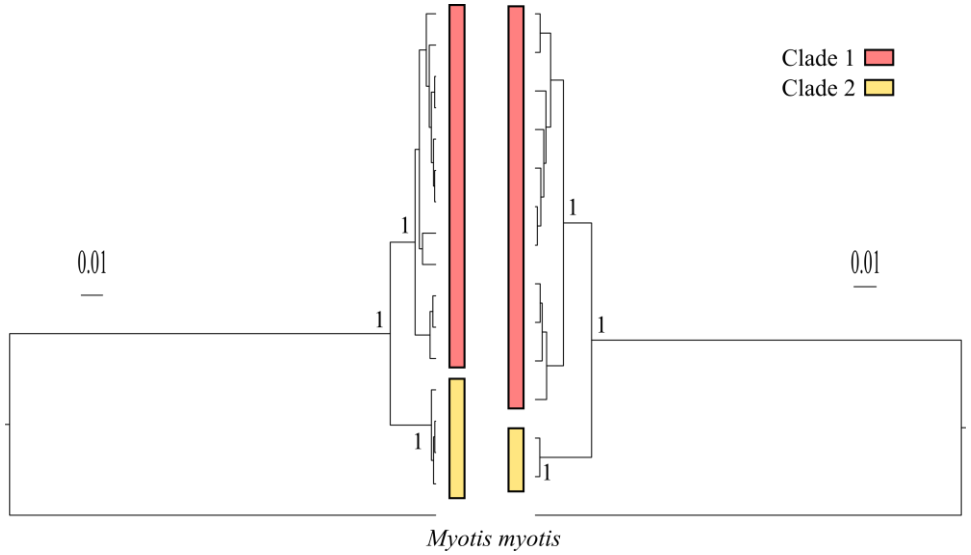

Figure B.12. *Miniopterus schreibersii*; ND1: HKY; Cytb: HKY.

APPENDIX C: NAKED-RUMPED TOMB BAT

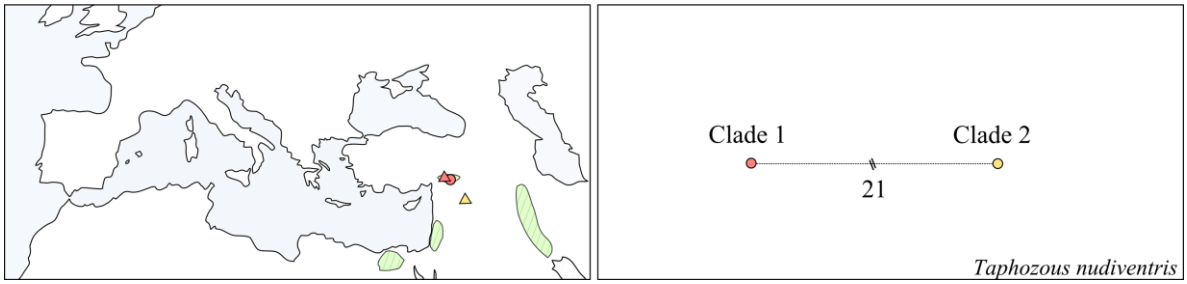

Figure C.1. Distribution of the identified clades in naked-rumped tomb bat, *Taphozous nudiventris* and statistical parsimony networks of unique ND1 haplotypes. Shaded areas show the range of the species, triangles indicate the location of samples with ND1 and Cytb sequences. The distribution map is taken from IUCN Red List of Threatened Species website (http://www.iucnredlist.org). Number in TCS network shows the base pair differences between the identified clades.

APPENDIX D: CRYPTIC SPECIES

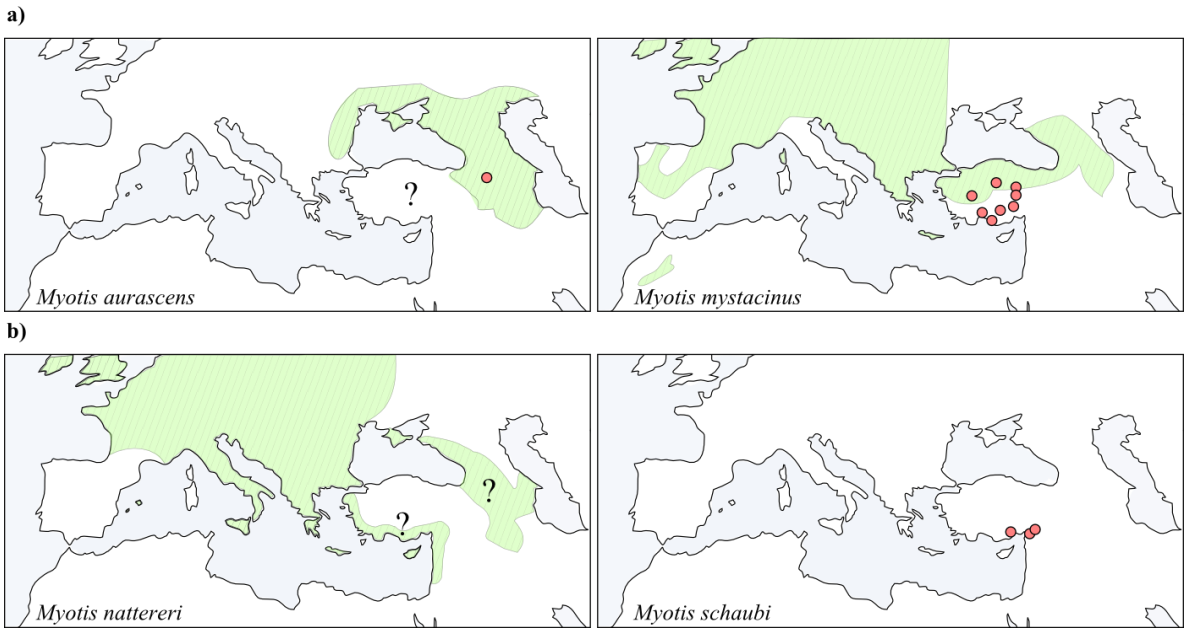

Figure D.1. Cryptic species pairs, *Myotis aurascens* and *M. mystacinus* (a) and *M. nattereri* and *M. schaubi* (b). Molecular identifications revealed the ranges of these cryptic species might be different than currently recognized. Green shaded areas show currently recognized distributions of species and circles indicate genetically confirmed samples from Anatolia. The distribution maps are taken from IUCN Red List of Threatened Species website (http://www.iucnredlist.org), *M. nattereri* map was updated based on the data from Puechmaille et al. (2011) and *M. aurascens* from Dietz et al. (2009). Question marks show areas where the presences of the species are doubtful.

