THE PHYLOGEOGRAPHIC HISTORY, TAXONOMY AND CONSERVATION OF TWO CAVE-DWELLING BAT SPECIES: *Miniopterus schreibersii* AND *Myotis capaccinii* (CHIROPTERA: VESPERTILIONIDAE)

by

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ABSTRACT

THE PHYLOGEOGRAPHIC HISTORY, TAXONOMY AND CONSERVATION OF TWO CAVE-DWELLING BAT SPECIES: *Miniopterus schreibersii* AND *Myotis capaccinii* (CHIROPTERA: VESPERTILIONIDAE)

Miniopterus schreibersii and *Myotis capaccinii* are two cave-dwelling bat species with sub-cosmopolitan distributions, which are well-suited for phylogeographic studies at large geographic scales. In this study, the social and genetic structure of *Miniopterus schreibersii* was investigated using nuclear microsatellite and mitochondrial DNA markers, and the taxonomy and phylogeography of *Myotis capaccinii* was examined using mitochondrial DNA data, using samples from the entire circum-Mediterranean range of these species. Local genetic structuring previously detected among populations of *Miniopterus schreibersii* using mitochondrial markers was not observed for microsatellite markers, indicating male-biased dispersal. Some support was found for the fifth paradigm of postglacial expansions in Europe, which states that Anatolia might have been a primary refugium during the last glacial maxima. Regarding *Myotis capaccinii*, all analyses revealed the existence of three haplogroups, B, C1 and C2. The divergence of haplogroup B from C haplogroups was dated to 350,000 years BP and divergence of C1 and C2 to 130,000 years BP, indicating isolation and differentiation during multiple glacial periods. Individuals from haplogroups B and C1 were found sympatrically in the same cave, suggesting they might represent distinct species. Similarly, the C1 – C2 divergence might indicate subspecific differentiation. However, analyses of nuclear markers and phenotypic characters are required to make further subspecific and specific recommendations. Species distribution models predicted a northward movement of potentially suitable areas for *Myotis capaccinii* in the near future due to climate change, thus the implementation of proper environmental measures is of utmost importance for the conservation of this species complex.

ÖZET

MAĞARADA YAŞAYAN İKİ YARASA TÜRÜNÜN, *Miniopterus schreibersii* VE *Myotis capaccinii*'nin (CHIROPTERA: VESPERTILIONIDAE) FİLOCOĞRAFİ TARİHİ, TAKSONOMİSİ VE KORUNMASI

Miniopterus schreibersii ve *Myotis capaccinii* geniş coğrafi ölçekli filocoğrafya çalışmalarına uygun, mağaralarda yaşayan ve yarı kozmopolit dağılım gösteren iki yarasa türüdür. Bu çalışmada bu türlerin tüm Akdeniz Havzası'ndaki dağılım alanından örnekler kullanılarak *Miniopterus schreibersii*'nin sosyal ve genetik yapısı çekirdek DNA mikrosatelit ve mitokondri DNA markörleri yardımıyla, *Myotis capaccinii*'nin taksonomisi ve filocoğrafyasıysa mitokondri DNA verilerinden faydalanılarak incelenmiştir. Daha önce *Miniopterus schreibersii* için mitokondri markörleri kullanılarak tespit edilen yerel genetik yapılanmalar mikrosatelit markörleri için gözlenmemiş olup bu durum erkek güdümlü dağılıma işaret etmektedir. Anadolu'nun son buzul maksimumunda birincil bir sığınak olduğunu öne süren, Avrupa'da buzul çağı sonrası genişlemeleri için önerilen beşinci paradigmaya destek bulunmuştur. *Myotis capaccinii* içinse tüm analizler B, C1 ve C2 haplogruplarının varlığını ortaya koymuştur. Haplogrup B'nin C haplogruplarından ayrışma zamanı günümüzden 350.000 yıl öncesi, C1 ile C2 haplogruplarının ayrışma zamanıysa günümüzden 130.000 yıl öncesi olarak belirlenmiş ve birden fazla buzul çağı sırasında gerçekleşen izolasyon ve farklılaşmaya işaret etmiştir. B ve C1 haplogruplarından bireylerin aynı mağarada simpatrik olarak bulunması bunların farklı türleri temsil ediyor olabileceğini gündeme getirmiştir. Benzer şekilde, C1–C2 ayrışması da başka bir alt tür farklılaşmasına işaret etmektedir. Ancak, daha fazla alt tür ve tür önermesi yapabilmek için çekirdek markörlerinin ve fenotipik karakterlerin analizi de gerekmektedir. Tür dağılım modelleri, yakın bir gelecekte *Myotis capaccinii* için potansiyel yaşanabilir alanların, iklim değişikliği sebebiyle kuzeye kayacağını ön görmüştür, bu yüzden de uygun çevresel tedbirlerin yürürlüğe konulması bu tür kompleksinin korunması için son derece önemlidir.

TABLE OF CONTENTS

ACKNOWLEDGEMENTS..... iii
ABSTRACT..... v
ÖZET..... vi
TABLE OF CONTENTS..... vii
LIST OF FIGURES..... ix
LIST OF TABLES..... xv
LIST OF SYMBOLS/ABBREVIATIONS..... xvii
1. INTRODUCTION..... 1
1.1. Phylogeography of *Miniopterus schreibersii*..... 4
1.2. Social Structure of Bats and Cytonuclear Discordance..... 7
1.3. Taxonomy and the Evolutionary History of *Myotis capaccinii*..... 9
1.4. Conservation and Contact Zones..... 11
1.5. Modeling the Future Distributions of Phylogenies: *Myotis capaccinii*..... 16
1.6. Comparative Ecology and Morphology of *Miniopterus schreibersi*i and *Myotis capaccinii*..... 21
1.7. The Objectives of the Study..... 23
2. MATERIALS AND METHODS..... 25
2.1. Analyses for *Miniopterus schreibersii*..... 25
2.1.1. Sampling..... 25
2.1.2. Laboratory Analyses..... 27
2.1.3. Statistical Analyses..... 27
2.2. Analyses for *Myotis capaccinii*..... 29
2.2.1. Sampling..... 29
2.2.2. Laboratory Analyses..... 30
2.2.3. Statistical Analyses..... 30
3. RESULTS..... 36
3.1. Analysis results for *Miniopterus schreibersii*..... 36
3.1.1. Genetic Differentiation and Clustering..... 36
3.1.2. Phylogeography..... 40
3.2. Analysis results for *Myotis capaccinii*..... 43
3.2.1. Phylogeography..... 43
3.2.2. Modelling the present, past and future distributions of *M. capaccinii*..... 58

4. DISCUSSION.....69
4.1. *Miniopterus schreibersii*.....69
4.1.1. Social Structure of *Miniopterus schreibersii*: A Continental Perspective.....69
4.1.2. Anatolia As The Single Refugium For *Miniopterus schreibersii*..... 71
4.2. *Myotis capaccinii*.....74
4.2.1. Phylogeography of *Myotis capaccinii*.....74
4.2.2. Taxonomy and conservation of *Myotis capaccinii*.....79
4.2.3. Future SDM Projections.....80
4.3. A comparison of postglacial expansion patterns of *Miniopterus screibersii* and *Myotis capaccinii* from an phylogeographic and ecological perspective..... 82
5. CONCLUSION..... 86
5.1. *Miniopterus schreibersii*.....86
5.2. *Myotis capaccinii*.....86
5.3. *Miniopterus schreibersii* & *Myotis capaccinii* comparison: How does biology affect phylogeography and conservation?.....87
REFERENCES.....88
APPENDIX A: A TABLE OF F_{IS} VALUES BETWEEN POPULATIONS OF *Miniopterus schreibersii*……..... 122
APPENDIX B: 692 BP LONG mtDNA CYT-B SEQUENCES OF *Myotis capaccinii* INDIVIDUALS SEQUENCED IN THIS STUDY……123
APPENDIX C: SUPPLEMENTARY FIGURES FOR *Myotis capaccinii* ANALYSES…….. 131

LIST OF FIGURES

Figure 1.1. The two bat species that will be investigated in this thesis study (a) The Common Bent-wing Bat, *Miniopterus schreibersii* and (b) the Long-fingered Bat, *Myotis capaccinii*.....4

Figure 2.1. Map of sampling locations for *Miniopterus schreibersii.* The numerical and color codes correspond to those on Table 2.1.....25

Figure 2.2. Map of sampling locations for *Myotis capaccinii*. The map and color codes correspond to those on Table 2.2.....31

Figure 3.1. F_{ST} values for 38 populations (excluding Sicily and Kocakuyu populations, from which only one individual was sampled) and 12 regions. The color codes of the regions correspond to those on Figure 2.1 & Table 2.1. The significance for these F_{ST} comparisons can be found in Figure 3.2.....37

Figure 3.2. Matrix of F_{ST} significance. The dark orange squares correspond to F_{ST} *P* values that are significant at 0.05 level.....37

Figure 3.3. Slatkin's linearized F_{ST}.....38

Figure 3.4. Matrix of F_{ST} significance. The dark orange squares correspond to FST P values that are significant at 0.05 level.....40

Figure 3.5. Allelic patterns across regions. Na is the number of different alleles, CA is the number of common alleles with a frequency ≥ 5%, Ne is the number of effective alleles, I is Shannon's Information Index, R is the allelic richness (N=17), RP is the private allelic richness (N=17) and He is the expected heterozygosity.....42

Figure 3.6. An ABC analysis of 172 *Miniopterus schreibersii* individuals genotyped at 10 microsatellite loci and one mtDNA locus. a) All 15 possible different scenarios (=rooted trees) were compared. The results of a logistic regression and the predicted probabilities of all 15 scenarios regarding four geographical regions, which are obtained using b) only mtDNA data and c) mtDNA and nuDNA data together, are shown…… 43

Figure 3.7. The haplotype network for 265 cyt-b sequences (692 bp) and their distribution based on geographical regions. The sizes of the circles are proportional to the frequency of the haplotypes they represent. The small dots stand for missing haplotypes. Another version of this network, which is based on 38 populations instead of geographical regions, is given in Appendix C……..…….....46

Figure 3.8. The split zone between haplogroups B and C1. The sizes of the circles are proportional to the population sizes…….. 47

Figure 3.9. The split zone between haplogroups C1 and C2. The sizes of the circles are proportional to the population sizes. The mountains extending along the France-Italy border, as well as the northern borders of Italy are the Alps, whereas the Apennine Mountains stretch from the northwest to the southeast of Italy. The Apennines, along with the Alps, act as a geographic barrier separating haplogroups C1 and C2. The mountains separating France from Iberia are the Pyrenees…….…….....47

Figure 3.10. The Bayesian phylogenetic tree of 17 cyt-b haplotypes of *M. capaccinii*. The posterior probabilities are given as percentages on the nodes. The units on the reverse scale axis are years……. 48

Figure 3.11. The neighbor-joining tree for *M. capaccinii*. The bootstrap support values are given on the nodes…….....48

Figure 3.12. The maximum likelihood tree for *M. capaccinii*. The bootstrap support values are given on the nodes……..……..49

Figure 3.13. The maximum parsimony tree for *M. capaccinii*. The bootstrap support values are given on the nodes……..……..49

Figure 3.14. The divergence tree of the most common haplotypes of three haplogroups of *M. capaccinii* obtained using a strict molecular-clock with a rate of 4.8% divergence per million years. The units on the reverse scale axis are years…….....50

Figure 3.15. The divergence tree of the most common haplotypes of three haplogroups of *M. capaccinii* obtained using an uncorrelated relaxed clock and *M. ricketti* as an outgroup. The units on the reverse scale axis are million years……..... 51

Figure 3.16. F_{ST} values for 38 populations and three haplogroups of *M. capaccinii*. The color codes of the haplogroups correspond to those on Figure 1……..... 52

Figure 3.17. The significance of the F_{ST} values. The color codes of the haplogroups correspond to those on Figure 1…… 53

Figure 3.18. Nei's distance and average number of pairwise differences within and between 38 populations of *M. capaccinii*……..53

Figure 3.19. The haplotype distance matrix of 265 *M. capaccinii* samples……... 54

Figure 3.20. The observed and expected mismatch distributions in constant size and expanding population models for *M. capaccinii* haplogroups B, C1 and C2. The x-axis shows the number of pairwise differences between cyt-b haplotypes, and the y-axis represents the frequency of these differences……... 55

Figure 3.21. Bayesian skyline plots for *M. capaccinii* haplogroups (a) B, (b) C1 and (c) C2…... 56

Figure 3.22. An ABC analysis of all 273 *M. capaccinii* individuals genotyped at cyt-b. a) All three possible scenarios (=rooted trees) regarding the ancestral relationship of haplogroups B, C1 and C2 were compared. The probabilities predicted by (b) direct estimation and (c) logistic regression are shown……. 57

Figure 3.23. The predicted current distribution of haplogroup B……...59

Figure 3.24. The predicted current distribution of haplogroup C1…….....60

Figure 3.25. The predicted current distribution of haplogroup C2……....60

Figure 3.26. The predicted distribution of haplogroup B during the LGM according to the Community Climate System Model (CCSM) general circulation model (GCM)……. 61

Figure 3.27. The predicted distribution of haplogroup B during the LGM according to the Model for Interdisciplinary Research on Climate Earth System Model (MIROC) GCM…….. 61

Figure 3.28. The predicted distribution of haplogroup C1 during the LGM according to the CCSM GCM……...62

Figure 3.29. The predicted distribution of haplogroup C1 during the LGM according to the MIROC GCM……...62

Figure 3.30. The predicted distribution of haplogroup C2 during the LGM according to the CCSM GCM……...63

Figure 3.31. The predicted distribution of haplogroup C2 during the LGM according to the MIROC GCM……...63

Figure 3.32. The predicted distribution of haplogroup B in 2070 according to the CCSM GCM……...64

Figure 3.33. The predicted distribution of haplogroup B in 2070 according to the MIROC GCM……...64

Figure 3.34. The predicted distribution of haplogroup C1 in 2070 according to the CCSM GCM……...65

Figure 3.35. The predicted distribution of haplogroup C1 in 2070 according to the MIROC GCM……...65

Figure 3.36. The predicted distribution of haplogroup C2 in 2070 according to the CCSM GCM……...66

Figure 3.37. The predicted distribution of haplogroup C2 in 2070 according to the MIROC GCM……...66

Figure 3.38. Three Maxent models for the potential distribution of three haplogroups of *M. capaccinii*. Maps in the first panel are calculated using bioclimatic variables for the current climatic conditions. Maps of suitable regions in the last glacial maximum (LGM) are obtained using the Model for Interdisciplinary Research on Climate (MIROC) or the Community Climate System Model (CCSM) for (a) haplogroup B, (b) haplogroup C1 and (c) haplogroup C2.……...67

Figure 3.39. Three Maxent models for the potential distribution of three haplogroups of *M. capaccinii*. Maps in the first panel are calculated using bioclimatic variables for the current climatic conditions. Maps of suitable regions in 2070 are obtained using the Model for Interdisciplinary Research on Climate (MIROC) or the Community Climate System Model (CCSM) for (a) haplogroup B, (b) haplogroup C1 and (c) haplogroup C2..... 67

Figure 3.40. A reclassified map of the potential distribution of *M. capaccinii*. The green areas indicate areas where suitable conditions exist in the present alone, whereas blue areas stand for areas where suitable conditions existed in the LGM (or will exist in 2070) alone. The red areas are stable regions where suitable areas are predicted for both the present and the LGM (or 2070)…….……..... 68

Figure 4.1. Ice age terminations. The green columns represent the time of termination for each glacial period, whereas the periods just before them (on their right), where the peaks are at minimum are the glacial maxima just before the termination of that period (Cheng et al., 2009)..... 76

Figure 4.2. The potential postglacial expansion routes of *Miniopterus screibersii* after the LGM. The possible location of glacial refugia were drawn according to the phylogeographic hypothesis suggested in Bilgin et al. (2016). The dark pink area represents the east of the Levantine Sea, the primary refugium for *M. schreibersii* during the LGM. Part of Anatolia, which might have acted as a secondary refugium as suggested in Bilgin et al. (2016), is shown in light pink. Bidirectional arrows were used, where the direction of expansion was not certain……..... 83

Figure 4.3. The potential postglacial expansion routes of *Myotis capaccinii* after the LGM. The possible location of glacial refugia were drawn according to the MAXENT predictions. The coloring of the potential refugia is random, whereas the color codes of the sample haplogroups correspond to those in Table 3.4. As mentioned in the text, the primary refugia for the *M. capaccinii* haplogroup C2 might have been Italy or Iberia, even though Italy is more likely to be so, and that is why both are shown as potential candidates using bidirectional arrows..... 84

LIST OF TABLES

Table 2.1. Sampling sites for 12 defined regions of *Miniopterus schreibersii*. N stands for the number of samples……..... 26

Table 2.2. Sampling sites for *Myotis capaccinii*. N stands for the number of samples. The color codes representing different haplogroups correspond to those on Table 3.3……31

Table 2.3. The climatic predictors that were used in SDMs. All were downloaded from Worldclim (www.worldclim.org) with a 5 arc-minute resolution. The variables that were used in the final SDMs are color-coded with green……..…… 34

Table 2.4. A list of all major analyses and the software used for them..... 35

Table 3.1. The pairwise F_{ST} values between regions, with those significantly greater than zero ($P<$ 0.05) shown in bold. The first table, (a), was produced using mtDNA data, whereas in (b), microsatellite data were used. Different individuals from the same populations were sampled for the mtDNA and microsatellite analyses. T & B, stands for the Trace & Balkans region, B. Sea for the Black Sea and N. Afr. for North Africa..... 39

Table 3.2. Descriptive statistics of *Miniopterus schreibersii* in 12 regions. N is the sample size, Na is the number of different alleles, CA is the number of common alleles with a frequency ≥ 5%, Ne is the number of effective alleles, I is Shannon's Information Index, R is the allelic richness (N=17), R_P is the private allelic richness (N=17), F_{IS} is the inbreeding coefficient and H_E is the expected heterozygosity. * indicates regions that are not in HWE and ** indicates the statistical significance of F_{IS} ($P<0.05$)……..... 41

Table 3.3. HP-Rare analysis results. N is the sample size, R is the allelic richness; R_P is the private allelic richness. * indicates regions that are not in HWE..... 42

Table 3.4. Descriptive statistics for the *Myotis capaccinii* samples. HG is the haplogroup, h is the haplotype diversity, π is the nucleotide diversity, SV is the number singleton variable sites, PI is the number of parsimony informative sites and r is Harpending's raggedness index. Koufovouno is not included in Thrace B and Thrace C1 geographical regions. The significant values are in bold, where one asterisk signifies (P<0.05) and two asterisks signify (P<0.02). The color codes representing different haplogroups correspond to those in Table 2.2.…… 44

LIST OF SYMBOLS/ABBREVIATIONS

Symbol	Explanation	Unit
µl	Microliter	
π	Nucleotide Diversity	
F_{ST}	Fixation Index	

Abbreviation	Explanation
A	Adenine
bp	Base pairs
C	Cytosine
Cyt-b	Cytochrome B
D-loop	Displacement Loop
DNA	Deoxyribonucleic Acid
DNTP	Deoxyribonucleotide Triphosphate
G	Guanine
G	Gamma Distribution
GTR	General Time Reversible
h	Number of Haplotypes
Hd	Haplotype Diversity
HKY	Hasegawa-Kishino-Yano
I	Proportion of Invariable Sites
IPCC	Intergovernmental Panel for Climate Change
IUCN	International Union for Conservation of Nature
K	Average Number of Nucleotide Differences
kyr	Thousand years
mtDNA	Mitochondrial Deoxyribonucleic Acid
MU	Management Unit
n	Number of Tissue Samples
nM	Nanomolar
PCR	Polymerase Chain Reaction
Sd	Standard Deviation
T	Thymine

1. INTRODUCTION

The study of phylogeography deals with the genetic relationships within and among species in a context of the geographical history and tries to explain the effects of historical events on the current distribution and genetic structuring of populations (Avise 1989, 1994, 2000; Knowles and Maddison, 2002). Glacial periods are among the most significant geographical events that drastically affect the phylogeography of species, and glacial refugia, in which many species have survived during and between glacial periods, have played a very important role in the evolution and distribution of many European taxa (Hewitt 1999, 2004a; Horacek, 2002; Veith et al., 2003; Graham et al., 1996; Rossiter et al., 2007). The populations' contraction during the glacial maxima and their postglacial expansions, accompanying differentiation in allopatric isolation from other populations and the following secondary contacts and possibility of gene flow, have been significant factors in shaping the genetic structure of these and many other species (Avise, 2000).