APPENDIX E: BIOCLIM VARIABLES

BIO1 = Annual Mean Temperature

BIO2 = Mean Diurnal Range (Mean of monthly (max temp - min temp))

BIO3 = Isothermality (BIO2/BIO7) (* 100)

BIO4 = Temperature Seasonality (standard deviation *100)

BIO5 = Max Temperature of Warmest MonthBIO6 = Min Temperature of Coldest Month

BIO6 = Min Temperature of Coldest Month

BIO7 = Temperature Annual Range (BIO5-BIO6)

BIO8 = Mean Temperature of Wettest Quarter

BIO9 = Mean Temperature of Driest Quarter

BIO10 = Mean Temperature of Warmest Quarter

BIO11 = Mean Temperature of Coldest Quarter

BIO12 = Annual Precipitation

BIO13 = Precipitation of Wettest Month

BIO14 = Precipitation of Driest Month

BIO15 = Precipitation Seasonality (Coefficient of Variation)

BIO16 = Precipitation of Wettest Quarter

BIO17 = Precipitation of Driest Quarter

BIO18 = Precipitation of Warmest Quarter

BIO19 = Precipitation of Coldest Quarter

APPENDIX F: FUTURE PROJECTIONS FOR CSRIO MK2 MODEL

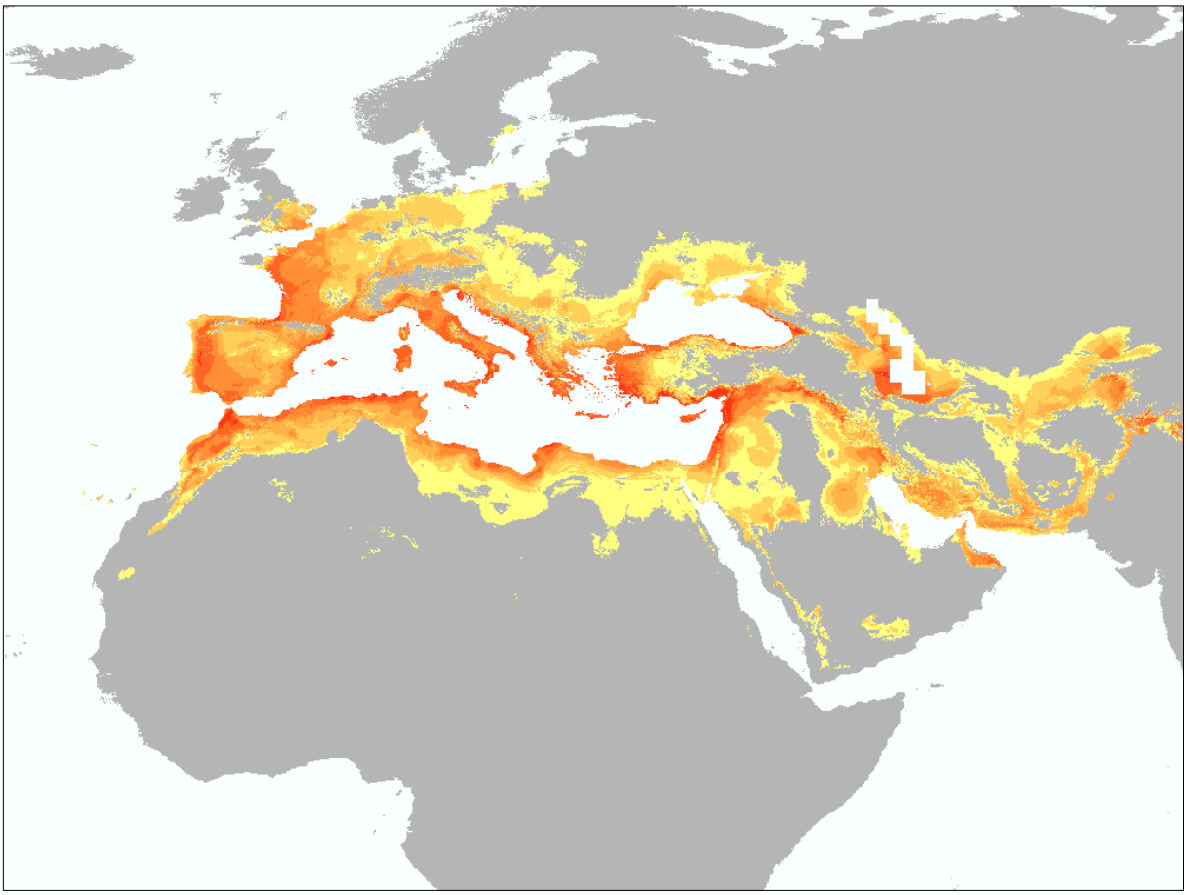

Figure F.1. Combined dataset 2020 projection for CSRIO mk2 ENM model.

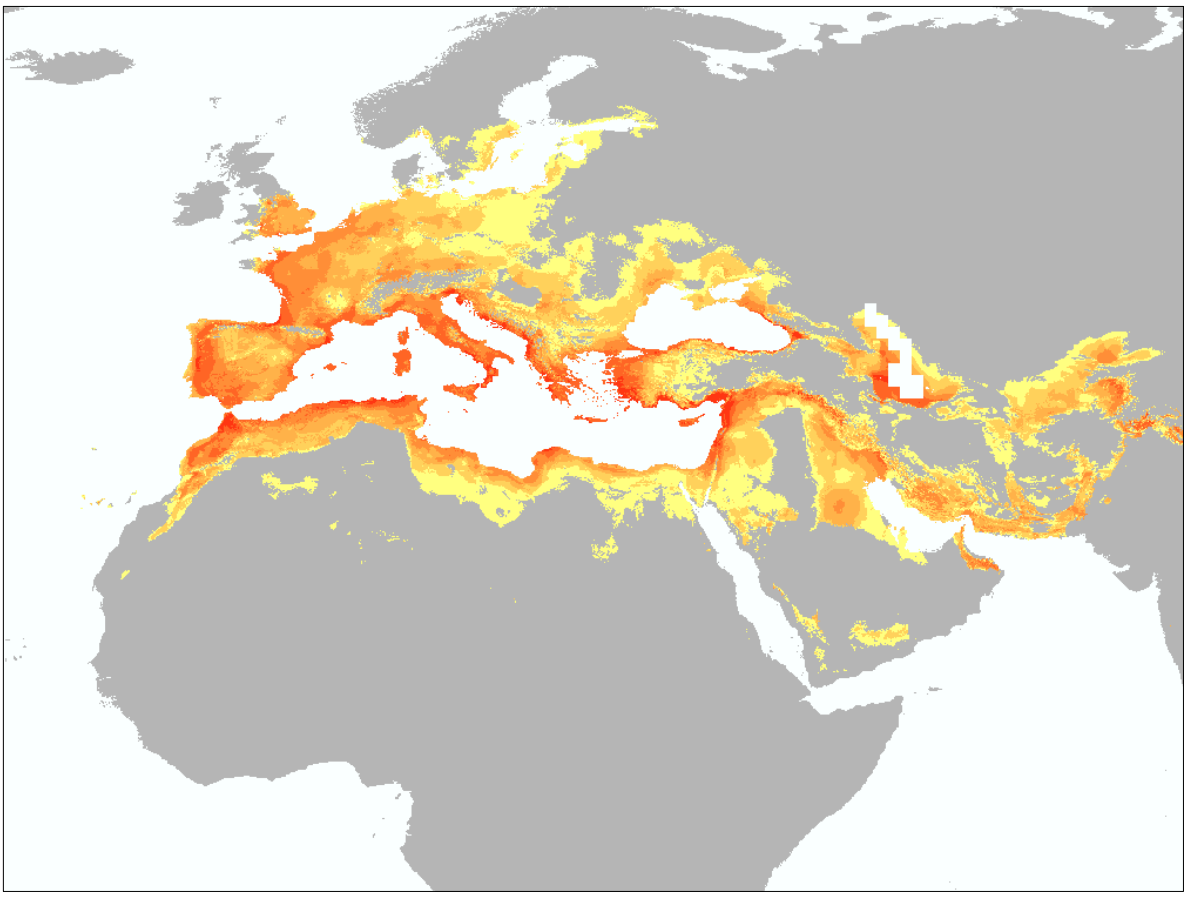

Figure F.2. Combined dataset 2040 projection for CSRIO mk2 ENM model.

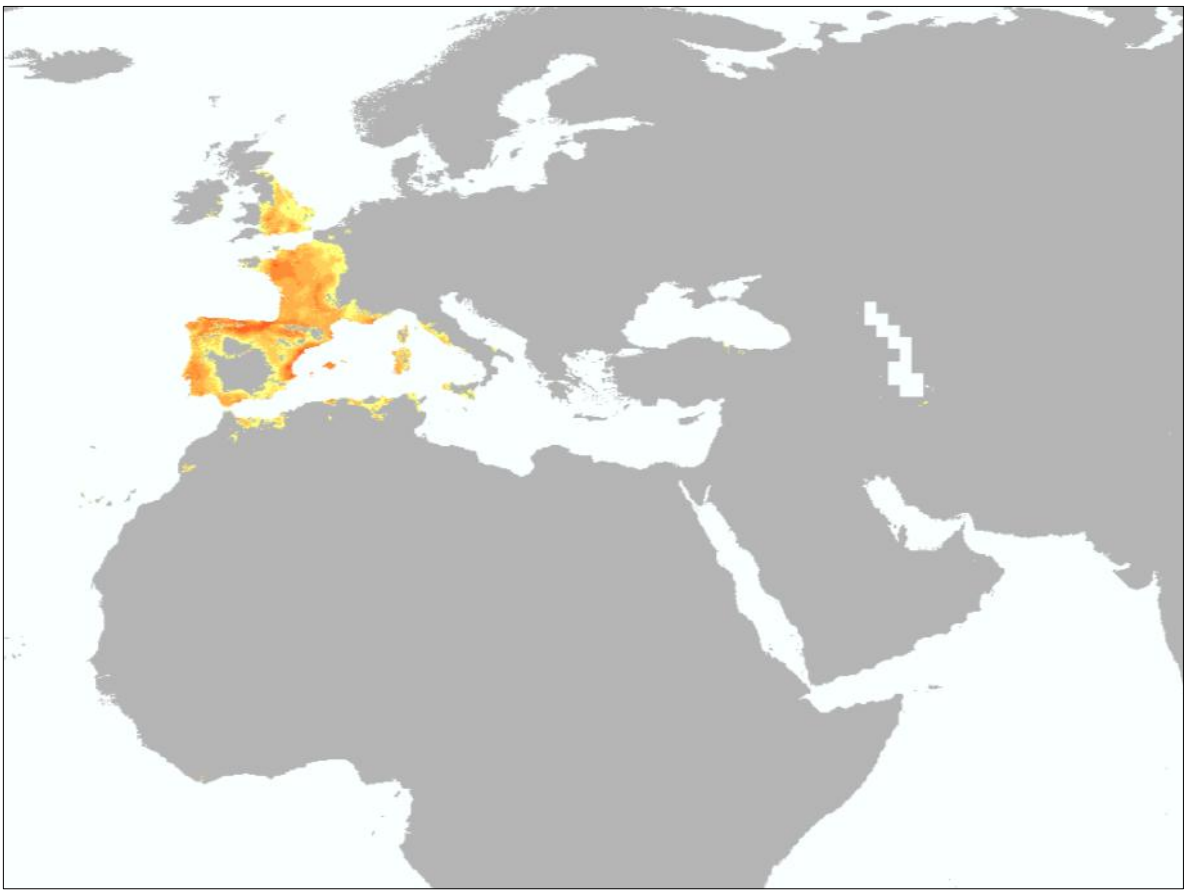

Figure F.3. Clade western 2020 projection for CSRIO mk2 ENM model.