The Iberian, Italian and Balkan peninsulas served as refugia for many species prior to their expansion into continental Europe after the Last Glacial Maximum (LGM; 23 000–18 000 years before present) (Rebelo et al., 2012). Because of the varying effects of geographical obstacles on the dispersal ability of different species, five different paradigms of repopulation were defined (Hewitt, 1999; Habel et al., 2005; Bilgin et al., 2016). According to the grasshopper paradigm, the refugial populations in the Balkan Peninsula recolonized Europe, whereas the Iberian and Italian populations were trapped by the Pyrenees and Alps, respectively. The hedgehog paradigm, on the other hand, states that all refugial population from these three peninsulas expanded rather simultaneously. The bear paradigm describes a pattern, where the refugial populations from Iberian and Balkan peninsulas expand after the LGM and meet in Central Europe, as the Italian populations are confined to their own peninsula by the Alps (Hewitt, 1999). The butterfly paradigm generalizes those species, whose Iberian populations were not able to expand because of the Pyrenees, whereas their Balkan and Italian populations recolonized the rest of Europe (Habel et al., 2005). These four paradigms, account for the expansion patterns of multiple species, including bats (Schmitt, 2007; Ruedi & Castella, 2003; Rossiter et al., 2007; Puechmaille et al., 2012; Dool et al., 2013). Finally, a fifth paradigm, oak gallwasp, states another pattern, where instead of the Iberian, Italian or Balkan peninsulas, Anatolia was the primary refugium for the repopulations of Europe after the LGM, while populations of the

species that fit this paradigm went extinct during the LGM in other European refugia (Bilgin et al., 2016).

Like many other species, many bat species also survived in these three southern European refugia and Anatolia during the LGM (Petit et al., 1999a; Ruedi & Castella, 2003; Rossiter et al., 2007; Puechmaille et al., 2012; Dool et al., 2013; Razgour et al., 2013, Bilgin et al., 2016). Many studies showed that the postglacial expansion patterns of bats were in consistence with the five expansion paradigms. As mentioned above, the continental scale study of Bilgin et al. (2016) on the phylogeography of the common bent-wing bat, *Miniopterus schreibersii* provided support for and suggested the fifth (oak gallwasp) paradigm using predominantly mitochondrial markers. The Natterer's bat, *Myotis nattereri* species complex, on the other hand, was shown to fit the grasshopper paradigm (Salicini et al., 2013), yet there are minor differences from the paradigmatic model (see discussion). Other bat species that clearly fit the grasshopper paradigm, in which the postglacial colonization originates in the Balkan Peninsula are the lesser horseshoe bat, *Rhinolophus hipposideros* (Dool et al., 2013), the Bechstein's bat, *Myotis bechsteinii* (Kerth et al., 2008; Rebelo et al., 2012) and the common noctule bat, *Nyctalus noctula* (Petit et al., 1999a; Ruedi & Castella, 2003). Unlike these species, the barbastelle bat (*Barbastella barbastellus*) fits the hedgehog paradigm, where all three European refugia contribute to the postglacial expansion significantly (Rebelo et al., 2012). The greater horseshoe bat (*Rhinolophus ferrumequinum*) is also a likely fit to the hedgehog paradigm (Rossiter et al., 2007). However, the exact pattern is less clear for this species, and the bear and butterfly paradigms are also possible, meaning only one of the two western refugia, namely Iberia and Italy might have contributed to the postglacial colonization alongside the Balkans (Flanders et al., 2009; Rebelo et al., 2012). Finally, the greater mouse-eared bat (*Myotis myotis*) likely follows the bear paradigm, where the two main contributors of its genetic constitution in Europe at both mitochondrial and nuclear level are the Iberian and Balkan peninsulas (Ruedi & Castella, 2003; Ruedi et al., 2008).

There are, of course exceptions to these patterns of distribution and postglacial expansion. A clear example of this is the grey long-eared bat, *Plecotus austriacus*, for which Iberia alone was the refugium from which all European populations of the species emerged (Razgour et al., 2013). Additionally, for some bat species it is difficult to determine if any of the postulated paradigms fits the postglacial distribution pattern of the species, and it is very likely that none of them are in fact applicable, because some species respond to climatic oscillations in novel ways (e.g., the alpine long-eared bat, *Plecotus macrobullaris*, pipistrelle bats including the common pipistrelle, *Pipistrellus*

pipistrellus, the soprano pipistrelle, *Pipistrellus pygmaeus* and Hanaki's dwarf bat, *Pipistrellus hanaki*) (Alberdi et al., 2015; Hulva et al., 2010).

In this thesis study, the taxonomy and the evolutionary history of two bat species, namely, the common bent-wing bat, *Miniopterus schreibersii* and the long-fingered bat, *Myotis capaccinii* were investigated (Figure 1.1) using molecular approaches that employ both mitochondrial and nuclear markers (Bilgin et al., 2008a, 2008b, 2008c; Furman et al., 2010a, 2010b, 2010c). Furthermore, a species distribution model (SDM) was utilized in order to deduce possible phylogeographic changes of *M. capaccinii* in the near future using mitochondrial data and available projections of climate change (Knowles et al., 2007; Cordellier & Pfenninger, 2009). A comprehensive study on a species requires a thorough investigation of its phylogeography, and its relations with its abiotic and biotic environment, (Bohmann et al., 2011; Clare et al., 2009). Such studies, as well as the studies that mainly focus on the contact and transition zones of species, are crucial for a reasonably accurate determination of the future distributions of species and to be able to design and implement much more efficient conservation strategies (Simberloff et al., 1998; Bangert et al., 2005). Another significant contribution of this study will be to provide insights for conservation planning efforts by detecting sites/roosts of high genetic diversity.

Of the two bat species that were investigated in this study, *Miniopterus schreibersii* is a species, whose evolutionary history was already thoroughly studied, previously (Appleton et al., 2004; Bilgin et al., 2006, 2008a, 2012, 2016; Furman et al., 2009, 2010a, 2010b, 2010c). There are still some questions that need to be answered regarding its phylogeography in some parts of its known distribution area (Furman et al., 2010a, 2010c). The outcomes of this thesis regarding *Miniopterus schreibersii* are a natural follow-up to previous research, whose details are discussed below. *Myotis capaccinii*, on the other hand, is a relatively less intensively studied species (Albayrak and Aşan, 2002; Bilgin et al., 2008b) and mitochondrial data were analyzed to obtain results that will partly fill the gap in the relevant literature on this bat species, again discussed in greater detail below. In the following sections of the introduction, firstly, the literature on the phylogeography and taxonomy of *Miniopterus schreibersii* and *Myotis capaccinii* will be explained and the possible contributions of this thesis study will be discussed. Next, the social structure of mammals, and specifically that of the bats, and its effect on their genetic structure will be described briefly, and the literature on the SDMs and their conservation implications will be reviewed. After a brief comparison of several aspects of the ecology and morphology of these two bats, which may drastically affect their dispersal ability and

thus their phylogeographic histories, finally, in the last section of the introduction, the main objectives of the study will be presented.



Figure 1.1. The two bat species that will be investigated in this thesis study (a) The common bent-wing bat, *Miniopterus schreibersii* and (b) the long-fingered bat, *Myotis capaccinii*.

1.1. Phylogeography of *Miniopterus schreibersii*

In the first part of this thesis study, the taxonomy, the evolutionary history and conservation of the cave-dwelling bat species, the common bent-wing bat, *Miniopterus schreibersii* (terra typica: Kolumbacs cave, Romania – Kuhl 1817) will be investigated. This species has a sub-cosmopolitan distribution, however cryptic species of *M. schreibersii* have been recently described, effectively decreasing its population size (Appleton *et al.*, 2004; Tian *et al.*, 2004; Simmons, 2005; Miller-Butterworth *et al.*, 2005; Furman *et al.*, 2010b; Bilgin *et al.*, 2012; Puechmaille *et al.*, 2014). Two of these species are *Miniopterus pallidus* (terra typica: South coast of Caspian Sea, Iran - Thomas, 1907) and *Miniopterus maghrebensis* (terra typica: Keff Aziza Cave, Morocco - Puechmaille *et al.*, 2014), and *M. schreibersii* is known to live in the same caves sympatrically with *M. pallidus* in Southern Anatolia and *M. maghrebensis* in North Africa.

M. schreibersii was once thought to be a single species with an exceptionally high dispersal capability and a resulting subcosmopolitan distribution (Simmons, 2005). However, more recent molecular studies, in addition to more thorough morphological analyses of some phenotypic features,

including the forearm length and echolocation calls, showed that *M. schreibersii* was in fact a group of distinct taxa, some of which were species themselves (Appleton et al., 2004; Miller-Butterworth et al. 2005; Bilgin et al. 2006, 2008a, 2012; Furman et al. 2009, 2010a, 2010b). For instance, *M. oceanensis*, which was once classified as *M. schreibersii oceanensis* is now a separate species (Tian et al., 2004; Appleton et al. 2004; Appleton, 2008). Similarly, the Natal long-fingered bat (*M. natalensis*, Smith, 1834) was once regarded as a subspecies of *M. schreibersii*, but now it is considered a distinct species, namely *M. natalensis* (Simmons, 2005; Miller-Butterworth et al., 2005). Another example is *M. pallidus*, a taxon that is phenotypically very similar to *M. schreibersii.* Recent phylogenetic and morphological studies have shown, however, that this taxon is significantly different from *M. schreibersii*, both genetically and phenotypically (Furman et al., 2010a, 2010b) and the discovery of caves, in which individuals from these two lineages were living in sympatry, was the final line of evidence required to designate these taxa as two separate species (Bilgin et al., 2012). The same is true for *M. maghrebensis*, individuals of which are found inhabiting the same caves in North Africa along with individuals of *M. schreibersii*, and it was shown recently that there are sufficient genetic and morphological divergence between the two taxa to accept *M. maghrebensis* as a distinct species (Puechmaille et al., 2014). Bearing in mind the possibility of similar taxonomic changes in the near future, it can be stated that the current range of the nominotypical, Palearctic-Ethiopian *M. schreibersii* complex inhabits Europe, Northern Africa and Anatolia (Hutson et al., 2008a).

There are several possible scenarios regarding the phylogeographic history of *M. schreibersii* (Bilgin et al., 2008a; Bilgin et al., 2016; Ramos Pereira et al., 2009; Furman et al. 2010c). According to Ramos Pereira et al. (2009), the western populations of *M. schreibersii* expanded northward from Portugal, an expansion that had begun in the Late Pleistocene and was completed before the Last Glacial Maximum (LGM; 23 000–18 000 years before present) (Rebelo et al., 2012). Furthermore, they noted the possibility that the populations in southern Iberia might not have survived the former glacial periods and thus they suggested that the actual glacial refugium from which the expansion of the Western European populations had begun might have been Northern Africa. Furman et al. (2010c), however, suggested that the Northwestern Anatolian, specifically Thracian, population was the ancestral stock and that the expansion of this ancestral population took place shortly after the LGM. The higher haplotype diversity in the Anatolian populations and the relatively small overall genetic differentiation between the western (Portugal) and eastern (Bulgaria, Georgia, Greece, Portugal, and Turkey) colonies supports these conclusions (Furman et al., 2010c). They also suggest that the Asia Minor was recolonized by the populations from Thrace, furthermore, they underline the

possibility of the existence of two other Anatolian refugia, where *M. schreibersii* might have survived the LGM, and that it might have recolonized the Asia Minor from these refugia, namely Central Anatolia & Mediterranean and the Black Sea, in addition to Thrace. The previous study of Bilgin et al. (2008a) also deals with the eastern populations of the species and it also suggests that the Thracian populations might be the ancestral populations for the eastern colonies and the timing of the expansion was around 15.5 kyr before present (BP). Yet, in this study, the authors concluded that the most ancestral haplotype was restricted to the Balkans for 6 kyr and then expanded and dispersed to the coasts of the Black Sea and the Mediterranean, differing from the suggestions of Furman et al. (2010c), which stated that the same haplotype expanded immediately after the LGM.

In the most recent study on the phylogeography of *M. schreibersii*, and also using an environmental niche modelling (ENM) approach, Bilgin et al. (2016) concluded that, contrary to the suggestions of previous studies, an area to the east of the Levantine Sea (coasts of Syria, Lebanon, Israel and north of Egypt) might have been the primary refugium for *M. schreibersii* during the LGM. Their results suggest that Anatolia might have been a stepping stone after the end of the LGM, where the species genetically diversified before recolonizing Europe, the Caucasus and North Africa. They also hypothesized that the *Miniopterus* presence during the LGM, which is suggested by the fossil record, is related to *M. maghrebensis*, or possibly a third *Miniopterus* species, rather than *M. schreibersii*, and populations of this Maghrebian lineage might have since gone extinct, possibly because of competitive exclusion after the expansion of *M. schreibersii* (Bilgin et al., 2016). The findings and conclusions of this study once again show the advantages and importance of combining several approaches, such as molecular ecology and ENM, in order to obtain more reliable and conclusive results.

In summary, the details of the phylogeographic history of *M. schreibersii* have not been deduced conclusively yet. In the recent study of Bilgin et al. (2016), a dense sampling from around the Mediterranean basin and the Caucasus was used, yet the nuclear markers had not been utilized. Hence, the validity of the model presented in that study was not tested, and potential gene flow mediated by male members of the populations was not investigated. To get a better, more conclusive picture of the phylogeographic history of the species, samples from a wide range of geographical locations must be examined and phylogenetic analysis using both mitochondrial and nuclear markers should be carried out, which was one of the main objectives of this study.

1.2. Social Structure of Bats and Cytonuclear Discordance

When studying mammal species, a multi-marker approach is essential for making inferences about the social structure and the resulting population structure. Using only one type of molecular marker in phylogeographic and social structure studies may generate questionable results, because factors such as homoplasy, incomplete lineage sorting, mode of inheritance and sex-biased dispersal all drastically affect the genealogy of each marker, which may differ from and/or be consistent with the species history to varying degrees (Hewitt 2004a, Flanders et al., 2009). After the naming of the field in 1987 (Avise et al., 1987), for many years, most phylogeographic studies made use of only mitochondrial DNA (mtDNA) markers (Avise, 1998). This was due to these markers diverging slowly (or just fast) enough, at a rate which is more suitable for inferring species genealogies, compared to that of nuclear autosomal genes (Moore, 1995; Hewitt, 2001), and due to their other advantages, such as their lack of recombination and high copy numbers (Ingman et al., 2000). However, in most mammals mtDNA is only maternally inherited and especially susceptible to interspecific gene flow, or introgression (Petit & Excoffier, 2009; Furman et al., 2014; Dool *et al.*, 2016a). In addition, some mtDNA markers, such as the D-Loop in the control region, are prone to homoplasy, due to their relatively high mutation rates (Lloyd, 2003; Ingman et al., 2000). On the other hand, the rate of mutations and divergence might still be not fast enough for studying more recent events like those that occurred in the last several millennia, where incomplete lineage sorting may be an issue, and more variable markers might be needed (Hewitt, 2004b; Bogdanowicz et al., 2009). Hence, mtDNA markers, as with all other types of markers, may lead to wrong assumptions and deductions regarding the evolutionary history of species, if they are used alone (Flanders et al., 2009).

To overcome the drawbacks of depending solely on mtDNA, nuclear microsatellite markers can be used simultaneously with mtDNA (e.g., Brito, 2007; Furman et al., 2010b; Puechmaille *et al.*, 2011; Bilgin et al., 2012; Puechmaille et al., 2014; Bilgin et al., 2016). Even though microsatellites suffer from homoplastic mutations and may generate ambiguous genealogies, they can be very useful for population genetics and phylogeography studies, when used with caution (Hewitt, 2001). In many studies using both mtDNA and nuclear DNA (nuDNA) markers, the resulting patterns obtained by both types of markers were consistent, and checking the nuDNA in addition to mtDNA provided support for inferences that were made using only mtDNA (Zink & Barrowclough, 2008; Furman et al., 2010b; Bilgin et al., 2012). Yet, the case of different genealogies for different markers may also be encountered when mtDNA and nuDNA markers are used, where the difference is referred to as cytonuclear discordance (Toews & Brelsford, 2012; Furman et al., 2014). Potential causes of

cytonuclear discordance include incomplete lineage sorting (Funk & Omland, 2003; Zink & Barrowclough, 2008), variation among mtDNA markers due to geographically distinct selection pressures (Cheviron & Brumfield, 2009; Irwin, 2012), and differential movement of molecular markers among populations via intraspecific gene flow and introgression (Toews & Brelsford, 2012). Upon secondary contact and subsequent range overlap, introgression of mtDNA may occur among species that have female- or male-biased dispersal (Toews & Brelsford, 2012; Petit & Excoffier, 2009).

In mammalian species, the most commonly observed form of social structure embodies male-biased dispersal and female natal philopatry (Greenwood, 1980; Lawson Handley & Perrin, 2007). In many cases, it was the detection of cytonuclear discordance that revealed this type of social structure of several mammal species, such as in African elephants (Nyakaana et al., 1999), deer mice (Yang & Kenagy, 2009) and orangutans (Nietlisbach et al., 2012). In these studies, it was concluded that gene flow between populations of a single species with male-biased dispersal resulted in intraspecific cytonuclear discordance, where the differentiation of the mtDNA among populations was not accompanied by a corresponding differentiation of the nuDNA.

In bats, like in most other mammals, male-biased dispersal is the norm (Arnold, 2007; Vonhof et al., 2008; Piaggio et al., 2009). However, in some bat species, there is female-biased dispersal (Nagy et al., 2007, 2013), or both sexes can be philopatric (Miller-Butterworth et al., 2003; Burland et al., 2006). *M. schreibersii* is a bat species that exhibits philopatric behavior in females. Female philopatry and male-dispersal were detected among its populations in Portugal (Ramos Pereira et al., 2009; Rodrigues et al., 2010), and a more recent study by Bilgin et al. (2016) suggested philopatry, using mtDNA markers and samples from its entire circum-Mediterranean range. In this study, the same continental populations were analyzed with nuclear microsatellites, with the intention of combining the results with previous findings based on mtDNA, to get a better understanding of the social structure of the species across its entire range, and to understand the effects of the social structure on the continental genetic make-up of *M. schreibersii*. The mtDNA data were already used by Bilgin et al. (2016) to study the broad scale phylogeography of the species. Accordingly, the above-mentioned conclusions were drawn, which suggest that an area to the east of the Levantine Sea (coasts of Syria, Lebanon, Israel and north of Egypt) was the primary refugium for *M. schreibersii* during the LGM, and Anatolia might have been a stepping-stone after the end of the LGM, where the species diversified before recolonizing Europe, the Caucasus and North Africa. In the current study,

the microsatellite markers were used to test some of these hypotheses, which were previously tested with mtDNA markers only.

1.3. Taxonomy and the Evolutionary History of *Myotis capaccinii*

The biological species concept (BSC) that was proposed by Mayr (1940) defines species as "groups of actually or potentially interbreeding populations that are reproductively isolated from other such groups." However, the definition of a species is not an easy task, and several alternative species concepts have been proposed (Templeton, 1989; Ehrlich, 1961; Raven, 1977). Nevertheless, the definition of Mayr is still widely used today. The main assumption of the BSC is the emergence of reproductive isolation mechanisms between distinct species that occupy distinct ecological niches (O'Brien & Mayr, 1991). One strength of the BSC is that it acknowledges the possibility of hybridization between separate species, and the existence of genetic diversity, which may give rise to so-called subspecies if they are allopatric, i.e., if they are reproductively separated because of geographical barriers. Mayr (1940) defined subspecies as "a geographically defined aggregate of local populations which differ taxonomically from other subdivisions of the species." The most important aspect of populations representing different subspecies is that they live allopatrically, meaning they are geographically separated and that they are phylogenetically distinct, usually with the presence of accompanying phenotypic characters (O'Brien & Mayr, 1991)

The secondary contact of species as a result of the recolonization events, which take place after allopatric separations, such as the ones that occur during the glacial periods, gives rise to the contact zones, multiple ones of which help define the so-called suture zones (Hewitt, 1999). Some species, whose genetic differentiation was mainly affected by such isolation and recolonization cycles and the presence of related refugia and suture zones include the bats *Miniopterus schreibersii*, *Rhinolophus euryale*, *Rhinolophus ferrimequinum* (Bilgin et al., 2008c), the greater mouse-eared bat (Ruedi & Castella, 2003), the brown hare (Kasapidis et al., 2005) and the Anatolian mountain frog (Veith et al., 2003). According to Bilgin et al. (2008b), there exist a similar pattern of differentiation when it comes to *Myotis capaccinii*, which is another species that is distributed along an eastern-western axis with a suture zone in southeastern Europe. Bilgin et al. (2008b) showed that there were indeed two different clades occupying east and west of this suture zone. These two clades were mitochondrial, and there was no significant differentiation between the nuclear DNA of these two mitochondrial clades, implying that although there is some genetic differentiation among the members of the eastern and western populations of *Myotis capaccinii*, these two groups still belong to the same species.

Furthermore, in the thesis / dissertation of Andrea Viglino, where samples from the whole distribution range of *Myotis capaccinii* were analyzed for the first time, a second mitochondrial break was detected around Italy, increasing the number of mtDNA clades to three (Viglino, 2012).

As explained by Bilgin et al. (2008b), the intraspecific taxonomy of *M. capaccinii* is controversial. The species has a relatively wide distribution, spanning Northern Africa in the south, the eastern parts of the Iberian Peninsula in the west and Mesopotamia in the east (Hutson et al., 2008b; Koopman, 1994). Yet, there was a difference of opinion regarding the possible subspecies occupying this distributional area. Corbet (1978), as well as Spitzenberger and Helversen (2001) had claimed that the species was monotypic, whereas Ellerman and Morrison-Scott (1966) and Koopman (1994) hypothesized the presence of two subspecies in both sides of a contact zone in southeastern Europe: one subspecies, *M. c. capaccinii*, was distributed in the west, from northeastern Africa to the former Yugoslavia, and the other, *M. c. bureschi*, was found in the east, from Bulgaria to Iran. Additionally, Albayrak and Aşan (2002) suggested that the nominate form was distributed in Turkey and Europe, whereas the Middle Eastern form was in fact another subspecies.

One of the most important factors giving rise to intraspecific differentiation is the isolation of different populations of a species during ice age glacial maxima (Bilgin et al., 2011). This isolation, followed by genetic differentiation results in so-called “speciation pumps” (Brown & Lomolino, 1998). The subsequent post-glacial expansions of these previously isolated populations are the main driver of the formation of intraspecific contact zones such as the ones encountered in *M. capaccinii* (Bilgin et al., 2011). The time of the onset of expansion for *M. capaccinii* in this study was dated to 11 kyr before present (BP), and considering that the deglaciation events at the end of the Pleistocene were dated to 15 kyr BP (Walter, 1995), the estimated date of population expansion seems to have happened after the Late Pleistocene. In the study of Bilgin et al. (2008b), the differentiation and formation of two separate clades was dated to about 500 kyr BP. This date corresponds to the Middle Pleistocene, when populations were subjected to repeated glaciations, which were much more severe than those in the Late Pleistocene and had more profound effects (Astakhov, 2004), consequently isolating populations from each other for varying periods of time. Even though there was also some evidence for male-biased dispersal, the timing of the splitting of the two mitochondrial clades suggests that the reason for the genetic differentiation was the isolation of the populations in the past, and not sex-biased dispersal (Bilgin et al., 2008b).

Consequently, the study of Bilgin et al. (2008b) supports the hypothesis of two different subspecies of *Myotis capaccinii*, considering the geographical overlap of the two clades recognized in this study around southeastern Europe, with the formerly proposed subspecies, *Myotis capaccinii capaccinii* and *Myotis capaccinii bureschi* (DeBlase, 1980; Koopman, 1994). The results are also consistent with those of Albayrak and Aşan (2002), at least to some degree, that is, one of the clades was only found in Europe. However, the members of the eastern clade were also found in some western caves, and the degree of the overlap of two clades has not been thoroughly investigated, yet.

The contribution of this thesis study to the scientific knowledge regarding *Myotis capaccinii* will be similar to its contribution regarding *Miniopterus schreibersii*, meaning that this study will provide a much more complete and/or fine-tuned picture of the phylogeographic history of *Myotis capaccinii*. In fact, *M. capaccinii* is a much less well-studied species, compared to *Miniopterus schreibersii*. Samples collected from the entire global distribution range of *Myotis capaccinii* were analyzed once before for the thesis of Andrea Viglino (Viglino, 2012), however, more samples were used in this study, allowing the making of much more comprehensive inferences regarding its evolutionary history and genetic differentiation. Especially, the higher number of samples obtained from the two contact zones of the three mitochondrial clades allows a more fine-tuned investigation of their ranges. The limits to the distribution of the eastern clade individuals and the range of overlapping of the two clades in southeastern Europe is especially important in this respect, an important aspect of the phylogeography of *Myotis capaccinii*, which is not investigated sufficiently yet (Bilgin et al., 2008b). Similarly, the overlap between two clades around Italy and the extent of their ranges will be better studied, using a higher number of samples from populations located in this more recently discovered contact zone.

1.4. Conservation and Contact Zones

The conservation of species, in addition to obviously being of vital importance, is a very complex, difficult and controversial issue, and there are many different approaches to and theories of conservation, which in many cases seem to be quite contradictory (Smith et al., 2001; Araujo, 2002; Simberloff, 1998). Unfortunately, the resources for conservation have been and still are scarce, and thus, strategies that primarily concentrated on the conservation of a single species had been proposed in the past, whose common goal was to determine and prioritize the conservation of a single species, which was considered of being of utmost importance in some way, and consequently could be used as a shortcut to increase the efficiency of conservation efforts (Simberloff, 1998). Such species

concepts included indicator, flagship, umbrella and keystone species concepts, all with their own advantages and disadvantages.

Indicator species are species, whose presence is hoped to reflect the presence of others, or species, which are thought to reflect the physicochemical changes in the environment (Simberloff, 1998; Landres, 1988). Yet the definition of the health of the system that is supposed to be indicated is not at all clear and even if there was a consensus on this definition, choosing the best indicator would still be an extremely difficult issue. Umbrella species, on the other hand, are species, whose protection would supposedly serve for the protection of many others, because they demand relatively large habitats to flourish, within which many other species are sheltered as well (Fleishman et al. 2000). However, this concept is also problematic, because the actual effective range of the so-called umbrella is usually not easy to determine scientifically and even the species that fall under the umbrella may not benefit from the special treatment for the umbrella species (Simberloff, 1998). Flagship species are species that are popular among the public and thus can be used in promoting a conservation campaign, but these species (usually large, charismatic vertebrates) are rarely indicator or umbrella species, their conservation is usually expensive, and sometimes the conservation efforts for two flagship species can conflict with each other (Simberloff, 1998). Finally, the keystone species are species that affect many others in the same ecosystem beyond expectations, due to some not easily recognizable interspecific interactions in the system (Paine, 1969; Menge et al., 1994). This concept, although very promising, has also its own shortcomings, for instance, the existence of keystone species in a given ecosystem is extremely difficult to determine experimentally (Simberloff, 1998).

The above-mentioned problems associated with single species conservation strategies gave rise to the development of more general hypotheses dealing with multi-species conservation. One such approach is the habitat conservation hypothesis, which suggests the focusing of protection efforts (e.g., the U.S. Endangered Species Act) on whole habitats, so that the efficiency of conservation can be increased (Carroll et al., 1996). Another approach brings forward the biodiversity hotspots, which are areas rich in species, endemic species and/or endangered species (Dobson et al., 1997; Reid, 1998, Myers et al., 2000). This approach may not be very useful at smaller, local scales, since the hotspots do not seem to be overlapping for many taxa and also rare species may not be present in the hotspots (Prendergast et al., 1993), but at larger, continental or global scales, environmental variation may be represented fairly with hotspots that contain both marginal and core populations of species, and transition and non-transition zones together (Aroujo, 2002). These transition zones are in fact another important concept of the multiple species conservation approach. A transition zone is an area that

exhibits an environmental gradient (e.g., the change in altitude along a hillside) and is extremely important in the adaptive radiation of species and the generation of biodiversity. Moreover, this area does not necessarily have to be also a biodiversity hotspot (Smith et al., 2001). Smith et al. (2001) claim that for the conservation of species, the transition zones are much more important than the biodiversity hotspots, stating that the location of these hotspots may change because of climatic shifts and that the preservation of adaptive radiation is the most significant issue, whereas Araujo (2002) objects to this argument by emphasizing the importance of both concepts in fulfilling the need for increasing the efficiency of conservation efforts, a need, which stems from the scarcity of conservation resources, and he underlines the role of large, core populations in relatively stable, non-transition zones in preserving the biodiversity of ecosystems (Araujo, 2002; Lawton, 1993). It should also be noted that the climatic changes might also change the transition zones in the long-term.

Another hypothesis regarding the multiple species conservation efforts is called the biogeographic crossroads (Spector, 2002). Biogeographic crossroads are the relatively narrow intersections of large habitats, where species turnovers take place, and such areas satisfy many criteria that are considered significant in conservation science, such as having a high biodiversity and the potential for driving long-term speciation events. Yet another suggestion for improving the efficiency of conservation is integrating the evolutionary thinking into conservation planning, considering the existence of some contemporary evolution incidents that have been documented, such as those observed under captive management, in unspoiled habitats, or as a result of species introduction events (Ashley et al., 2003). According to this view, wrong management decisions with severe consequences can be made and unexpected evolutionary responses to even the slightest disturbances may be observed, if evolutionary thinking is avoided.

The integration of molecular biology techniques with population genetics gave rise to the study of molecular ecology (Freeland, 2005), which have proven to be useful for making more informed conservation decisions as shown in numerous studies (e.g., Keim et al., 1989; Bouzat et al., 1998; Bellinger et al., 2003). One of the most significant contributions of the application of the molecular techniques to conservation biology is that they render the detection of various genetic clades and even cryptic species, and thus sites with a relatively high heterozygosity much easier, enabling the researchers to determine high priority conservation zones more accurately (Prior et al., 1997; Furman et al., 2010b).

The concepts of contact zones, suture zones, hybrid zones and transition zones are all very important for the conservation science, and their definitions and actual physical extent mostly coincide, so that in a manner, these zones are nested. A contact zone is any area in which the ranges of two or more populations coincide, where they may or may not overlap (Martinez-Freiria et al., 2008). A stable hybrid zone is where divergent genomes meet and continuously produce hybrids that are unfit compared to their parent populations (Hewitt 1996, 1999). A suture zone, on the other hand, was at first defined as the clustering of such hybrid zones (Remington, 1968; Hewitt, 1996), however, it is proposed that the definition should be expanded to include the clusters of contact zones and intraspecific phylogeographic breaks, so that the areas where different species that do not hybridize and areas where different clades of the same species meet are also covered, a proposal, which is justified when the similarity of the processes, such as the postglacial range expansions, that bring all these differentiated taxa together, are taken into account (Swenson & Howard, 2005; Bilgin, 2011).

In some research articles, the suture zone and transition zone are used as synonyms (e.g., Maggs et al., 2008), yet in this text, the definition of transition zones, given above will be used, that is, an environmental gradient that promotes adaptive variation and speciation. In fact, such gradients might give rise to yet another mechanism for the formation of some hybrid zones, where the fitness differences of the members of the parent populations and hybrids stem from the environmental differences along the gradient zone (Fritsche & Kaltz, 2000).

Usually all of the above-mentioned ecological zones have a relatively high heterogeneity and diversity, which are investigated at many different studies regarding different species. For instance, in a study on cottonwoods, the effect of the diversity in a cottonwood hybrid zone on the diversity of a dependent arthropod community was evaluated, and it was shown that the diversity of the arthropods was highest in the cottonwood hybrid zone, where the cottonwood-cross-type diversity was highest (Bangert et al., 2005). In another study, Walter and Epperson (2005) showed that the highest diversity for the red pine (*Pinus resinosa*) was found in a contact zone of two populations, which was actually a transition zone between two glacial refugia, where these two populations persisted during glacial periods. Maggs et al. (2008) also showed that a secondary contact zone, where descendants from two glacial refugia have met, had higher diversity, in their study regarding eight marine taxa in the North Atlantic. Petit et al. (2003) had reached the same conclusion when studying 22 tree and shrub species from the forests of Europe. They also stated that the highest diversity was seen in the transition zone (at intermediate latitudes) located between glacial refugia. In another study, Seeb and Crane (1999) showed that the highest heterogeneity for 64 populations of the Chum Salmon

was found in western Alaska, the contact zone of two lineages, which were again descendants of populations from two glacial refugia.

In summary, the contact, suture and transition zones are very important for the conversation planning efforts, because of the high biodiversity they exhibit. Especially, the exact locations of habitats, or biodiversity hotspots, which host many species, including cryptic species and subspecies that live in sympatry or parapatry are of utmost importance (Bangert et al., 2005). The interspecific and intraspecific interactions and competition are other significant factors that must be taken into account, when developing future strategies, considering the fragility of most ecological systems.

The species, which will be investigated in this thesis study, namely *Miniopterus schreibersii* and *Myotis capaccinii*, are species that are listed in the Red List of the International Union for Conservation of Nature and Natural Resources (IUCN) as Near Threatened and Vulnerable, respectively (Hutson et al., 2008a, 2008b). Also because of the possible discovery of new cryptic species among populations of both of these bat species that may drastically reduce their population sizes, one of the most significant considerations in conservation (Shaffer, 1981; Furman et al., 2010b; Bilgin et al., 2012, Puechmaille et al., 2014). In addition, climate change and other negative anthropogenic influences may change the conservation status of these two species for the worse in the near future. Hence, it is crucial to carry out further scientific research on these two species and to get a clearer picture of their actual phylogeographic structure. Improving the knowledge on their current distribution, biodiversity, evolutionary history and interspecific, as well as intraspecific interactions, will enable the scientific community to make better suggestions regarding their conservation. This will also render the proper application of the hypotheses and concepts of conservation, explained before, more easily and efficiently.

The sites mentioned in the previous sections, where different clades of *Miniopterus schreibersii* or *Myotis capaccinii* are living in sympatry are contact and (as long as no complete reproductive isolation is established) hybrid zones (Bilgin et al., 2008b, 2012, 2016). Furthermore, these contact and hybrid zones coincide with known suture zones for various species, including these two bats (Bilgin, 2011). According to Bilgin (2011), one such suture zone is located between Anatolia and the Balkans, which has hybrid zones for at least ten species, one of which is *Myotis capaccinii*, and yet another suture zone is an Intra-Anatolian one, with hybrid zones for at least eight different species, one of which is *Miniopterus schreibersii*.

Besides sites, in which members of the intraspecific clades and cryptic species live together in sympatry, there are also many caves that host populations of both of these species, namely *Miniopterus schreibersii* and *Myotis capaccinii*, who belong to different genera. For example, they dwell in caves in Thrace and around Istanbul together (Furman & Ozgul 2002, 2004). Yet there are almost no proper comparative studies that deal with the interactions of these two cave-dwelling bats. One exception is the study of Amengual et al. (2007), in which the seasonal oversea migration of these two bat species between Balearic Islands in Spain are compared, and using the numbers from cave colonies, where they are living sympatrically, deductions about their migration routes and habits are made.

To summarize briefly, the main contribution of this study, regarding the conservation of the two species investigated, will be the confirmation and possible discovery of new ecological sites that are crucial for the conservation efforts. Such sites include local biodiversity hotspots, as well as contact and transition zones. Until now, the conspecific sympatry of these two species is investigated mostly in relatively small parts of their whole distribution range (Bilgin et al., 2008b, 2012; Furman et al., 2010b). In this thesis study, the collection and analysis of samples from the global distribution range of *Miniopterus schreibersii* and *Myotis capaccinii*, along with the investigation of their social structure and modelling the effects of future climate changes on their distribution, will help increasing the efficiency and reliability of the conservation planning efforts regarding these two species.

1.5. Modeling the Future Distributions of Phylogenies: *Myotis capaccinii*

According to the most up-to-date assessment reports of the Intergovernmental Panel for Climate Change (IPCC), the average global annual temperature, which has increased about 0.6°C over the last 100 years, especially between 1910 and 1945, and then from 1976 onwards, is going to increase a further 4°C until the end of 2100 (IPCC, 2014, Parts A & B; Quintero & Wiens, 2013; Walther et al., 2002). The rate of warming in the last 40 years has been higher than it has been at any other time during the last millennium and at the end of the century, and subsequently the Earth may end up becoming warmer than it has ever been in the last 40 Myr (Thomas et al., 2004). Furthermore, in addition to the increase in the average temperatures, the rainfall patterns will also be significantly affected, with up to 30% increase or decrease in local annual precipitation (Quintero & Wiens, 2013). Regional changes in climatic parameters, such as temperature and precipitation tend to be spatially heterogeneous (Walther et al., 2002), and many local populations of species are already shifting their ranges because of these changes (Parmesan & Yohe, 2003). It has long been established that the

distribution of a given species is profoundly influenced by the climate, as shown by the vast cumulative evidence from fossil data (Woodward, 1987; Huntley, 1999; Davis & Shaw, 2001; Overpeck et al., 2002; Benton & Twitchett, 2003) and some recent trends in biogeographic distributions (Hughes, 2000; McCarty et al., 2001; Walther et al., 2002; Pounds et al., 1999; Root et al., 2003). The correlation between the climate change, the phenological events, and the shift in species' boundaries, which occurs towards the poles longitudinally and upwards in elevation, is clearly observed (Root et al., 2005; Parmesan, 2007). For instance, the range boundaries of species moved towards and away from the poles during warming and cooling periods, respectively (Parmesan et al., 2011). Hence, it is only natural to expect that the future changes in climate will also affect the distributions of the species significantly.

The concept of ecological niche, which is used in correlation of a species' behavior and the environmental conditions in its habitat, has been used in ecology since Grinnell (1917). According to Hutchinson, the fundamental ecological niche is an n-dimensional hypervolume, axes of which consist of the environmental conditions that are required for that species to exist (Hutchinson, 1957) and later, a bioclimate envelope was defined as the part of the fundamental ecological niche, the multi-dimensional space that constitutes only of its climatic components, which is also called the climatic niche (Pearson & Dawson, 2003). Yet, modeling the past, the present and the future distributions of species has become possible, only after the definition of a set of climatic parameters that are biologically meaningful (Nix, 1986). When they were first utilized, the so-called species distribution models (SDMs), were used to investigate the present distribution of the species, however, later on, the time dimension was added to the models and the SDMs were used to get past and future projections regarding the distribution of the species in question (Stanton et al., 2012). There are many algorithms that are applied for different SDMs, including those based on machine-learning approaches, community models and generalized additive methods (Elith et al., 2006), yet the basic premise is always the same: several environmental variables, such as the temperature, precipitation and soil-type are used as the independent variables, whereas the presence, and also sometimes the absence localities of the species is used as the dependent variables as the study area is divided into grids at a specific resolution (Hirzel et al., 2002). If only the climatic variables that define a species' bioclimatic envelope are used and other environmental variables, e.g. the land cover or the soil type are excluded, the model may simply be called a bioclimate envelope model, which seems to be decently precise at large-scale studies, at least for predicting the potential future distributions under potential climate change, if not the real changes in distribution that will come to pass exactly (Pearson & Dawson, 2003; Hamann & Wang, 2006). One of the earliest empirical approaches that were used

to develop SDMs is BIOCLIM, which uses a set of climatic variables that were determined by statistical tools like discriminant function, multiple regression and principle components, and investigates the correlation of these variables with the species' ranges (Nix, 1986). Another approach is using a model that incorporates some kind of decision process to adapt to local habitats by defining different rules for different habitats throughout the range, which can be accomplished, for instance, by using classification and regression trees, or CARTs (Parmesan et al., 2005). More sophisticated machine-learning techniques that make use of artificial intelligence are also used, as in the case of Genetic Algorithm for Rule-set Prediction, or GARP (Stockwell & Noble 1992), which detects the variables that provide the most information and adapt accordingly. Logistic regression and multivariate analyses are also utilized in order to find the best, most informative variables (Parmesan et al., 2005). As new algorithms and new methods are developed, the models become increasingly complex.