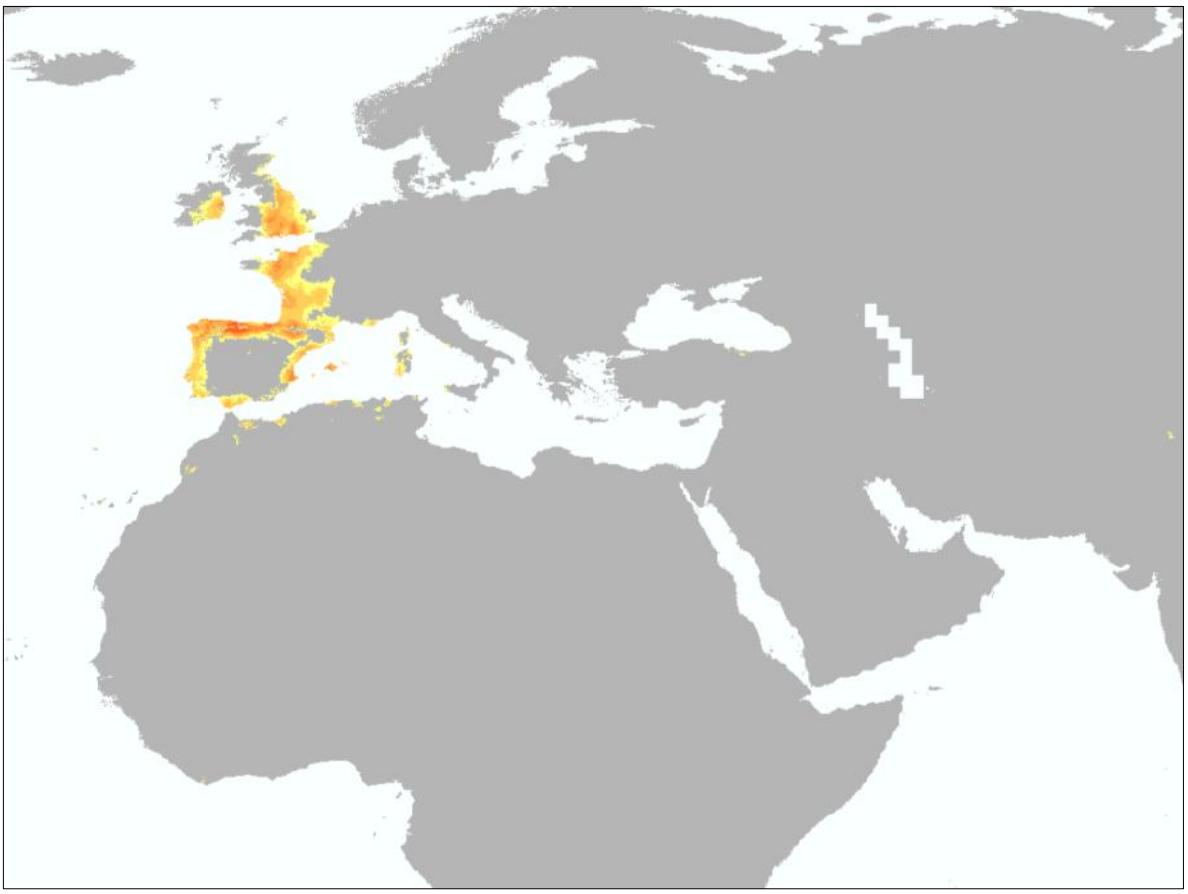

Figure F.4. Clade western 2040 projection for CSRIO mk2 ENM model.

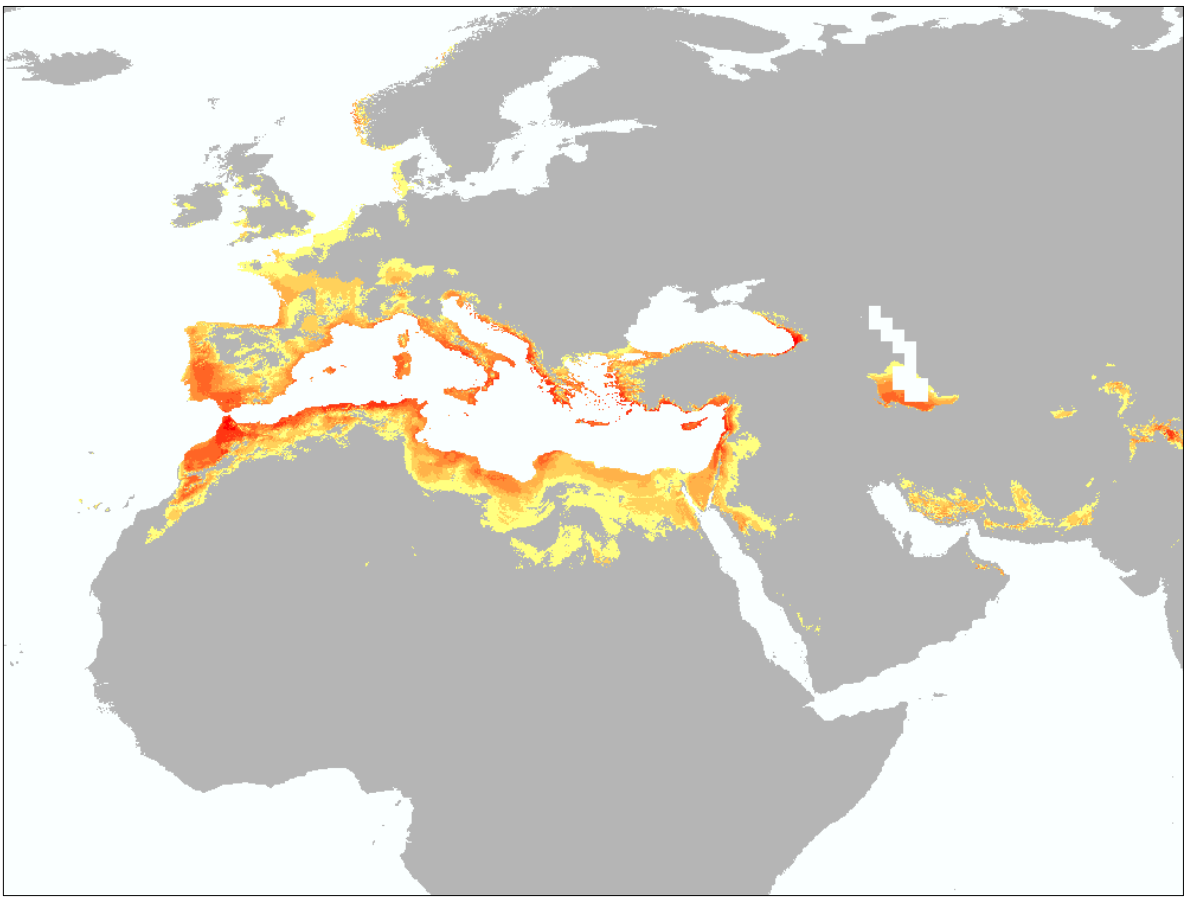

Figure F.5. Clade kuhlii 2020 projection for CSRIO mk2 ENM model.

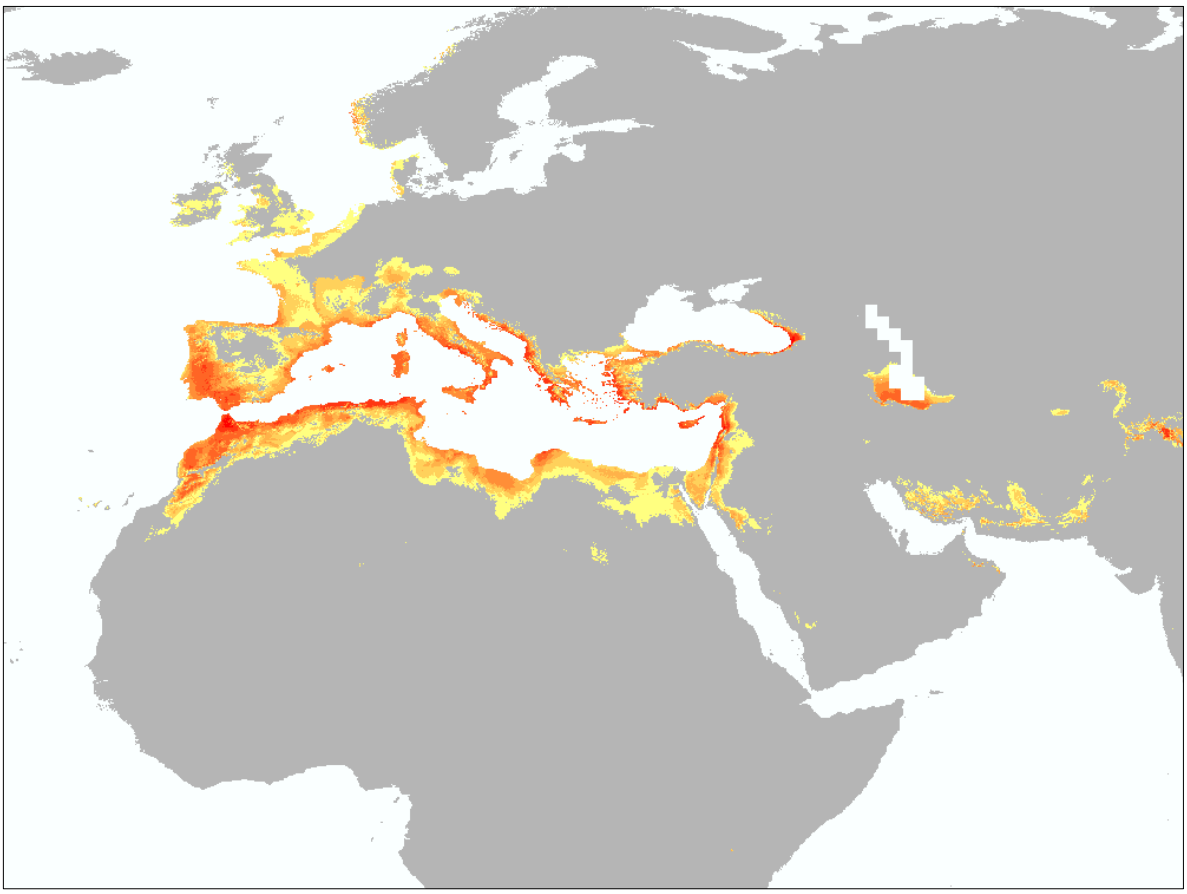

Figure F.6. Clade kuhlii 2040 projection for CSRIO mk2 ENM model.