Even though the modeling algorithms are continuously improved, as new data sets for the Geographic Information System become available, and the IPCC scenarios regarding the future climate change are renewed, predicting the future distribution of the species is far from being an easy task (Parmesan et al., 2005, Balint et al., 2011). First of all, there is the intrinsic uncertainty of SDMs, which stems from the use of a single model algorithm and the use of general circulation model as the lone source of climate forecasting (Buisson et al., 2010; Nogues-Bravo, 2009). Also the distribution of populations is affected by many factors, including the biotic interactions among individuals and populations (Davis et al., 1998a, 1998b), different dispersal abilities of different species (Lawton et al., 2000), and the effects of anthropogenic land-use and the accompanying habitat destruction (Hof et al., 2011), all of which render making accurate predictions very difficult. Detecting the significance of anthropogenic influence when it comes to the responses and range-shifts of a given population is not possible in many cases, especially at local scales, because the climate change that is caused by human activities is detectable only at large scales. Furthermore, even the responses of different species to local climate change may vary greatly, and there are other human influences on the habitat of species, caused by activities that do not necessarily increase gas emissions, such as the abovementioned land-use, which results in habitat fragmentation and destruction (Parmesan et al., 2011). Only in some rare instances, extinction of a species due to climate change that is caused by human activities is clearly observed, e.g. the case of the golden toad and also potentially the white lemuroid possum (Pounds & Crump, 1994; Parmesan et al., 2011).

In the last three decades, many studies have been carried out that have investigated the possible past, present and future distributions of species using SDMs with locality (presence, and in some cases absence) data as the dependent variable, including studies on tree species (Hamann & Wang, 2006), amphibians (Pounds et al., 1999), insects (Parmesan et al., 1999), marine invertebrates (Sagarin et al., 1999), fish (Holbrook et al., 1997), birds (Thomas & Lennon, 1999) and mammals (Hersteinsson & MacDonald, 1992). The high number of different methods utilized causes a high variability in the projections, making them less reliable (Pereira et al., 2010; Bellard et al., 2012) Although the models obviously need to be improved to overcome their weaknesses, the studies that take into account only the possible changes in the climatic variables, the bioclimatic envelope models (BEMs), seem to be quite useful tools. As the possibility of climate change to become the dominant reason of a possible decrease in global biodiversity increases, surpassing even the effect of habitat destruction, the use of BEMs seems to be valuable, at least for first impressions and approximations regarding the possible changes in species distributions (Pearson & Dawson, 2003; Leadley et al., 2010). The studies that make use of BEMs, as well as other SDM studies that may also take other parameters such as the changes in land-cover and soil-types into account, may be used to affect the decision-makers' approaches regarding emissions, land use and other anthropogenic factors (Bellard et al., 2012).

SDMs that use locality data, can in fact also be used in concordance with statistical phylogeography, which investigates the correlations between the geographic distributions and genetic variations of populations, utilizing genetic data, i.e. molecular markers, so that the hypotheses derived from these two different approaches can be compared and reciprocally tested against each other (Avise, 2009, Chan et al., 2011). However, there are a relatively and surprisingly small number of studies that combine SDMs and phylogeography, even though both SDM and phylogeography studies are very active fields of research (Scoble & Lowe, 2010; Forester et al., 2013). Some of these studies have only looked into the past to examine historic species responses to climate change (Rissler et al., 2006; Bond et al., 2006; Richards et al., 2007; Theissinger et al., 2011; Wielstra et al., 2013; Dalmaris et al., 2015). For instance, an SDM was used to examine the relationships of endemic areas with geographic barriers to dispersal, which in turn were determined by using mitochondrial markers and detecting phylogeographic patterns among Californian amphibians and reptiles (Rissler et al., 2006). Phylogeographic data, which again is obtained using mitochondrial markers can also be utilized to find signs of population extinction and then SDM can be used to predict areas, where the individuals of a population would still be present if it was not for the habitat destruction caused by urban development or other anthropogenic factors, which was done for the *Apomastus* spiders around Los

Angeles (Bond et al., 2006) In another study, Wielstra et al. (2013) have investigated the phylogeography of nine *Triturus* species (marbled and crested newts), and they made use of both mitochondrial markers, and species distribution modeling, the combined use of which provided a more clear understanding of the species' phylogeography. SDMs can also be used to develop alternative hypothesis regarding the phylogeographic history of a species, which was done for *Ameletus inopina*tus, an organism that was used as a model for similar aquatic insects (Theissinger et al., 2011). Using SDMs as a tool for developing alternative phylogeographic hypotheses is an approach that was used in several other similar studies (Knowles et al., 2007; Waltari et al., 2007; Shepard & Burbrink, 2008; Jakob et al., 2009; Buckley et al., 2010).

With available projections of climate change in the near future, possible phylogeographic changes in the near future can also be deduced using SDMs, as was done in studies that only made use of the occurrence data. In some studies that also made use of mitochondrial markers, the possible impact of climate change in the future has already been assessed. For instance, using COI mitochondrial DNA data and Maxent modeling, Cordellier & Pfenninger (2009) checked the possible future distribution of phylogenies of the pond snail *Radix balthica*, after determining the phylogeographic history and two refuges of the species during the LGM, which they sampled over its entire range, and predicted a northward shift of its range towards the pole, and a significant loss of habitats in Southern Europe. Galbreath et al. (2009), on the other hand, examined the possible past and future distributions of the American pika *Ochotona princeps*, an alpine specialist, which like some other similarly cold-adapted species, is usually neglected in phylogeographic history studies. They detected a recent decline for the species demographically and using mitochondrial markers and environmental niche modeling (ENM, a synonym for SDM), they predicted a further change of genetic diversity of the species. Similar predictions for range contractions and loss of genetic diversity were made for nine aquatic insect species of European mountains, again in a study that combined the uses of mitochondrial markers and SDM (Balint et al., 2011).

Phylogeographic studies that use genetic markers other than mitochondrial markers can also be integrated into SDM studies. In fact, they should be used, so that the differences between predictions for past, present and future distributions, and the different results for conservation decisions can be detected and compared, when different kinds of markers are used for defining phylogenic lineages (Rissler et al., 2006). Dalmaris et al. (2015) detected a recent decline in a tree species, *Eucalyptus wandoo*, due to climate change with a relatively high certainty, through the combined use of SDMs and genetic markers, in this case, restriction fragment length polymorphism analysis of chloroplast

DNA. In Forester et al. (2013), phylogenies of *R. integrifolia* that were estimated using the sequence data of five nuclear loci were used for testing predicted divergences that were in turn based on SDM predictions for past distributions of the species. The future projections for the distribution of the species that were made subsequently were dependent on the agreement of both approaches (Forester et al., 2013). Not only sequence data from nuclear DNA, but also microsatellites can be used in phylogeographic studies that are coupled with SDMs. For instance, in their abovementioned study, Theissinger et al. (2011) also used previously published (Taubmann et al., 2011) microsatellite data, in addition to the mitochondrial data they generated. A comprehensive study by Quintero & Wiens (2013) made use of phylogenies that were produced with both mitochondrial and nuclear DNA data. They used climatic niche values and IPCC scenarios for climate change to estimate the rates for climatic niche evolution and compared them with rates of climatic change for more than 500 extant species that represent all major tetrapod clades. When they compared these rates to predicted rates of climate change until 2100, they concluded that adaptation to projected changes between 2000 and 2100 would require rates of evolution that have never been reached in the history of most species. There are other SDM studies that use different kinds of genetic markers, yet the number of SDM studies that investigate the intraspecific genetic diversity, which is one of the most important driving forces of evolutionary progress, is still relatively low (Pauls et al., 2013).

In this thesis study, samples of *M. capaccinii* from the whole Mediterranean Basin and the Near Eastand Caucasus were analyzed. The possible past distribution of the species was investigated using SDMs and mitochondrial data and the results were compared to the results of former studies, and using the same tools and the same approach, the future distribution of *M. capaccinii* was also predicted. The findings were used to test and possibly validate the presence of three different mitochondrial clades that may correspond to subspecies or even cryptic species, to investigate the ranges of their overlap, to detect biodiversity hotspots, and to provide input for the decision-making processes regarding the conservation of this species.

1.6. Comparative Ecology and Morphology of *Miniopterus schreibersii* and *Myotis capaccinii*

The feeding habits and wing morphology of a bat significantly affect its habitat preferences and migration (Norberg & Rayner, 1987). Bats have a large variety of feeding behaviors and although the ancestral form of insectivorous feeding is still the most common strategy for many Chiroptera, bats have adapted to include fruit, nectar, pollen, plants, blood, fish and other small vertebrates in their

diet (Norberg & Rayner, 1987; Aizpurua et al., 2013). Similarly, there is great variation in their morphological characteristics, which determine the flight capacity and migratory range of a given bat species (Norberg & Rayner, 1987). The two bats investigated in this thesis study differ in many aspects of their ecology and morphology, specifically in their foraging strategy and flight ability (Norberg & Rayner, 1987; Gardiner et al., 2014; Amengual et al., 2007).

Myotis capaccinii is an insectivorous and piscivorous bat, feeding on insects and small fish (Aihartza et al., 2003, 2008; Aizpurua et al., 2013, Gardiner et al., 2014). It has a very specialized foraging strategy and hunts mainly on ponds, lakes and streams by trawling and gaffing its prey from surface waters by flying low above the water and using its hind feet (Aizpurua et al., 2013, Gardiner et al., 2014). Even though *M. capaccinii* is categorized as a vulnerable species, which is likely to become endangered unless proper measures are taken, not much is known about its habitat requirements (Aihartza et al., 2003). Faecal analysis revealed the presence of Culicidae (mosquitos), Chironomidae (flies), Trichoptera (caddisflies) and Lepidoptera (moths) remains in their feces (Medard & Guibert, 1990). The detection of moths in its diet, indicates that *M. capaccinii* may in fact be able to hunt in habitats other than wetlands (Aihartza et al., 2003), yet the main source of food for the species seems to be water bodies. The more recent detection of fish scales and bones in faecal pellets supports this argument (Aihartza et al., 2003, 2008; Aizpurua et al., 2013). Furthermore, preying on fish seems to be a constant activity of *M. capaccinii* that may be observed throughout the year, and is not related to seasonal precipitation as was previously thought (Aizpurua et al., 2013). Levin et al. (2006) suggested that anthropogenic influences such as the introduction of exotic fish to water bodies might have been a significantly contributing factor of this relatively recent observation, yet it is not clear whether the fishing behavior is new for *M. capaccinii* or this finding only shows that human activities have recently favored fish over insects (Aizpurua et al., 2013). Whatever the case, it is safe to say that even though its diet contains fish species as well as many insects, *M. capaccinii* is a specialized species, which is able to hunt in aquatic environments for the most part (Voigt et al., 2015; Whitaker & Karataş, 2009).

Miniopterus schreibersii, on the other hand, is a different story. Even though *Miniopterus schreibersii* individuals are found living in sympatry with *Myotis capaccinii* individuals, their foraging behavior and feeding preferences differ greatly. *Miniopterus schreibersii* is a bat that is able to fly in high altitudes thanks to its long wings with a relatively high aspect ratio; yet it is also a fast and swift flier (Norberg & Rayner, 1987; Harrison, 1964). Whereas *Myotis capaccinii* is a trawling bat, *Miniopterus schreibersii* is an aerial-hawker, hunting species from several families and orders of

insects in open areas at high altitudes (Gardiner et al., 2014). These taxa include Coleoptera (beetles), Gryllidae (crickets), Lepidoptera (moths), Hemiptera (bugs) and Siphonaptera (fleas) (Whitaker & Karataş, 2009). Unlike *Myotis capaccinii*, it is a generalist species able to prey on many types of insects at relatively large habitats (Voigt et al., 2015; Whitaker & Karataş, 2009).

Both *Miniopterus schreibersii* and *Myotis capaccinii* are able to fly over relatively long distances over water (Amengual et al., 2007). The recorded distances flown between summer and winter roots are usually higher for *Miniopterus schreibersii* with an average of 120 km (Amengual et al., 2007). *Myotis capaccinii*, on the other hand, is a short to middle range migrant with average distances of 40-50 km covered between summer and winter shelters (Amengual et al., 2007). *Miniopterus schreibersii* is known to use landmarks such as cliffs and rivers for navigational purposes (Voigt et al., 2015; Di Salvo et al., 2009). It was recorded, however, that both *Miniopterus schreibersii* and *Myotis capaccinii* were able to find their way to their roosts over the sea without making use of such landmarks, as they may be utilizing the Earth's magnetic field (Amengual et al., 2007). In any case, the differences and similarities in their flight ability, as well as their habitat preferences may have a significant effect delineating the differences and similarities in their evolutionary history.

1.7. The Objectives of the Study

The main objective of this study was to answer questions regarding the phylogeographic history of these two relatively under-studied species, namely *Miniopterus schreibersii* and *Myotis capaccinii*, separately. For *Miniopterus schreibersii*, the main focus was to check for structuring at nuDNA level and make deductions regarding the dispersal strategy and phylogeography of the species by determining and comparing genetic differentiation at both mitochondrial and nuclear DNA. For this purpose, microsatellite data from the whole distribution area were obtained and analyzed for the first time for this species. For *Myotis capaccinii*, a high number of samples from the whole distribution area of the species were analyzed to improve previous studies, and investigate the phylogeography and taxonomy of the species. In fact, one of the aims of the study was to see if it was possible to make specific and subspecific suggestions. Another concern was the conservation of these species. In this respect, sites of high biodiversity were detected for *Miniopterus schreibersii*. For *Myotis capaccinii*, SDMs were utilized to study the possible effects of climate change on the distribution of the species. Finally, the phylogeographic histories, postglacial expansion patterns, levels of genetic structuring and taxonomies of these two bat species were compared. The differences were assessed in the context of their ecological and morphological characteristics, in order to get a better understanding of the

correlation of factors such as habitat preference and flight ability with the genetic make-up and evolution of species.

2. MATERIALS AND METHODS

2.1. Analyses for *Miniopterus schreibersii*

2.1.1. Sampling

A total of 333 tissue samples from 17 countries and 40 populations were newly analyzed (Figure 2.1 & Table 2.1). These populations were grouped together in 12 phylogeographically defined regions (Bilgin et al., 2016). Tissues were obtained from the wing-membranes of the bats using biopsy-punches and stored in 80% ethyl alcohol or dried with silica gel until the DNA extraction stage (Worthington Wilmer & Barratt, 1996; Puechmaille et al., 2011).

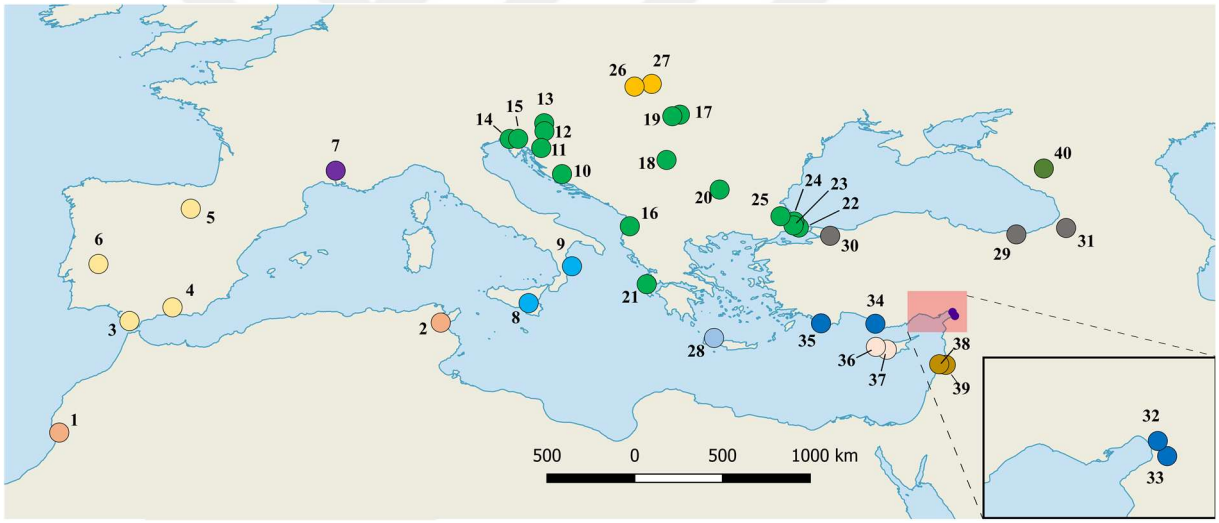


Figure 2.1. Map of sampling locations. The numerical and color codes correspond to those on Table 2.1.

Table 2.1. Sampling sites for 12 defined regions of *Miniopterus schreibersii* (Bilgin et al., 2016). N stands for the number of samples.