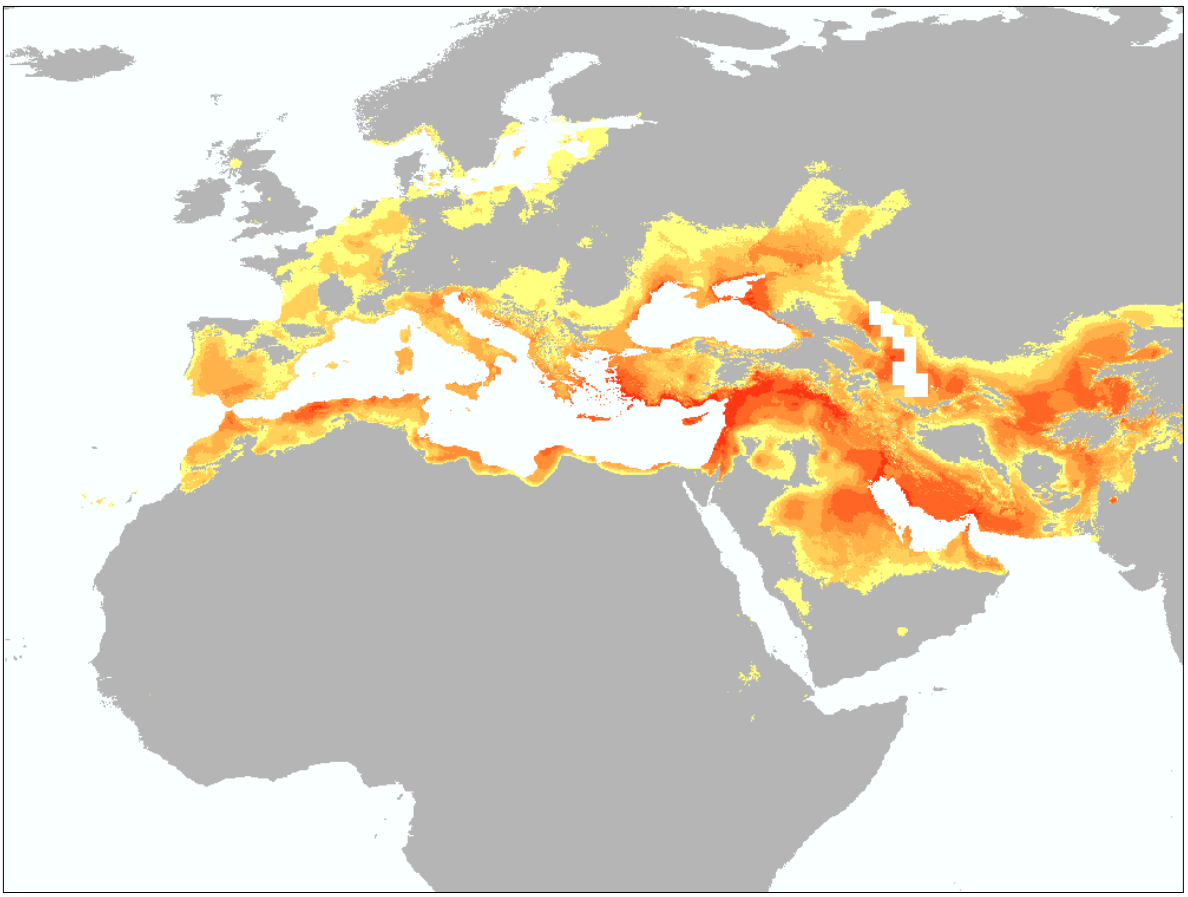

Figure F.7. Clade lepidus 2020 projection for CSRIO mk2 ENM model.

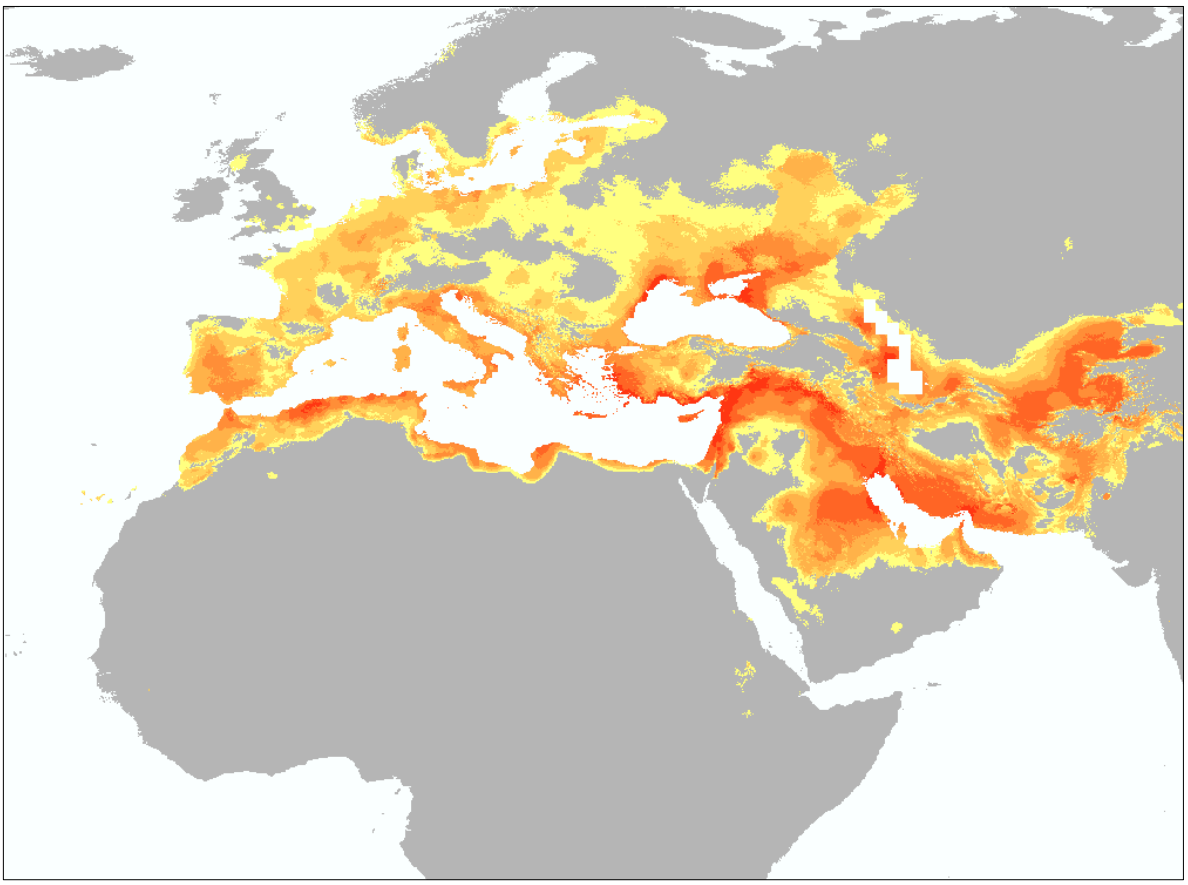

Figure F.8. Clade lepidus 2040 projection for CSRIO mk2 ENM model.