Region	Code	Country	Location	N	Latitude	Longitude
North Africa	1	Morocco	Win-Timdouine	2	30.6806	-9.3465
	2	Tunisia	Mines de Zaghouan	16	36.3997	10.1472
Iberia	3	Spain	Mesas de Algar Cave	4	36.3561	-5.9102
	4	Spain	Silo del Carmen de los Catalanes	3	37.1767	-3.5905
	5	Spain	Jorge's Cave	8	42.2230	-2.6596
	6	Portugal	Marvao	18	39.4061	-7.3762
France	7	France	Piolenc	18	44.1790	4.7681
Italy	8	Italy	Sicily	1	37.3939	14.6424
	9	Italy	Verzino	16	39.2817	16.8616
Balkans	10	Croatia	Ćulumova	15	43.9892	16.3772
	11	Croatia	Dragina	18	45.3470	15.2949
	12	Slovenia	Cerkev Mati	10	46.2260	15.4475
	13	Slovenia	Cerkev Device	2	46.5526	15.4262
	14	Slovenia	Jama v Doktorjevi ogradi	4	45.7793	13.7901
	15	Slovenia	Predjamski sistem	11	45.7793	14.1313
	16	Albania	Treni Cave	9	41.3321	19.8119
	17	Romania	Astilev Cave	15	47.0330	22.3830
	18	Romania	Gaura cu Musca Cave	9	44.7200	21.7100
	19	Romania	Betfia Cave	2	46.9816	22.0194
	20	Bulgaria	Parnitzite	2	43.2003	24.4321
	21	Greece	Kefalonia	23	38.2231	20.5722
	22	Turkey	Kocakuyu	1	41.2885	28.3180
	23	Turkey	Yaylacık	11	41.3573	28.2192
	24	Turkey	Çilingoz	10	41.5238	28.2216
	25	Turkey	Kız	9	41.8363	27.5573
Slovakia	26	Slovakia	Veľká Drienčanská	10	48.4891	20.0640
	27	Slovakia	Drienovská jaskyňa	18	48.6243	20.9523
Crete (GR)	28	Greece	Ag. loannou Cave	3	35.5911	24.1494
Black Sea	29	Turkey	Çatak	11	40.7984	39.5779
	30	Turkey	Gökçeören	4	40.8496	29.9956
	31	Turkey	Cehennemdere	2	41.1329	42.0483
Anatolia without Black Sea	32	Turkey	Güzelpınar	3	36.8741	36.3016
	33	Turkey	Demrek	2	36.6776	36.4243
	34	Turkey	Yalandünya Cave	5	36.2198	32.4022
	35	Turkey	Hıdırellez	9	36.1728	29.6445
Cyprus	36	Cyprus	Akamas	6	35.0500	32.3300
	37	Cyprus	Troodos Forest	3	34.9162	32.8755
Lebanon	38	Lebanon	Saleh Cave, Amchite	4	34.1500	35.6700
	39	Lebanon	Rouais Cave	2	34.1166	35.9167
Russia	40	Russia	Svetlaya Cave	14	44.1700	40.9200
		Total number of samples analyzed:		333		

2.1.2. Laboratory Analyses

For the DNA extractions, one biopsy punch from each bat was used and a PureLink® Genomic DNA Mini Kit (Invitrogen) was utilized, following the protocols of the manufacturer. Fourteen microsatellite primer pairs were used to amplify microsatellite loci: MM2, MM3, MM12, MM18, MM20, MM23, MM25, MM27, MM29, (Wood et al., 2011), and Mschreib2, Mschreib4, CH37, CHB12 and CHD2 (Miller-Butterworth et al., 2002; Han et al., 2008). Five of the loci, Mschreib2, Mschreib4, CH37, CHB12 and CHD2, were amplified using PCR conditions as described in Miller-Butterworth et al. (2002) and Han et al. (2008). For the other loci, the PCR amplifications were carried out using a QIAGEN Multiplex kit, following the manufacturer's protocols (QIAGEN, Valencia, CA), but the reaction volume was decreased to 10 µl to lower costs, and 0.5 µl of DNA was used. For the remaining nine loci that were amplified using the multiplex kit, two sets of microsatellite primer pairs were used in two multiplex solutions (Set 1 – MM20, MM27, MM29 and Set 2 – MM2, MM3, MM12, MM18, MM23 and MM25). All 14 loci were amplified in 321 samples, whereas 10 loci (except the monomorphic loci MM20, MM25, MM27 and MM29) were amplified in 333 samples. The fragment sizes were determined commercially at the Institute of Biotechnology at Cornell University (Ithaca, NY) and scored manually using the software GeneMarker v.2.7.0 (SoftGenetics, State College, PA). The raw allele length data were binned into allelic classes using the Excel macro FlexiBin (Amos et al., 2007). To determine the mtDNA identities of 18 new samples collected in this study from Tunisia (to make sure they are not *Miniopterus maghrebensis* individuals), a partial fragment of the mitochondrial cytochrome-b region (cyt-b) was also amplified, using the primers Molcit-F (Ibanez et al., 2006) and MVZ-16 (Smith & Patton, 1993) as described in Bilgin et al. (2016). Sequencing was done commercially at Macrogen Inc. (South Korea) and the sequences were aligned and edited using the software Sequencher v.4.7 (Gene Codes Corp.).

2.1.3. Statistical Analyses

The presence of null alleles, large allelic dropout and stutter was tested with Micro-Checker v.2.2.3 (Van Oosterhout et al., 2004). Plots of allele length frequencies were generated and observed homozygosity, expected heterozygosity (HE), number of alleles (Na) and effective number of alleles (Ne) were calculated with GenAlEx v.6.5 (Peakall & Smouse, 2012). HP-Rare was used to perform rarefaction to determine allelic and private allelic richness (R and RP respectively), while compensating for sampling bias (Petit et al., 1998; Kalinowski, 2005). The baseline number of samples was chosen as 6, 9, 14 and 17 for four different analyses; corresponding to the four minimum

sample sizes in the data set (Crete with a population size of three was excluded). Variation patterns were investigated by a principal component analysis (PCA) and the correlation between the genetic and geographic distances of the populations was evaluated through a Mantel test using GenAlEx. For the Mantel test, $F_{ST}/(1- F_{ST})$ was used as the measure of population distance, as suggested by Rousset (1997). Hardy-Weinberg equilibrium (HWE) and linkage equilibrium between loci were checked with Genepop v.4.5.1 (Rousset, 2008). To correct for multiple testing in the linkage equilibrium analysis, a standard Bonferroni correction was applied (Rice, 1989). Genetic subdivision among populations was calculated in Arlequin v.3.5 using pairwise estimates of F_{ST}, where the significance of F_{ST} values was tested through 50,000 permutations for each pairwise comparison (Excoffier & Lischer, 2010). Arlequin was also used to compute population specific inbreeding coefficients (F_{IS}). The F_{ST} over all 10 loci and 12 geographical regions was calculated using FSTAT v.2.9.3.2 (Goudet, 2001). Genetic differentiation among geographical regions was also determined by calculating F_{ST} values and their significance in Arlequin v.3.5, using microsatellite data, as well as previously obtained mtDNA sequence data, which were also used in the study of Bilgin et al. (2016). Heterozygosity excess or deficit was tested with Bottleneck (Cornuet & Luikart, 1997; Piry et al., 1999). The sign test and Wilcoxon's sign rank test were used under the two-phase mutation model (TPM). Proportion of single-step mutations in TPM and the variance for TPM were set to 90% and 12, respectively. For the analyses at the individual sampling-site level (i.e. not regional), only those with a sample size of eight or larger were included.

Population structure was investigated using STRUCTURE v.2.3.4 (Pritchard et al., 2000), TESS v.2.3.1 (Caye et al., 2016) and the R package Geneland v.4.0.3 (Guillot et al., 2012) on all 333 *Miniopterus schreibersii* samples. In STRUCTURE, K was set from one to eight, representing the minimum and maximum number of suspected populations, and for each value of K, 10 simulations were run. The admixture model with correlated allele frequency was assumed, and the model was run with a burn-in length of 50,000 and MCMC (Markov chain Monte Carlo) chains of 50,000 without prior population information. The ΔK statistic that reflects the most likely value of K, as developed by Evanno et al. (2005) was determined with Structure Harvester Web v.0.6.94 (Earl & Vonholdt, 2012). CLUMPP v.1.1.2 (Jakobsson & Rosenberg, 2007) was used to align the 10 repetitions and the results were displayed graphically with Distruct v1.1 (Rosenberg, 2004). When the most likely value for K was determined as 2, this visualized STRUCTURE output was examined to check if the real value of K was 1 or 2, as the Evanno method can only be applied when K is 2 and bigger (Evanno et al., 2005). In CLUMPP, the FullSearch algorithm was used to calculate the individual ancestry coefficients, which were also weighted by the number of individuals in each population. In order to

incorporate the spatial coordinates of each individual in the clustering procedure, the R package Geneland was used. The population number was set to 1-10 in ten different runs of 100,000 MCMC iterations with a thinning value of 100, thus storing 1000 iterations for each run, of which 200 were used as burn-in. The Poisson maximum was set to 400, whereas the Poisson-Veroni tessellation was set to 1200. Correlated allele frequencies and null alleles were allowed for these analyses. R package maptools was used to project the resulting cluster memberships to a shapefile of the Palearctic. The software TESS, which can incorporate spatial data in its analysis and does not assume HWE, was also used to investigate structuring among populations. Ten simulations were run for each value of K, which were set from 2 to 10. The CAR model of admixture was used, and 50,000 sweeps of MCMC were run after 10,000 sweeps of burn-in. Again, CLUMPP was used to average multiple runs and correct for label switching, and Distruct for displaying the results graphically.

Finally, approximate Bayesian computations (ABC) were utilized to compare 15 possible scenarios (=rooted trees) regarding the phylogeographic history and ancestral relationships of *Miniopterus schreibersii* populations. For this purpose, microsatellite data and mtDNA sequence data (a partial fragment of HV1; Bilgin et al., 2016), for the four regions with the highest genetic diversities (Anatolia, Balkans, Slovakia and Iberia) were analyzed with the software DIYABC v.2.1.0 (Cornuet et al., 2014); these four regions resulted in the 15 rooted tree combinations mentioned above. The ABC was also undertaken for the mtDNA only data set. Out of 1,000,000 simulated data sets for each scenario, 100,000 closest to the observed data set were selected for the computation of posterior probabilities of all scenarios via logistic regression. In the logistic regression run, 126 summary statistics were replaced by components of a linear discriminant analysis in total, and 10 intermediate values were used.

2.2. Analyses for *Myotis capaccinii*

2.2.1. Sampling

A total of 273 tissue samples from 10 countries and 40 populations (including two unknown populations from Turkey and Greece) were analyzed. However, another set of 265 samples (excluding eight samples from the two unknown populations) was used for most analyses (Figure 2.2 & Table 2.2). Wing-punches were stored in 80% ethyl alcohol or dried with silica-gel until further processing (Worthington Wilmer & Barratt, 1996; Puechmaille et al., 2011).

2.2.2. Laboratory Procedures

For the DNA extractions, one biopsy punch from each bat was used and a PureLink® Genomic DNA Mini Kit (Invitrogen) was utilized, following the protocols of the manufacturer. A partial cytochrome-b region (cyt-b) was amplified using the primers Molcit-F (Ibanez et al., 2006) and MVZ-16 (Smith and Patton, 1993). The PCR products were sequenced commercially at Macrogen Inc. (South Korea). Sequencer v.4.7 (Gene Codes Corp) was used to align and edit the sequences.

2.2.3. Statistical Analyses

PopArt v.1.7 (Leigh & Bryant, 2015) was used to construct a haplotype network of 265 samples with the TCS network option (Clement et al., 2000). The relationship between haplotypes was also investigated by constructing phylogenetic trees with MEGA v.7.0.26 (Kumar et al., 2016). The substitution model for the maximum likelihood analysis was determined as the Hasegawa-Kishino-Yano (HKY) model (no Gamma distributed rate or invariant sites) with jModeltest v.0.1.1 (Posada, 2008; Guindon & Gascuel, 2003). For the neighbor-joining tree, maximum composite likelihood distances were used. For the maximum parsimony tree, 10 initial trees were used and Tree-Bisection-Reconnection search algorithm was applied to obtain a proper set of cladograms (Kitching et al., 2000). For all three methods, nodal support was evaluated by 1000 bootstrap replicates and gaps were treated as missing data. Another phylogenetic tree was constructed with Bayesian inference using BEAST v.1.8.4 (Drummond et al., 2012). For this analysis the HKY substitution model, Yule process, and a strict molecular-clock with a rate of 4.8% divergence per million years for the cyt-b gene (Bilgin et al., 2008; Ruedi & Mayer, 2001) were used. The analysis was run for 50,000,000 generations, logging every 5000 states and discarding the first 10% states as burn-in, resulting in effective sample sizes (ESS) greater than 1000 for all traces. The results were visualized using Tracer 1.6 (Rambaut et al., 2014).

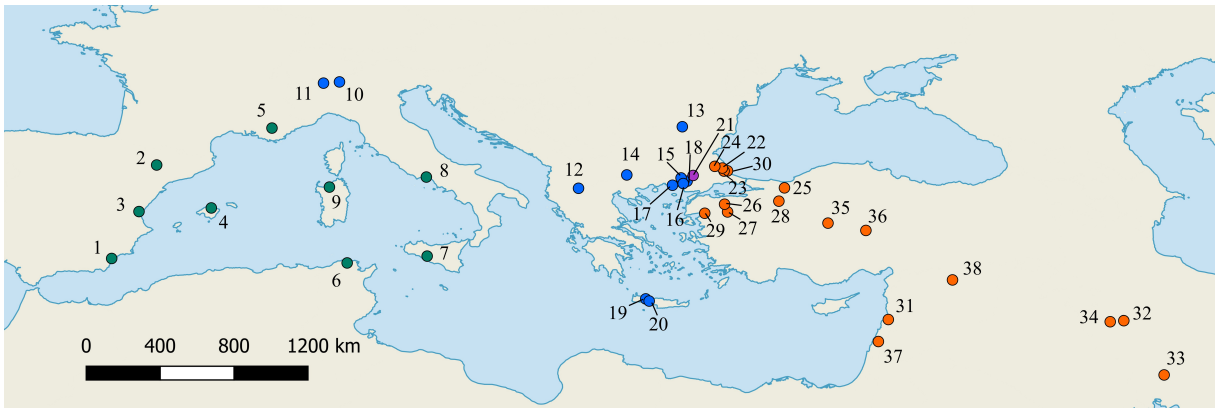


Figure 2.2. Map of sampling locations. The map and color codes correspond to those in Table 2.2.

Table 2.2. Sampling sites for *Myotis capaccinii*. **N** stands for the number of samples. The color codes representing different haplogroups correspond to those in Table 3.4.

Region	Population	Country	N	Map Code	Latitude	Longitude
Iberia	Almeria	Spain	10	1	37.39	-1.76
	Huesca	Spain	8	2	41.9	0.43
	Valencia	Spain	8	3	39.66	-0.43
	Mallorca	Spain	10	7	39.83	3.1
France	France	France	10	4	43.71	6
N. Africa	Tunisia	Tunisia	27	5	37.14	9.67
Italy	Sicily	Italy	5	6	37.48	13.55
	Lazio	Italy	5	8	41.31	13.49
	Sardegna	Italy	10	9	40.82	8.81
Italy	Lombardia	Italy	10	10	45.96	9.3
	Piemonte	Italy	2	11	45.89	8.53
Thrace - Balkans	Albania	Albania	10	12	40.78	20.91
	General Todorov	Bulgaria	1	13	41.47	23.27
	Krasen	Bulgaria	2	14	43.74	25.98
	Katratzides	Greece	3	15	41.13	26.16
	Lefkimis	Greece	1	16	41.07	26.19
	Maronia	Greece	18	17	40.93	25.5
	Tsoutouro	Greece	31	18	41.15	26.19
	Dramia	Greece	1	20	35.28	24.32
	Argyroupoli	Greece	1	21	35.35	24.25
Thrace - Balkans	Koufovouno	Greece	31	19	41.35	26.45
Thrace - Balkans	Kav	Turkey	1	22	41.71	27.91
	Yenasu	Turkey	3	23	41.62	27.97
	Kuru	Turkey	1	24	41.84	27.56
Anatolia	Aksu	Turkey	5	25	40.76	30.96
	Boğazpınar	Turkey	7	26	40.02	28.02
	Nusret Düdeni	Turkey	3	27	39.62	28.16
	Beyyayla	Turkey	7	28	40.13	30.7
	Karan	Turkey	14	29	39.56	27.1
Thrace - Balkans	Kıyıköy	Turkey	1	30	41.64	28.09
Middle East	Haqel el Azim	Lebanon	1	31	34.4	36.02
	Bisotun	Iran	1	32	34.38	47.43
	Si Mili	Iran	1	33	31.7	49.4
	Eslamabad	Iran	3	34	34.27	46.79
Anatolia	Kulu	Turkey	1	35	39.09	33.08
	Sarıhıdır Nevşehir	Turkey	10	36	38.74	34.93
Middle East	Beaufort Castle	Lebanon	1	37	33.32	35.53
	Syria	Syria	1	38	36.33	39.08
Total number of samples analyzed:			265			

BEAST was also used to prepare Bayesian skyline plots (BSPs) separately for the three haplogroups B, C1 and C2 that were determined during the haplotype network construction (see results) to detect recent changes in their effective population sizes (Drummond et al., 2012). The same parameters that were used in Bayesian phylogenetic construction were used, except Bayesian skyline coalescence was selected instead of Yule process. The final analysis was run for 200 million, 250 million and 250 million generations for haplogroups B, C1 and C2, respectively, logging every 20,000 generations for B and every 25,000 generations for C1 and C2. Again, the first 10% of the states were discarded as burn-in.

The divergence time of these haplogroups was also estimated using BEAST v.1.8.4. The most common haplotypes from each of the three haplogroups (i.e., H11 from haplogroup B, H7 from haplogroup C1 and H1 from haplogroup C2) were analyzed together using two different methods. In the first approach, the three haplotypes were compared using a strict clock and Yule process once again with the same parameters as in BSPs for 100 million generations, logging every 10,000 generations and discarding the first 1000 states as burn-in. The second approach made use of an uncorrelated relaxed clock with a lognormal distribution and an outgroup species (i.e., *Myotis ricketti*, which is estimated to have diverged from *M. capaccinii* circa 8 million years ago according to Stadelmann et al., 2006). This analysis was run for 250 million generations, logging every 25,000 generations and discarding the first 1000 states as burn-in.

Descriptive statistics such as the number of haplotypes, haplotype (h) and nucleotide (π) diversities and the number of polymorphic sites (Nei and Tajima, 1981; Tajima, 1983; Graur and Li, 2000) were assessed using DnaSP v.5 (Librado and Rozas, 2009). Also Tajima's D (Tajima, 1989), Fu's F_S (Fu, 1997) and D (Fu & Li, 1993) and Harpending's raggedness index (r) (Harpending et al., 1993) were also determined with DnaSP. The significance of these statistics was assessed with 1000 coalescent simulations. DnaSP was also used to determine mismatch distributions for constant and expanding population sizes.

Differentiation in mtDNA among haplogroups was explored using analysis of molecular variance (AMOVA) (Excoffier et al., 1992) and the significance was evaluated using 1000 replications. The software Arlequin v.3.5 was used to carry out AMOVA ((Excoffier & Lischer, 2010). Arlequin was also used to determine F_{ST} values among populations and carrying out an exact test of population differentiation, using 100,000 steps in Markov chain and 10,000 dememorization

steps. In addition, Mantel tests were carried out with Arlequin to check for isolation-by-distance (IBD), using three sample sets consisting of samples belonging to haplogroups B, C1 and C2.

The relationship between the three haplogroups was also investigated using ABC. Three possible rooted scenarios were compared with the software DIYABC v.2.1.0 (Cornuet et al., 2014). For each scenario, 1,000,000 simulated data sets were used, and 10,000 closest to the observed data set were selected for the computation of posterior probabilities of all scenarios via direct estimation, and 300,000 sets were selected for the logistic regression. In the logistic regression run, 39 summary statistics in total were replaced by components of a linear discriminant analysis, and 10 intermediate values were used.

For the SDM part of this study, a maximum entropy modelling technique as implemented in MAXENT v.3.4.1 (Elith et al., 2011) was used, where probability densities were calculated using presence data and environmental predictors to estimate the current distribution of the species, as well as to project possible past and future distributions of the species. 19 environmental predictors were downloaded from WorldClim (Hijmans et al., 2005) (Table 2.3). A five arc-minutes (ca. 10 km) resolution was selected. These variables are associated with the metabolic rates, water loss and gestation times of animals and their effects on the distribution of bats are well established, which is also outlined in Table 2.3 (Ulrich et al., 2007; Adams & Hayes, 2008; Racey & Speakman, 1987).

The collinearity between the predictors was first checked with ENM Tools v.1.3 (Warren et al., 2008, 2010) to get more parsimonious models (Merow et al., 2013). Yet there were high correlations among all predictors (Pearson correlation coefficient > 0.9). To determine the relative contributions of the predictors to the models built, the jackknife test implemented in MAXENT was carried out, which calculated the variable importance by excluding all variables in turn and then also using all variables in isolation and determining the gain from each of them. Also response curves that show the effect of the variables on the Maxent prediction were generated in two ways. Firstly, each variable itself is varied, when keeping all the others at their average value. Secondly, each variable is used alone to build the prediction model. After assessing the results of these analyses, eight variables (BIO7, BIO8, BIO9, BIO12, BIO13, BIO16, BIO18 and BIO19) were selected for the final analyses.

Table 2.3. The climatic predictors that were used in SDMs. All were downloaded from Worldclim (www.worldclim.org) with a 5 arc-minute resolution. The variables that were used in the final SDMs are color-coded with green.