APPENDIX G: NICHE IDENTITIES

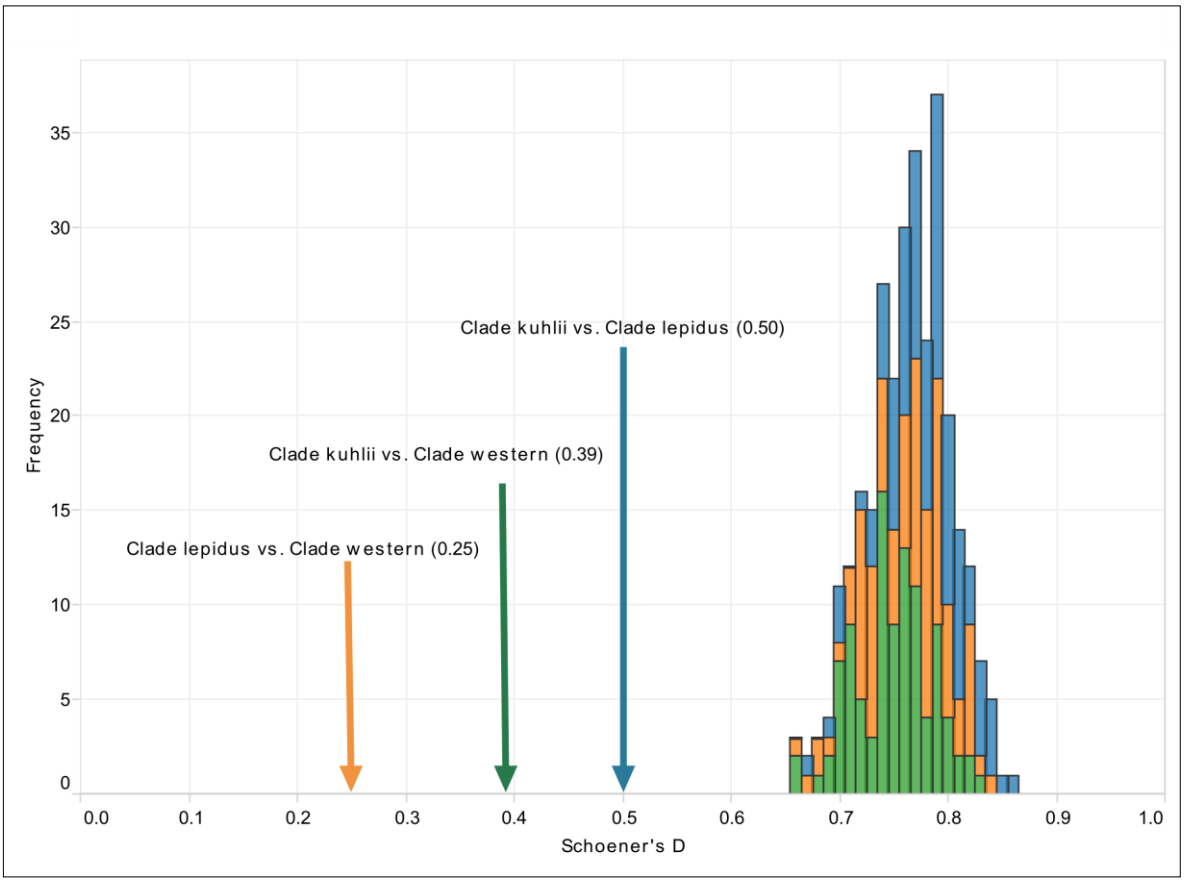

Figure G.1. Niche overlap for values for Schoener's *D* compared to the null distributions. Clade lepidus vs. Clade kuhlii comparisons are shown with green bars, Clade lepidus vs. Clade western with blue, and Clade western vs. Clade kuhlii with orange. The arrows show the similarity scores.

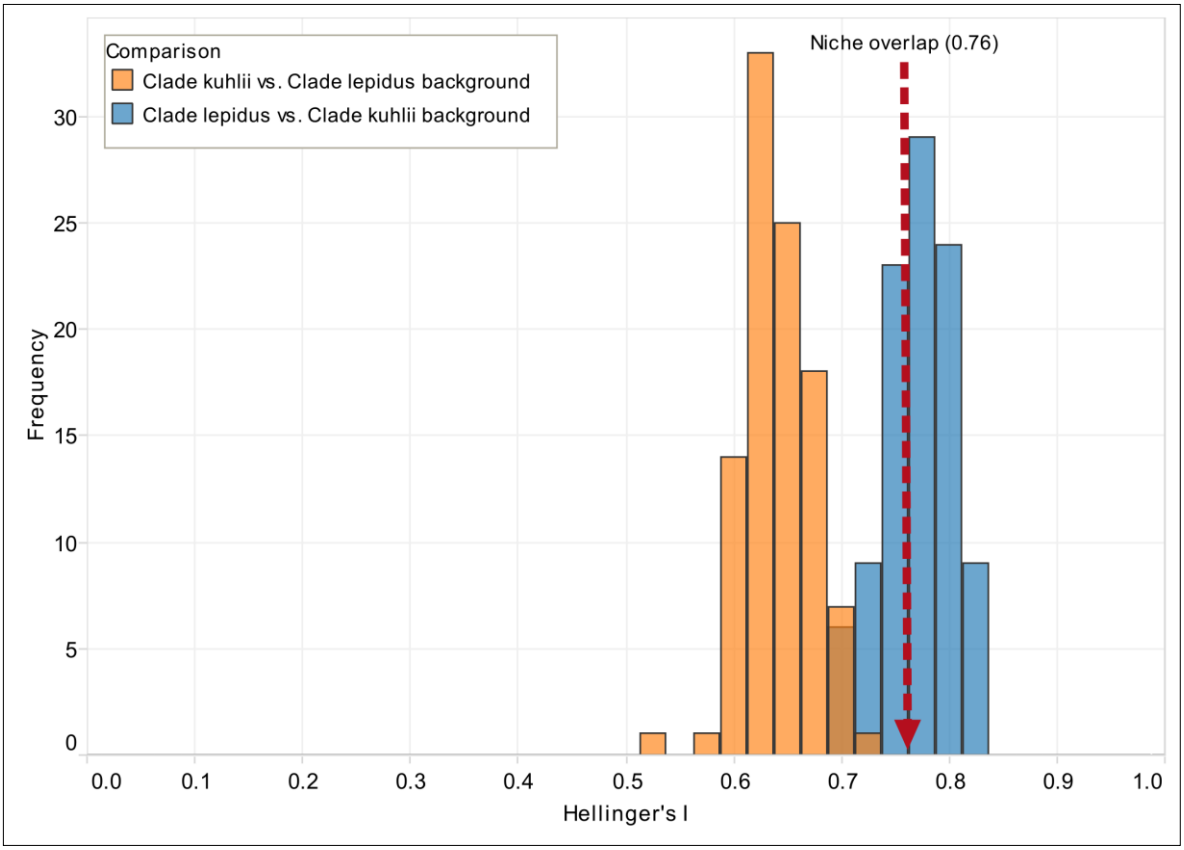

Figure G.2. Background tests for Clade kuhlii vs. Clade lepidus using Hellinger's *I*. Orange bars show Clade kuhlii model run in the Clade lepidus background and blue bars for the opposite. Dashed red line shows the similarity score.