Code	Variable Name	Influence on bats (Ulrich et al., 2007)
BIO1	Annual Mean Temperature	**Affects metabolic rate and water loss**
BIO2	Mean Diurnal Range (Mean of monthly (max temp - min temp))	
BIO3	Isothermality (BIO2/BIO7) (* 100)	
BIO4	Temperature Seasonality	
BIO5	Max Temperature of Warmest 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	**It is a proxy of water availability**
BIO13	Precipitation of Wettest Month	
BIO14	Precipitation of Driest Month	
BIO15	Precipitation Seasonality	
BIO16	Precipitation of Wettest Quarter	
BIO17	Precipitation of Driest Quarter	
BIO18	Precipitation of Warmest Quarter	
BIO19	Precipitation of Coldest Quarter	

In the subsequent analyses, presence-only data was used, because absences data of bats is highly unreliable because of their low detectability (Bilgin et al., 2016). Occurrence data of the three haplogroups of *M. capaccinii* were analyzed separately. A 10-fold cross-validation to test all models, and auto features option was checked. Regularization was set to zero and a maximum iteration of 1000 was used.

The model predictions regarding the current distribution of the haplogroups were projected to the last glacial maximum (LGM; 23 000–18 000 years BP) and to 2070, using raster layers of the same bioclimatic variables downloaded from WorldClim. For both the LGM and 2070 data, the same two general circulation models (GCM), namely the Community Climate System Model (CCSM4.0) and the Model for Interdisciplinary Research on Climate Earth System Model (MIROC-ESM) were preferred (Gent et al., 2011; Watanebe et al., 2011). For the future projections, the representative concentration pathway scenario 8.5 was selected (Riahi et al., 2011). For these projections, the 10th percentile training presence was used as the threshold value, above which occurrence was assumed (Raes et al., 2009; Rebelo & Jones, 2010). Two GCMs were used in order to compare different

projections and get more reliable predictions, because differences in the prediction between GCMs are possible (e.g., Diniz-Filho et al., 2009). These two GCMs were selected, because they were the only two scenarios available for both the past (i.e., the LGM) and the future (i.e., the year 2070). Furthermore, these two GCMs were successfully used in the environmental niche modelling of other bat studies (Rebelo et al., 2012; Bilgin et al., 2016; Flanders et al., 2011). Finally, the resulting models were overlapped in order to determine stable environments for the species, where it can survive during both glacial and interglacial periods as done previously for *Miniopterus schreibersii* (Bilgin et al., 2016), and to get more reliable, albeit more conservative projections. In order to determine these stable areas, predictions for the past and future distributions of *Myotis capaccinii* obtained from both models were assessed together, marking only the pixels that were suggested by both models as potential presence points. These areas were further overlapped with the predictions for the current distribution of the species, so that areas, which are suitable for *Myotis capaccinii* in the projected distributions (for the LGM or for 2070) as well as in the current climatic conditions, the so-called stable areas, were detected.

A list of all analyses is given in Table 2.4.

Table 2.4. A list of all major analyses and the software used for them.

Species	Software	Analysis
Miniopterus schreibersii	Micro-Checker v.2.2.3	To check for null alleles, large allelic dropout and stutter
	GenAlEx v.6.5	Descriptive statistics, allele frequency graphs, principle coordinate analysis, Mantel test
	HP-Rare	Allelic richness and private allelic richness by rarefaction
	Genepop v.4.5.1	To check for Hardy-Weinberg equilibrium (HWE) and linkage equilibrium betwen loci and populations
	Arlequin v.3.5	To determine FST & FIS values and their significance.
	FSTAT v.2.9.3.2	To determine the overall FST
	Bottleneck	To test for heterozygosity excess or deficit
	STRUCTURE v.2.3.4	To investigate structuring among populations and geographical regions
	TESS v.2.3.1	
	Geneland v.4.0.3	
	DIYABC v.2.1.0	To carry out approximate Bayesian computations
Myotis capaccinii	PopArt v.1.7	To generate a haplotype network
	jModeltest v.0.1.1	To determine the substition model for the maximum likelihood analysis
	MEGA v.7.0.26	To construct phylogenetic trees
	BEAST v.1.8.4	To construct a phylogenetic tree using Bayesian inference, to construct Bayesian skyline plots & to estimate divergence times
	Arlequin v.3.5	To carry out analysis of molecular variance, to determine FST values and their significance
	DIYABC v.2.1.0	To carry out approximate Bayesian computations
	MAXENT v.3.4.1	To generate species distribution models (SDMs)
	ENM Tools v.1.3	To check for collinearity between SDM predictors

3. RESULTS

3.1. *Miniopterus schreibersii*

Out of 40 populations, 10 did not have enough samples for Microchecker analysis, based on the requirements of the program (Van Oosterhout et al., 2004). After adjusting for null alleles according to van Oosterhout et al. (2004), only one population (Svetlaya Cave in Russia) still showed signs of null alleles at CHD2, because of homozygote excess at this locus. No evidence for stutter-bands or large allelic dropout was found. No linkage disequilibrium was detected between pairs of loci and according to the Genepop analysis, all loci were in Hardy-Weinberg equilibrium (Fisher's method; Bonferroni adjusted $P < 0.05$). Also 39 out of 40 populations were in Hardy-Weinberg equilibrium, with the exception of the Drienovská population in southern Slovakia (Fisher's method; $P < 0.05$). Among the 12 geographical regions defined in the study, three were not in HWE, namely Iberia, Slovakia and Thrace & Balkans.

3.1.1. Genetic Differentiation and Clustering

Relatively low levels of genetic differentiation were observed for *M. schreibersii* along its distributional range (Figures 3.1, 3.2 and 3.3). The overall F_{ST} was determined as 0.015. No pattern of IBD was found (Mantel test $R^2 = 0.024$, $P = 0.13$) and there was no clear pattern of variation in nuDNA among regions and populations based on the PCA (results not shown).

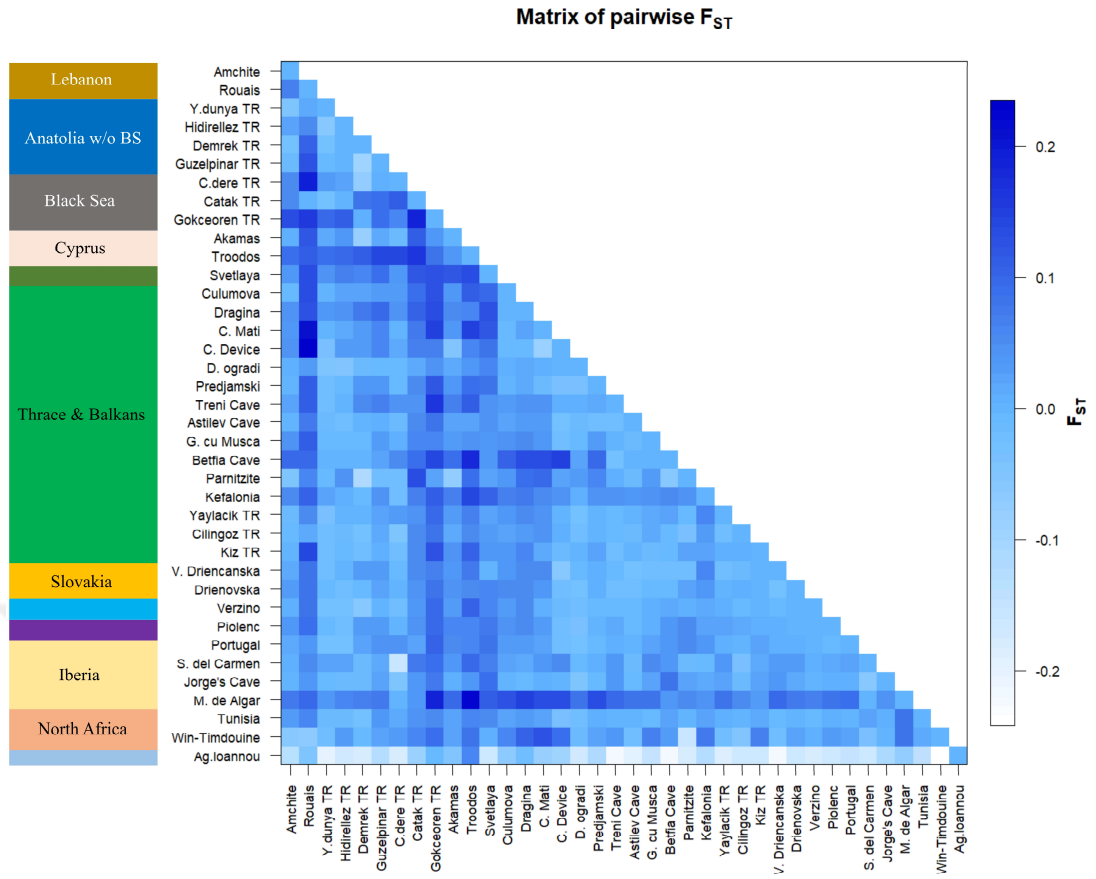


Figure 3.1. F_{ST} values for 38 populations (excluding Sicily and Kocakuyu populations, from which only one individual was sampled) and 12 regions. The color codes of the regions correspond to those in Figure 2.1 & Table 2.1. The significance for these F_{ST} comparisons can be found in Figure 3.2.

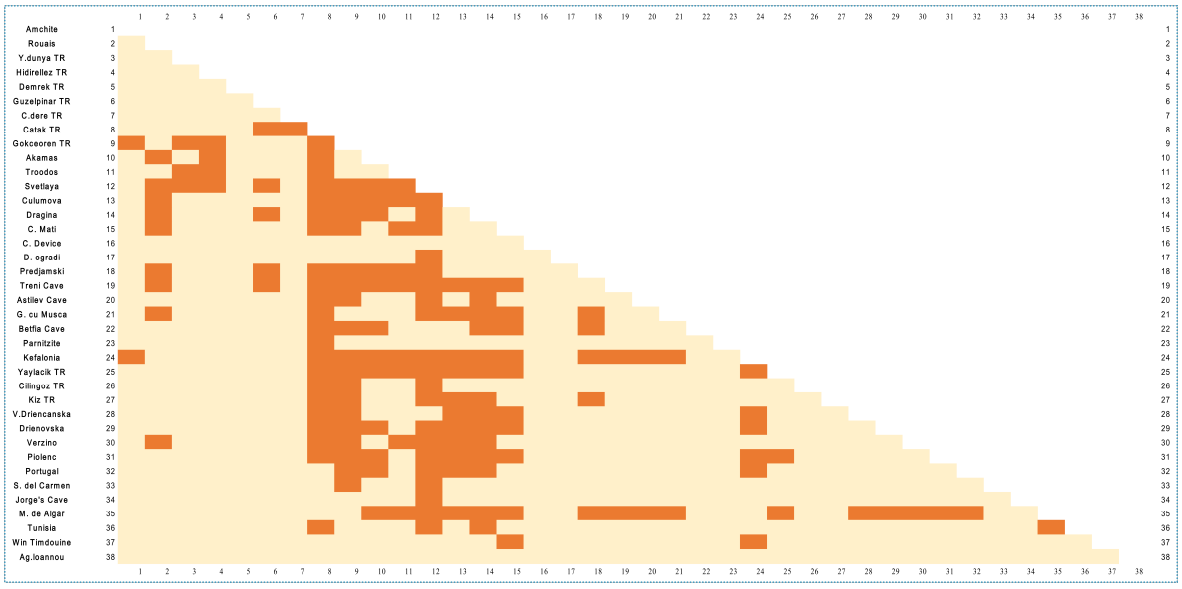


Figure 3.2. Matrix of F_{ST} significance. The dark orange squares correspond to F_{ST} *P* values that are significant at the 0.05 level.

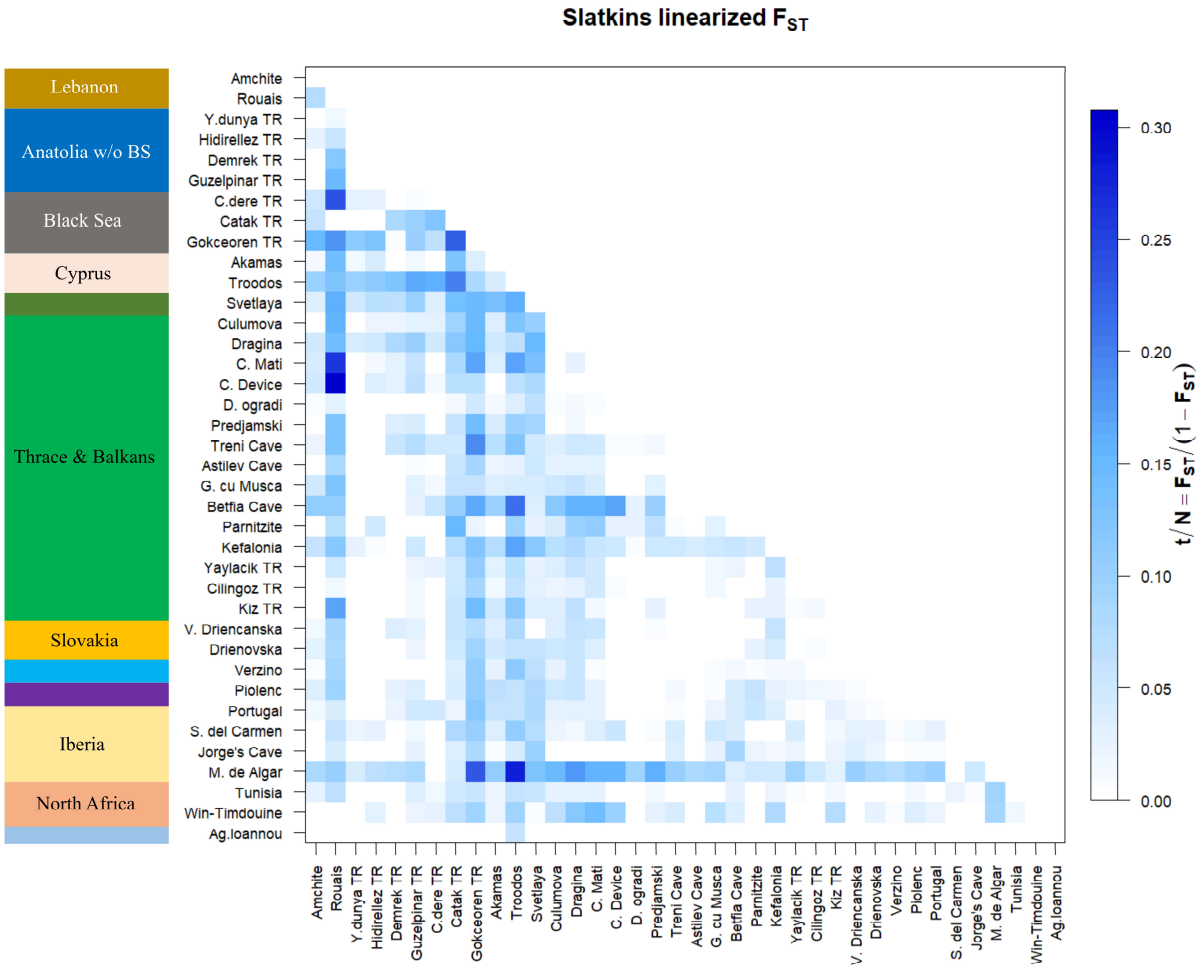


Figure 3.3. Slatkin's linearized F_{ST}.

When the geographical regions of *M. schreibersii* were compared using mitochondrial sequence data, very great (>0.25) (Wright, 1943; Hartl & Clark, 1997) and significant F_{ST} values, and thus genetic differentiation was detected among most regions (Table 3.1a) (see also Bilgin et al., 2016). However, the same pattern of differentiation was not detected, when microsatellite data were utilized (Table 3.1b). In this case, most F_{ST} values were not significant and even the significant ones had much smaller values (<0.05), indicating little genetic differentiation (Wright, 1943; Hartl & Clark, 1997). Consequently, cytonuclear discordance was detected for *M. schreibersii*, when comparing its populations from corresponding geographical regions, regarding its nuclear and mitochondrial data.

Table 3.1. The pairwise F_{ST} values between regions, with those significantly greater than zero ($P<$ 0.05) shown in bold. The first table, (a), was produced using mtDNA data, whereas in (b), microsatellite data were used. Different individuals from the same populations were sampled for the mtDNA and microsatellite analyses. T & B stands for the Trace & Balkans region, B. Sea, for the Black Sea and N. Afr., for North Africa.

(a)	Cyprus	T & B	B. Sea	Anatolia	France	Iberia	Italy	Lebanon	N. Afr.	Slovakia
Cyprus	0.00000									
T & B	**0.68334**	0.00000								
B. Sea	**0.60791**	**0.28126**	0.00000							
Anatolia	**0.41062**	**0.40765**	**0.36683**	0.00000						
France	**0.73009**	**0.27690**	**0.30572**	**0.42787**	0.00000					
Iberia	**0.81991**	**0.07331**	**0.34900**	**0.54646**	**0.41713**	0.00000				
Italy	**0.85596**	-0.08142	0.11516	**0.29991**	0.20458	0.00903	0.00000			
Lebanon	**0.73185**	**0.53963**	**0.42564**	**0.14623**	**0.58754**	**0.73940**	**0.63517**	0.00000		
N. Afr.	**0.95659**	0.01473	**0.26923**	**0.40586**	**0.33137**	**0.01152**	0.15323	**0.86581**	0.00000	
Slovakia	**0.83844**	-0.00761	**0.27343**	**0.35839**	**0.32002**	**0.05955**	-0.10564	**0.69813**	0.04320	0.00000

(b)	Cyprus	T & B	B. Sea	Anatolia	France	Iberia	Italy	Lebanon	N. Afr.	Slovakia
Cyprus	0.00000									
T & B	**0.03335**	0.00000								
B. Sea	**0.04737**	**0.01613**	0.00000							
Anatolia	**0.04317**	0.00517	0.00469	0.00000						
France	**0.04964**	0.00879	0.01440	0.00803	0.00000					
Iberia	**0.03833**	0.00228	0.00275	-0.00036	0.00334	0.00000				
Italy	**0.04660**	-0.00090	0.00417	-0.00962	0.00135	0.00418	0.00000			
Lebanon	0.04087	0.02351	0.00869	0.01383	**0.04513**	0.00918	0.02047	0.00000		
N. Afr.	0.03128	0.00534	0.00823	0.00936	0.00778	0.00811	-0.00815	0.02170	0.00000	
Slovakia	**0.03933**	**0.00990**	**0.02248**	0.01070	0.00817	**0.02058**	0.00615	**0.03930**	0.00658	0.00000

There was a relatively uniform distribution of genotypes among samples, which showed no strong signal of structuring as evidenced by STRUCTURE and TESS results (Figure 3.4). When the Evanno method was applied, the most likely value of K among K values 2-8, was found to be 2 (ΔK=21.14). Although TESS does not assume HWE for the different clusters, and makes use of spatial coordinates (unlike STRUCTURE), the output for all the tested values of K was very similar for both programs (Figure 3.4). To determine whether the real value was 1 or 2, the Distruct graphs were examined, and in both STRUCTURE and TESS outputs, most samples belonged to the same cluster and there was no clear separation of two different groups, so the real value of K was determined as 1 (Figure 3.4). Similarly, according to the Geneland analysis, the populations formed a single cluster (average posterior log probability after 200 burn-in = -4669.96).