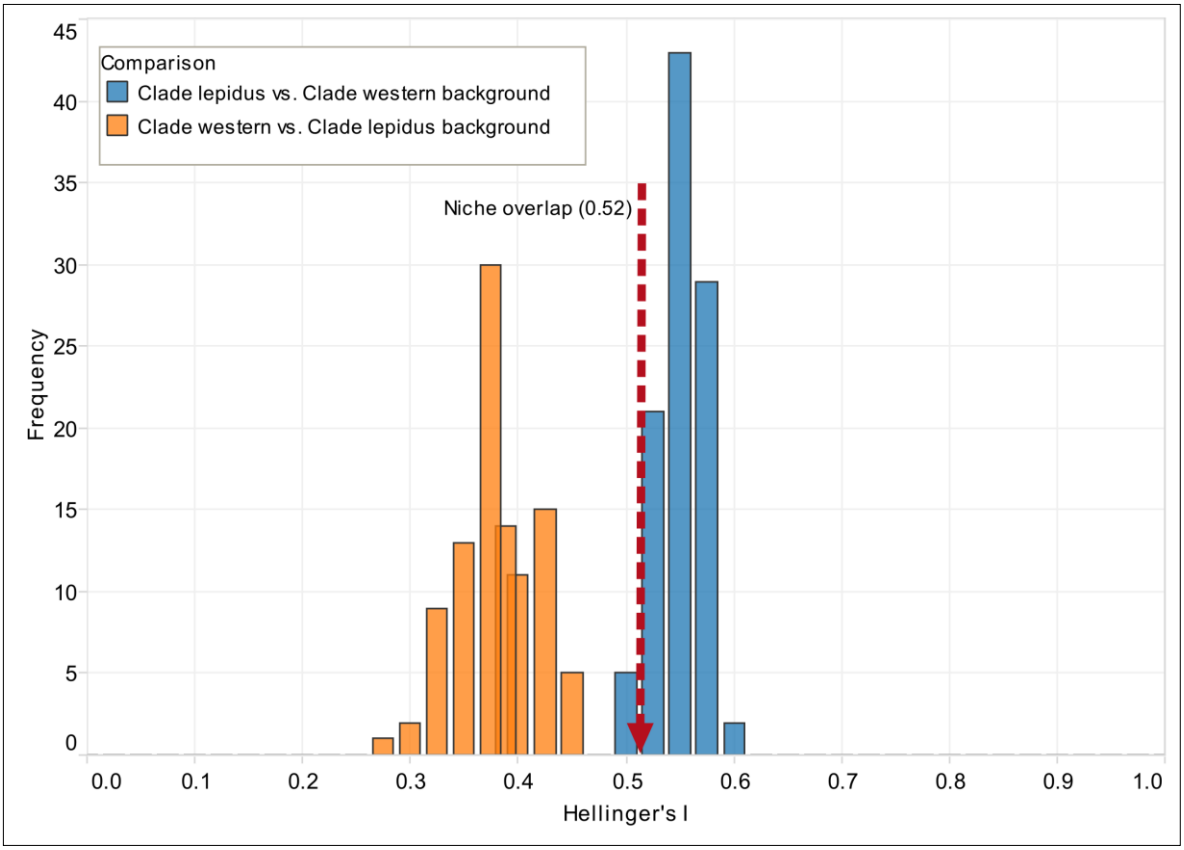

Figure G.3. Background tests for Clade lepidus vs. Clade western using Hellinger's *I*. Blue bars show Clade lepidus model run in the Clade western background and orange bars for the opposite. Dashed red line shows the similarity score.

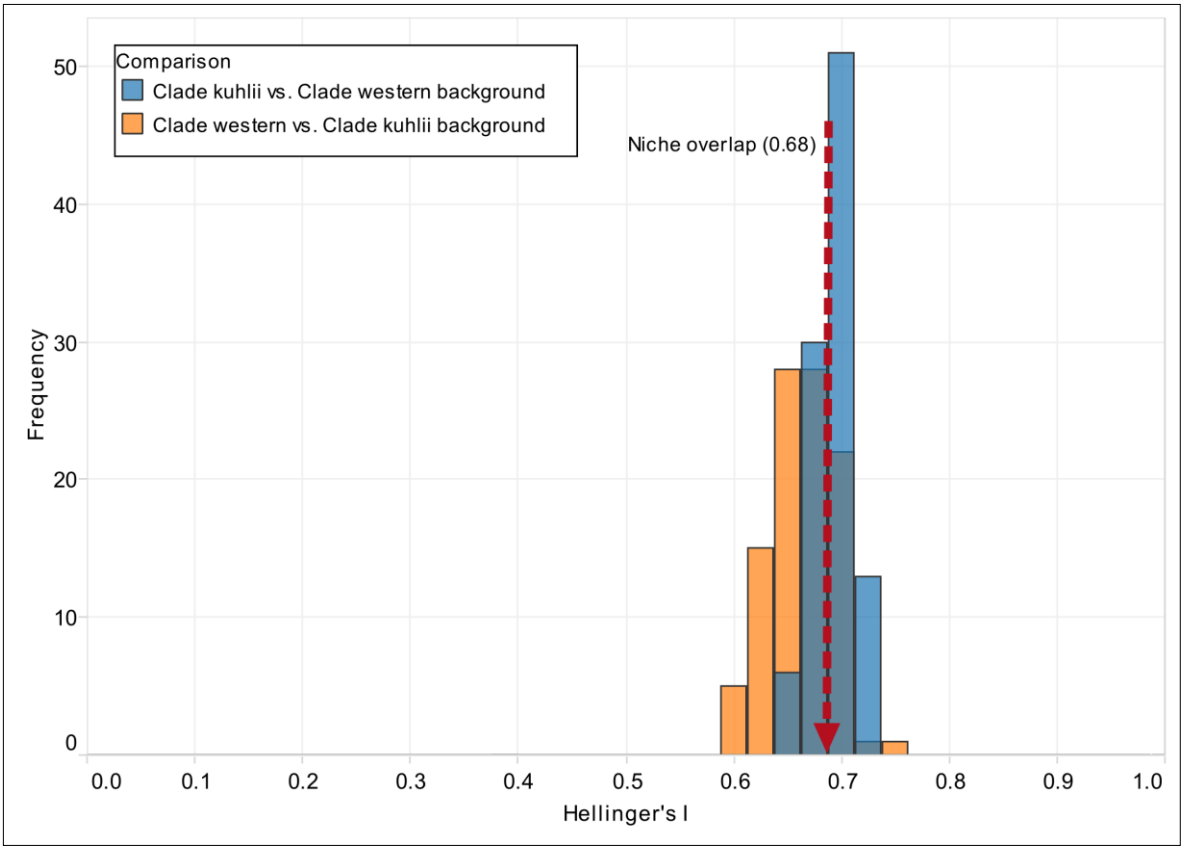

Figure G.4. Background tests for Clade kuhlii vs. Clade western using Hellinger's *I*. Blue bars show Clade western model run in the Clade kuhlii background and orange bars for the opposite. Dashed red line shows the similarity score.