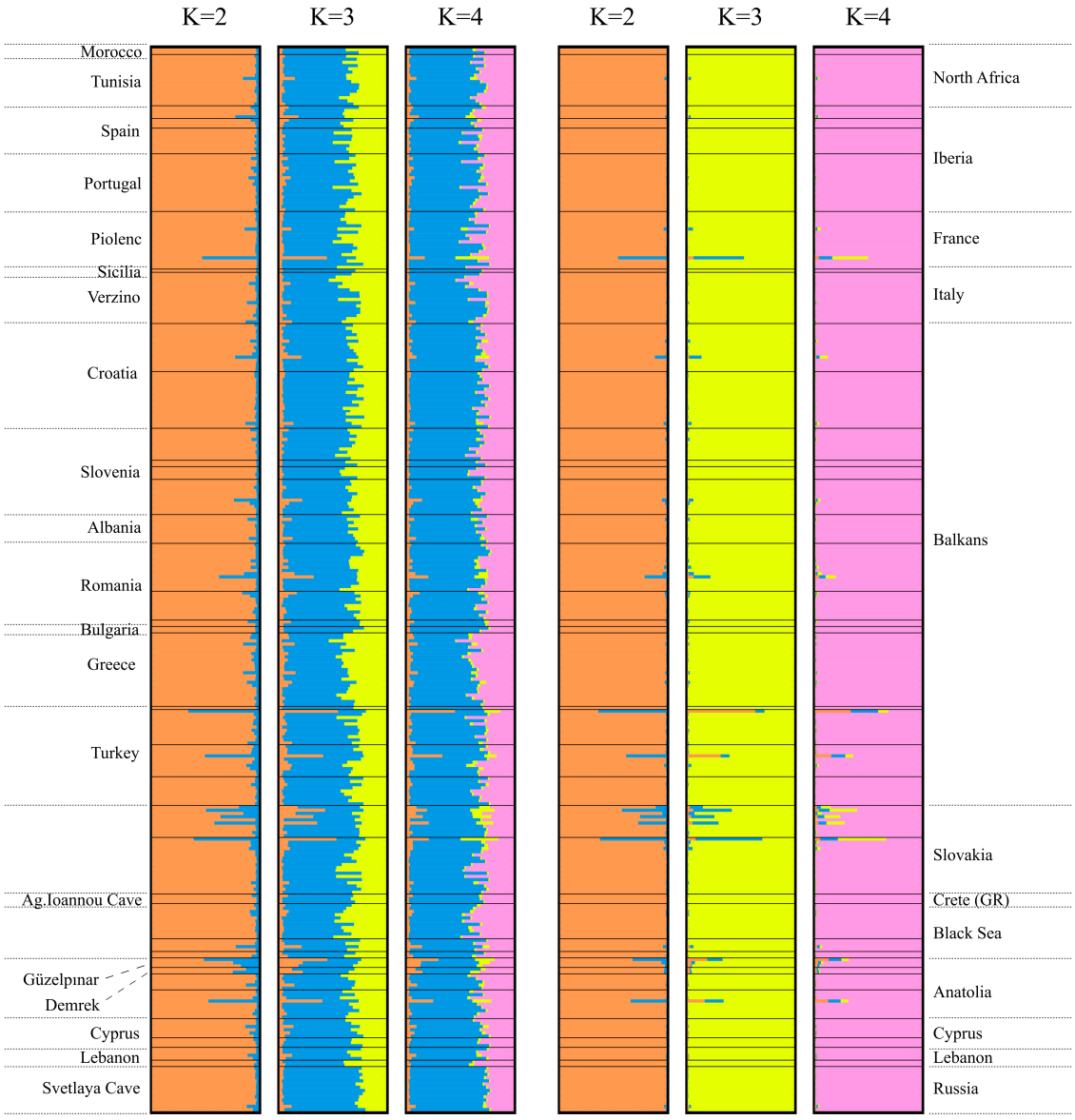


Figure 3.4. Inferred population memberships of *M. schreibersii* individuals according to (a) STRUCTURE and (b) TESS analysis of 10 microsatellite loci. The number of clusters, K, varied from two to four. Most important populations are listed on the left, and all 12 defined regions of *M. schreibersii* are listed on the right.

3.1.2. Phylogeography

Descriptive statistics are given in Figure 3.5 and Table 3.2. The Thrace & Balkans region had the highest number of alleles (N_a=7), followed by Slovakia (N_a=5.2) and Iberia (N_a=4.8). Yet the

region with the highest number of effective alleles was Slovakia (N_e=2.52), followed by Anatolia (N_e=2.5). Slovakia also had the highest value for Shannon's information measure (I=0.997). Shannon's information measure was 0.947 for the Thrace & Balkans region and 0.904 for Anatolia. The highest heterozygosity was again detected in Slovakia (He=0.489). For the population size of 17, the allelic richness (R) found by rarefaction was the highest for Slovakia (R=4.57), and was followed by Anatolia (R=4.55). The private allelic richness (R_P), on the other hand, was highest for Anatolia (R_P=0.26), followed by Iberia (R_P=0.18) and the Thrace & Balkans region (R_P=0.12). Similar results were found for population sizes of 6, 9 and 14 (Table 3.3). F_{IS} values were only significant for Iberia and the Thrace & Balkans, for values of 0.16 and 0.04, respectively, indicating heterozygote deficiencies in these regions. Under the TPM, heterozygote deficiencies were found in Anatolia, Crete and Thrace & Balkans with the sign test, and in Anatolia, Iberia and Thrace & Balkans with the Wilcoxon's test, whereas no heterozygote excess was detected in any region (P<0.05). Individual sampling-site based analyses under TPM suggested heterozygote deficiencies for Ag. loannou Cave (Crete, Greece), Kefalonia (Greece) and Portugal when the sign test was applied, and heterozygote deficiencies for Kefalonia (Greece) and Portugal, and once again a heterozygosity excess for Jorge's Cave (Spain), when Wilcoxon's test was applied (P<0.05).

Table 3.2. Descriptive statistics of *M. schreibersii* in 12 regions. N is the sample size, Na is the number of different alleles, CA is the number of common alleles with a frequency ≥ 5%, Ne is the number of effective alleles, I is Shannon's Information Index, R is the allelic richness (N=17), R_P is the private allelic richness (N=17), F_{IS} is the inbreeding coefficient and H_E is the expected heterozygosity. * indicates regions that are not in HWE and ** indicates the statistical significance of F_{IS} (P<0.05).

Region	N	Na	CA	Ne	I	R	R_P	F_{IS}	He
Anatolia	19	4.600	3.500	2.502	0.904	4.55	0.26	-0.067	0.432
Black Sea	17	3.900	2.800	2.228	0.807	3.9	0.08	0.089	0.417
Crete (GR)	3	2.100	2.100	1.615	0.540	NA	NA	0.053	0.317
Cyprus	9	3.300	3.300	2.387	0.824	3.3	0	-0.036	0.442
France	18	3.900	2.900	2.126	0.817	3.84	0.07	0.031	0.431
Iberia*	33	4.800	2.700	2.421	0.884	4.07	0.18	**0.156****	0.437
Italy	17	4.000	3.200	2.326	0.836	4	0.01	-0.095	0.422
Lebanon	6	3.000	3.000	2.223	0.737	3	0	0.167	0.400
North Africa	18	4.000	2.900	2.465	0.881	3.94	0.05	0.010	0.460
Russia	14	3.500	2.900	2.283	0.848	3.5	0.09	-0.097	0.461
Slovakia*	28	5.200	3.300	2.518	0.997	4.57	0.06	-0.012	0.489
Thrace & Balkans*	151	7.000	3.200	2.431	0.947	4.28	0.12	**0.041****	0.448
						Legend:	Highest	Second	Third

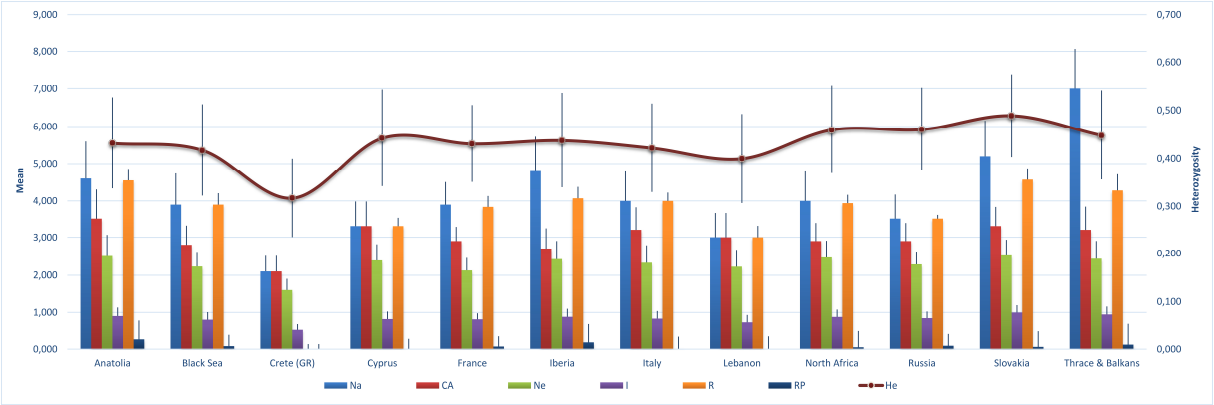


Figure 3.5. Allelic patterns across regions. Na is the number of different alleles, CA is the number of common alleles with a frequency ≥ 5%, Ne is the number of effective alleles, I is Shannon's Information Index, R is the allelic richness (N=17), RP is the private allelic richness (N=17) and He is the expected heterozygosity.

Table 3.3. HP-Rare analysis results. N is the sample size, R is the allelic richness; R_P is the private allelic richness. * indicates regions that are not in HWE.

		N=6		N=9		N=14		N=17	
Region	**N**	**R**	**R_P**	**R**	**R_P**	**R**	**R_P**	**R**	**R_P**
Anatolia	19	3.27	0.26	3.8	0.29	3.48	0.27	4.55	0.26
Black Sea	17	2.87	0.09	3.26	0.09	3.01	0.09	3.9	0.08
Cyprus	9	3	0.03	3.3	0.01	3.13	0.02	3.3	0
France	18	2.85	0.09	3.22	0.08	2.99	0.09	3.84	0.07
Iberia*	33	3	0.11	3.4	0.14	3.15	0.12	4.07	0.18
Italy	17	3.01	0.04	3.43	0.03	3.17	0.03	4	0.01
Lebanon	6	3	0.03	3	0.02	3	0.03	3	0
North Africa	18	3	0.05	3.34	0.05	3.13	0.05	3.94	0.05
Russia	14	2.9	0.05	3.22	0.06	3.02	0.06	3.5	0.09
Slovakia*	28	3.32	0.18	3.81	0.16	3.51	0.18	4.57	0.06
Thrace & Balkans*	151	3.09	0.11	3.54	0.12	3.26	0.11	4.28	0.12
						Legend:	Highest	Second	Third

Logistic regression of 15 scenario probabilities based on the deviations between simulated and observed summary statistics did not provide strong support for any scenario. When 10 microsatellite loci where analyzed together with the mtDNA data, the most probable scenarios were [[[Thrace & Balkans, Slovakia], Spain], Anatolia], [[Thrace & Balkans, Slovakia], [Spain, Anatolia]] and [[[Thrace & Balkans, Slovakia], Anatolia], Spain], where the region written before the rightmost bracket represents the most basal node. The posterior probabilities of the scenarios were low (P<0.5) and usually only around 0.2. The three theoretically most probable scenarios according to the mtDNA data only were [[[Thrace & Balkans, Slovakia], Spain], Anatolia], [[[Slovakia, Spain], Thrace & Balkans], Anatolia] and [[[Thrace & Balkans, Spain], Slovakia], Anatolia], respectively. As can be

seen, in all of these mtDNA scenarios, Anatolia is the most ancestral/basal region, yet the support for the most probable scenario was again relatively low, around 0.2 (Figure 3.6).

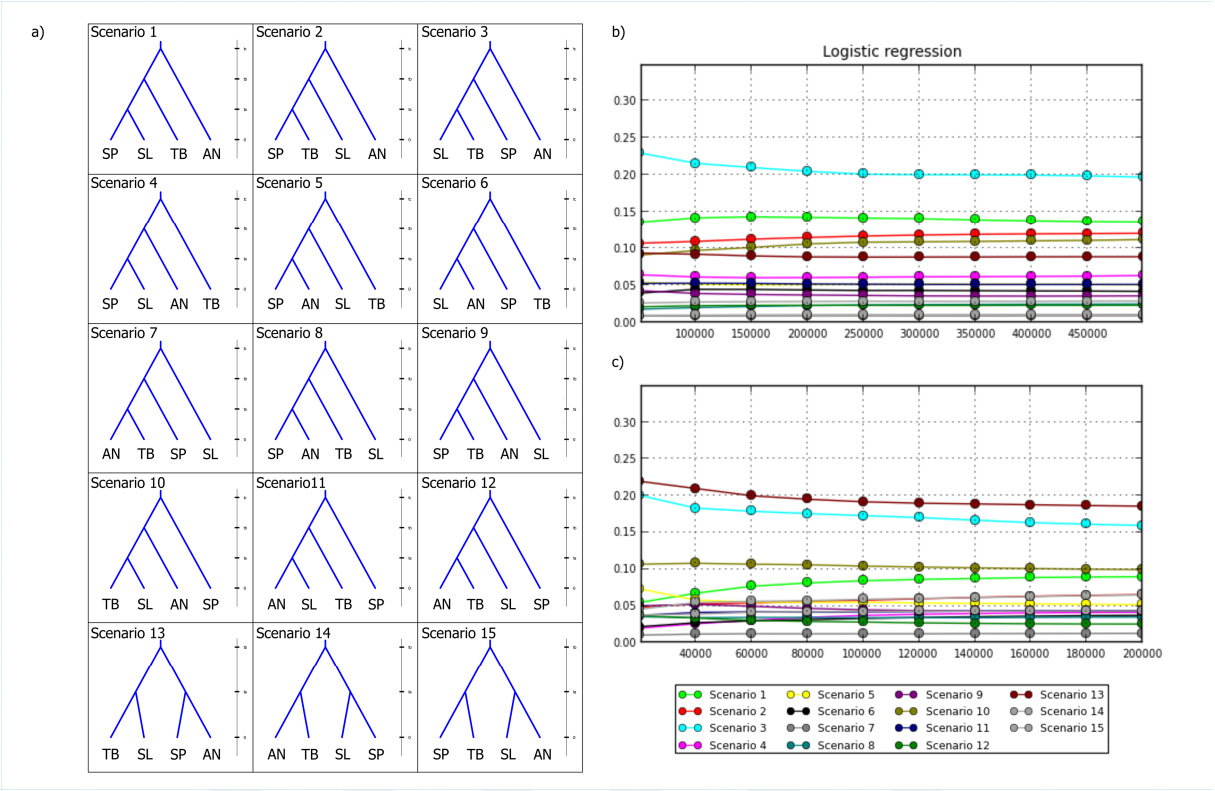


Figure 3.6. An ABC analysis of 172 *Miniopterus schreibersii* individuals genotyped at 10 microsatellite loci and one mtDNA locus. a) All 15 possible different scenarios (=rooted trees) were compared. The results of a logistic regression and the predicted probabilities of all 15 scenarios regarding four geographical regions, which are obtained using b) only mtDNA data and c) mtDNA and nuDNA data together, are shown.

3.2. *Myotis capaccinii*

3.2.1. Phylogeography

The final trimmed sequence of cyt-b was 692 base pairs long. The haplotypes are given in Appendix B. Descriptive statistics for the haplogroups, populations and geographical regions are given in Table 3.4.

Table 3.4. Descriptive statistics for the *M. capaccinii* samples. HG is the haplogroups category (B, C1 or C2, see below), h is the haplotype diversity, π is the nucleotide diversity, SV is the number singleton variable sites, PI is the number of parsimony informative sites and r is Harpending's raggedness index. Koufovouno is not included in Thrace B and Thrace C1 geographical regions. The significant values are in bold, where one asterisk signifies (P<0.05) and two asterisks signify (P<0.02). The color codes representing different haplogroups correspond to those in Table 2.2.

HG	Haplotypes	Size	Population	Country	Code	h	π	SV	PI	Tajima's D	Fu's F_S	Fu's D	r
C2	H1	10	Almeria	Spain	1	0	0	0	0	NA	NA	NA	NA
	H1	8	Huesca	Spain	2	0	0	0	0	NA	NA	NA	NA
	H1	8	Valencia	Spain	3	0	0	0	0	NA	NA	NA	NA
	H2	10	Mallorca	Spain	4	0	0	0	0	NA	NA	NA	NA
	H1	10	France	France	5	0	0	0	0	NA	NA	NA	NA
	H1, H2, H3	27	Tunisia	Tunisia	6	0.510	0.00078	1	1	0.068	-0.561	-0.702	0.211
	H1, H4, H5	5	Sicily	Italy	7	0.700	0.00289	2	2	0.273	0.278	0.273	0.710
	H2	5	Lazio	Italy	8	0.400	0.00058	1	0	-0.816	-0.772	-0.817	0.200
	H2	10	Sardegna	Italy	9	0	0	0	0	NA	NA	NA	NA
C1	H6	10	Lombardia	Italy	10	0	0	0	0	NA	NA	NA	NA
	H6	2	Piemonte	Italy	11	0	0	0	0	NA	NA	NA	NA
	H7	10	Albania	Albania	12	0	0	0	0	NA	NA	NA	NA
	H7	1	G. Todorov	Bulgaria	13	NA	NA	0	0	NA	NA	NA	NA
	H7	2	Krasen	Bulgaria	14	0	0	0	0	NA	NA	NA	NA
	H7	3	Katratzides	Greece	15	0	0	0	0	NA	NA	NA	NA
	H7	1	Lefkimis	Greece	16	NA	NA	0	0	NA	NA	NA	NA
	H7	18	Maronia	Greece	17	0	0	0	0	NA	NA	NA	NA
	H7, H9	31	Tsoutouro	Greece	18	0.065	0.00009	1	0	-1.145	-1.774	-1.693	0.763
	H8	1	Dramia	Greece	19	NA	NA	0	0	NA	NA	NA	NA
	H8	1	Argyroupoli	Greece	20	NA	NA	0	0	NA	NA	NA	NA
C1&B	H7, H10, H11	31	Koufovouno	Greece	21	0.185	0.00698	0	27	-1.021	0.972	**1.712****	0.679
B	H10	1	Kav	Turkey	22	NA	NA	0	0	NA	NA	NA	NA
	H10	3	Yenasu	Turkey	23	0	0	0	0	NA	NA	NA	NA
	H10	1	Kuru	Turkey	24	NA	NA	0	0	NA	NA	NA	NA
	H11, H12	5	Aksu	Turkey	25	0.400	0.00058	1	0	-0.817	-0.772	-0.817	0.200
	H11, H13	7	Boğazpınar	Turkey	26	0.286	0.00041	1	0	-1.006	1.101	-1.049	0.265
	H11	3	N. Düdeni	Turkey	27	0	0	0	0	NA	NA	NA	NA
	H11, H14	7	Beyyayla	Turkey	28	0.571	0.00083	0	1	1.342	1.101	0.953	0.347
	H11	14	Karan	Turkey	29	0	0	0	0	NA	NA	NA	NA
	H11	1	Kıyıköy	Turkey	30	NA	NA	0	0	NA	NA	NA	NA
	H11	1	H. el Azim	Lebanon	31	NA	NA	0	0	NA	NA	NA	NA
	H11	1	Bisotun	Iran	32	NA	NA	0	0	NA	NA	NA	NA
	H11	1	Si Mili	Iran	33	NA	NA	0	0	NA	NA	NA	NA
	H11	3	Eslamabad	Iran	34	0	0	0	0	NA	NA	NA	NA
	H15	1	Kulu	Turkey	35	NA	NA	0	0	NA	NA	NA	NA
	H16	10	Sarıhıdır	Turkey	36	0	0	0	0	NA	NA	NA	NA
	H16	1	B. Castle	Lebanon	37	NA	NA	0	0	NA	NA	NA	NA
	H17	1	Syria	Syria	38	NA	NA	0	0	NA	NA	NA	NA
C2	H1, H2	36	Iberia	NA	NA	0.413	0.0006	0	1	1.04744	1.369	0.57433	0.2008
	H1	10	France	France	NA	NA	NA	0	0	NA	NA	NA	NA
	H1, H2, H3	27	North Africa	Tunisia	NA	0.51	0.00078	1	1	0.06822	-0.56146	-0.70207	0.2111
	H1, H2, H4, H5	20	Italy C2	Italy	NA	0.432	0.00107	2	2	-1.003	-0.7	-0.75932	0.2209
C1	H6	12	Italy C1	Italy	NA	NA	NA	0	0	NA	NA	NA	NA
	H7, H8, H9	68	Thrace C1	NA	NA	0.086	0.00021	1	2	-1.48444	-1.81	-0.4935	0.7872
B	H10, H11	6	Thrace B	NA	NA	0.333	0.00048	1	0	-0.93302	-0.003	-0.95015	0.2222
	H11, H16, H17	8	Middle East	NA	NA	0.464	0.00145	4	0	-1.5347	0.204	-1.66525	0.2283
	H11-H16	47	Anatolia	Turkey	NA	0.702	0.00135	2	4	-0.80152	-1.304	-0.52004	0.1548
C1	H6 - H9	111	NA	NA	NA	0.242	0.00096	1	4	-0.62185	-0.27986	-0.04824	0.6136
C2	H1- H5	93	NA	NA	NA	0.534	0.00095	2	3	-0.70802	-1.11607	-1.06357	0.1661
B	H10 - H17	69	NA	NA	NA	0.718	0.00150	3	7	-1.35270	-0.99891	-0.58370	0.1288
All	H1- H17	273	NA	NA	NA	0.804	0.01777	5	38	**2.19087***	1.59991	0.64269	0.0606

There was a total of 18 haplotypes in all 273 sequences and a total of 43 polymorphic sites, 38 of which are parsimony informative. The haplotype diversity of all samples was 0.804 (± 0.015 = 1 SD), and the nucleotide diversity was 0,01777 (± 0,00077). The significant Tajima's D was 2.1909 (P<0.05). Fu's F_S and D and Harpending's raggedness index (r) were all insignificant.

The haplotype network clearly reveals the existence of three haplogroups, which can be termed as B, C1 and C2, in a similar vein to Bilgin et al. (2008b) (Figure 3.7). Among the 265 samples that were used to construct this network, the most common one is H7, which is encountered in 93 samples and a part of haplogroup C1. There are four haplotypes in total in this haplogroup (H6-H9). Haplogroup B, on the other hand, has the highest number of haplotypes, consisting of eight haplotypes (H10-H17), of which H11 is the most common haplotype found in 31 samples. The other seven haplotypes of haplogroup B differ from H11 by 1-3 bp and are clustered around it, forming a clear star-like pattern. There are five haplotypes in Haplogroup C2 (H1-H5), the most common of which are H1 and H2, encountered in 54 and 34 samples, respectively. No clear star-like pattern is observed in haplogroups C1 and C2. Haplogroup B is the most diverged one, differing from haplotypes of haplogroup C1 at least by 26 bp and from haplotypes of haplogroup C2 at least by 24 bp. C1 and C2 differ from each other at least by 6 bp.

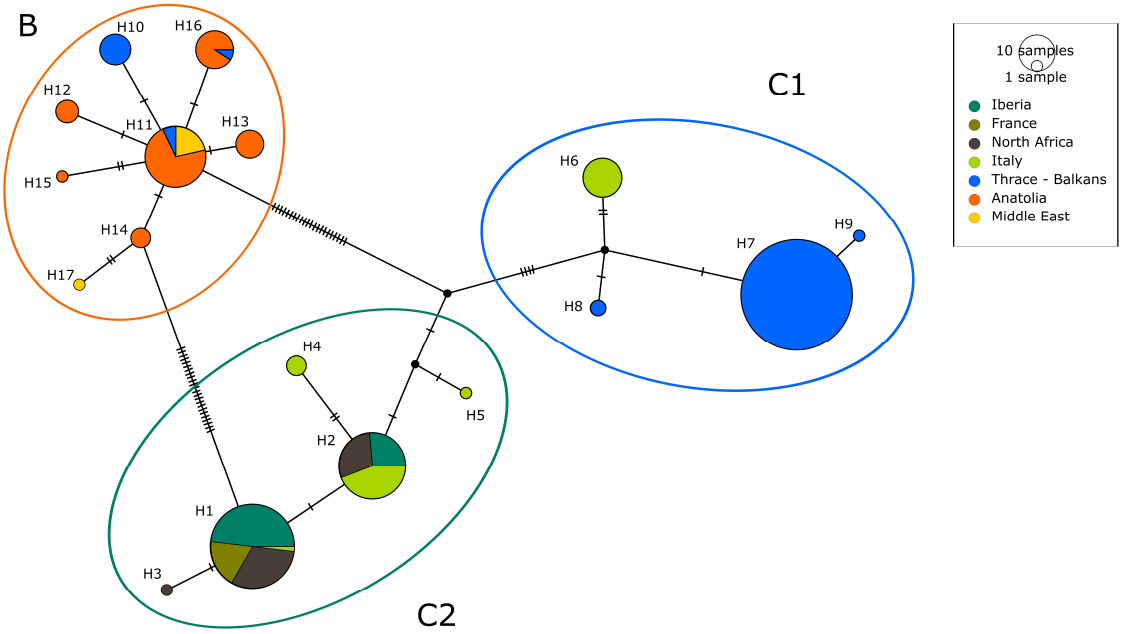


Figure 3.7. The haplotype network for 265 cyt-b sequences (692 bp) and their distribution based on geographical regions. The sizes of the circles are proportional to the frequency of the haplotypes they represent. The small dots stand for missing haplotypes. Another version of this network, which is based on 38 populations instead of geographical regions, is given in Appendix C.

Haplotype diversities of haplogroups B, C1 and C2 are 0.718 (± 0,049), 0,242 (± 0,050), 0,534 (± 0,031) and the nucleotide diversities were 0,00150 (± 0,00019), 0,00096 (± 0,00021), 0,00095 (± 0,00012), respectively. None of the other descriptive statistics were significant for any haplogroups or populations, except Fu and Li's D for Koufovouno population in Greece, because all 27 polymorphic sites of this population are parsimony informative and there are no singleton variable sites. Tajima's D was negative for all haplogroups, indicating expansion, yet none of the values were significant.

The geographical split zone between haplogroups B and C1 is in Thrace, around the Turkish border with Greece. In fact, haplotypes from both haplogroups were found in the same population in Koufovouno, Greece (Figure 3.8). The split zone between haplogroups C1 and C2, on the other hand, is around northern Italy (Figure 3.9).

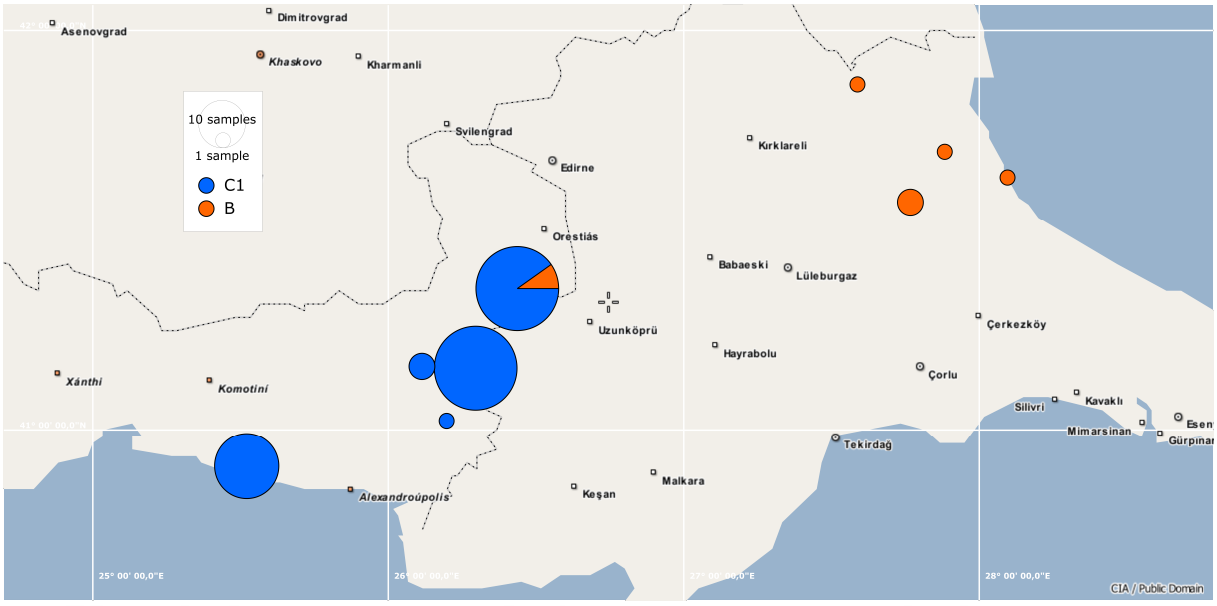


Figure 3.8. The split zone between haplogroups B and C1. The sizes of the circles are proportional to the population sizes.

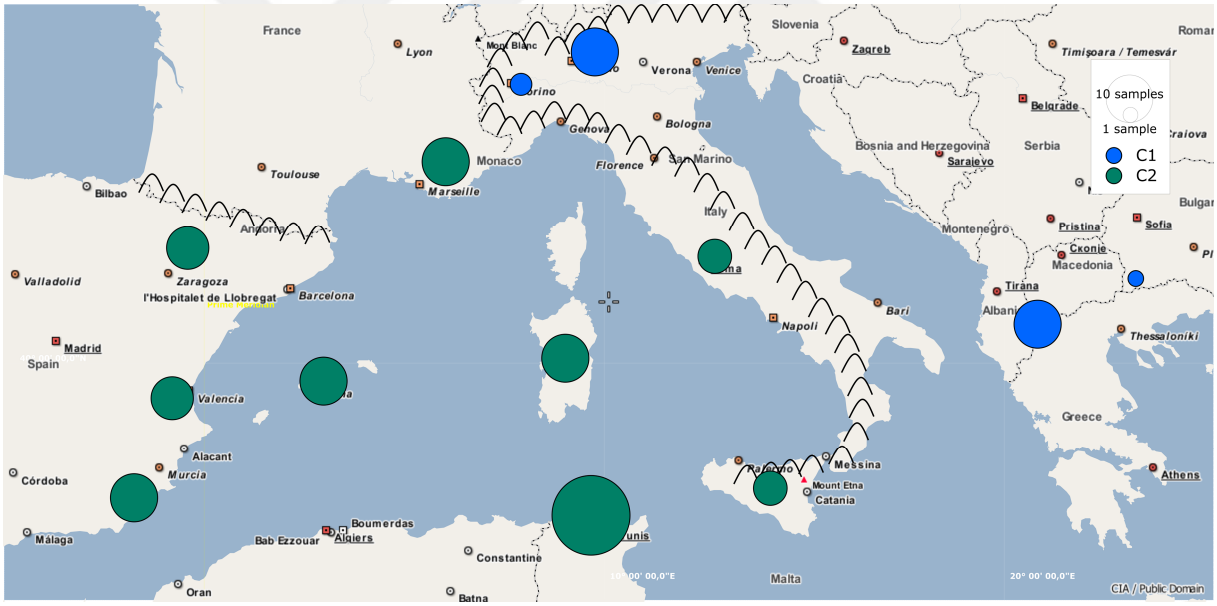


Figure 3.9. The split zone between haplogroups C1 and C2. The sizes of the circles are proportional to the population sizes. The mountains extending along the France-Italy border, as well as the northern borders of Italy are the Alps, whereas the Apennine Mountains stretch from the northwest to the southeast of Italy. The Apennines, along with the Alps, act as a geographic barrier separating haplogroups C1 and C2. The mountains separating France from Iberia are the Pyrenees.

When the 17 haplotypes were analyzed together, three out of four phylogenetic tree construction methods, namely Bayesian inference, neighbor-joining and maximum likelihood, showed the

presence of the same three haplogroups, or clades, with high bootstrap support (Figures 3.10-3.13). The maximum parsimony analysis also separated clade C haplotypes from the rest of the haplotypes and differentiated between clades C1 and C2 with high bootstrap support, yet it did not cluster the clade B haplotypes together (Fig 8). The tree topologies constructed using the other three methods, however, were very similar, resulting in reoccurring subclades within haplogroups, such as (H1, H3) in haplogroup C2, (H7, H9) in haplogroup C1, and (H14, H17) in haplogroup B.

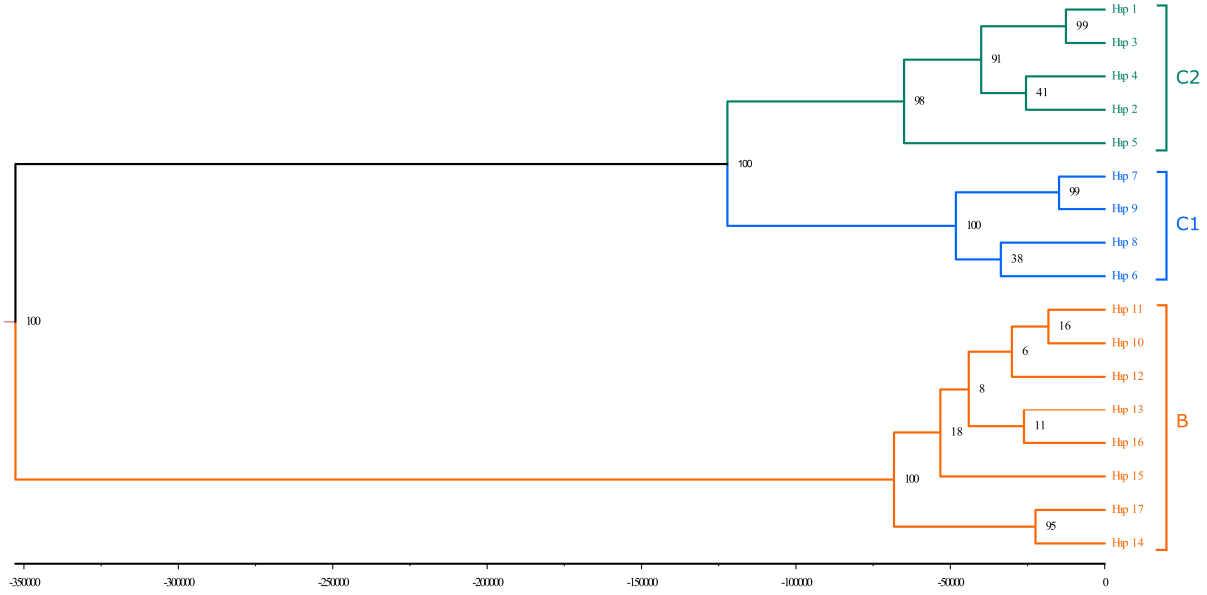


Figure 3.10. The Bayesian phylogenetic tree of 17 cyt-b haplotypes of *M. capaccinii*. The posterior probabilities are given as percentages on the nodes. The units on the reverse scale axis are years.

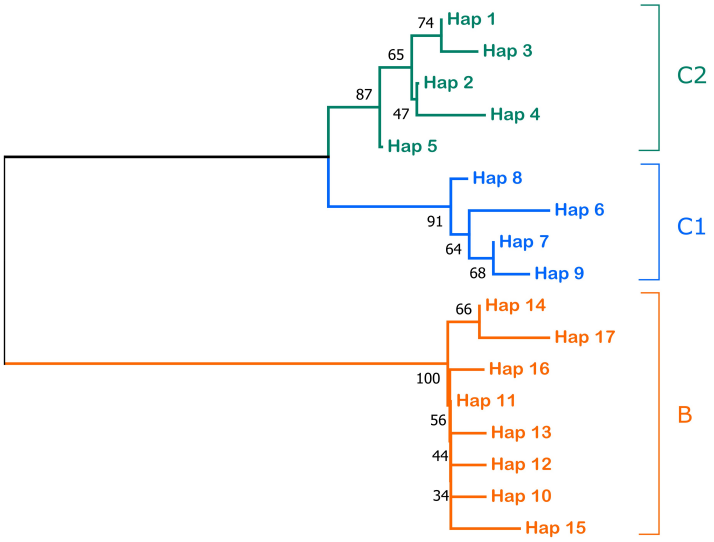


Figure 3.11. The neighbor-joining tree for *M. capaccinii*. The bootstrap support values are given on the nodes.

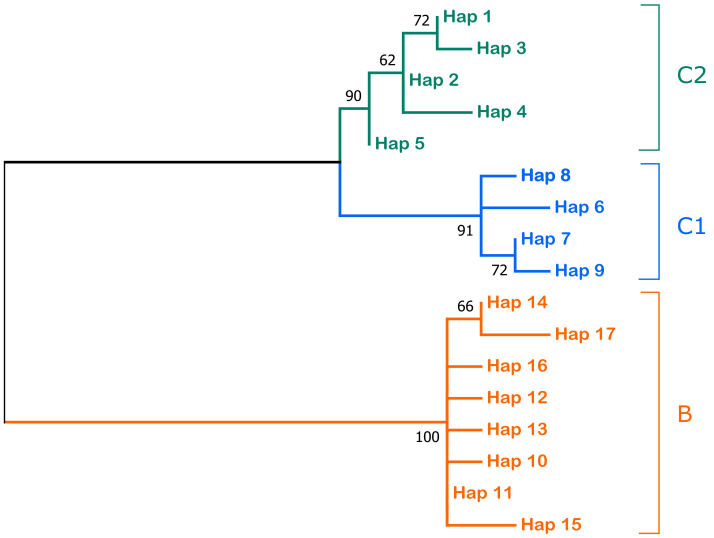


Figure 3.12. The maximum likelihood tree for *M. capaccinii*. The bootstrap support values are given on the nodes.

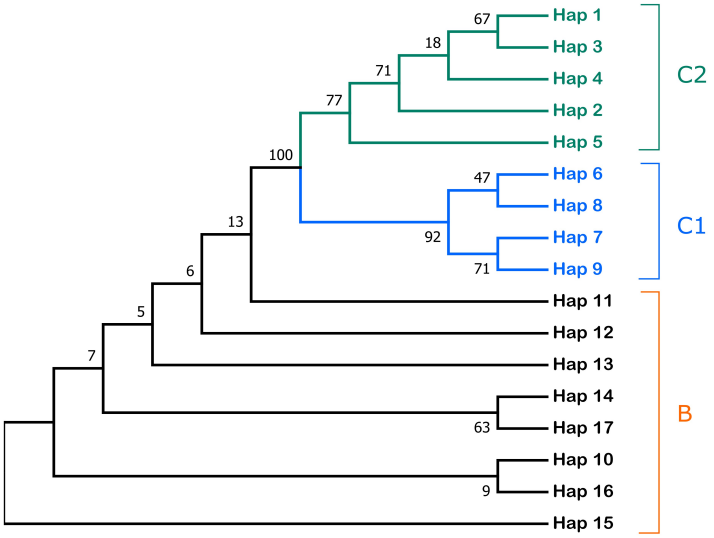


Figure 3.13. The maximum parsimony tree for *M. capaccinii*. The bootstrap support values are given on the nodes.

The divergence times for the B and C clades, as well as the C1 and C2 clades determined with two different methods using the most common haplotype from each group were similar. When a strict molecular-clock with a rate of 4.8% divergence per million years was used, the divergence time of clades B and C was calculated as 387,520 years (95% HPD Interval = 255,180 – 543960 years) (Figure 3.14). The divergence of C1 and C2 was determined as 126,360 years (95% HPD Interval =

50,638 – 212790 years). With the second method, where an uncorrelated relaxed clock was selected and *M. ricketti* was used as an outgroup, the divergence time of *M. capaccinii* and *M. ricketti* was adjusted by the program as 7,743,300 years (95% HPD Interval = 5,613,400 – 9,633,600 years) (Figure 3.15). In this case, the divergence time of clades B and C was calculated as 370,400 years (95% HPD Interval = 87,450 – 877,900 years). The divergence of C1 and C2 was determined as 129,000 years (95% HPD Interval = 21,464 – 333,400 years).

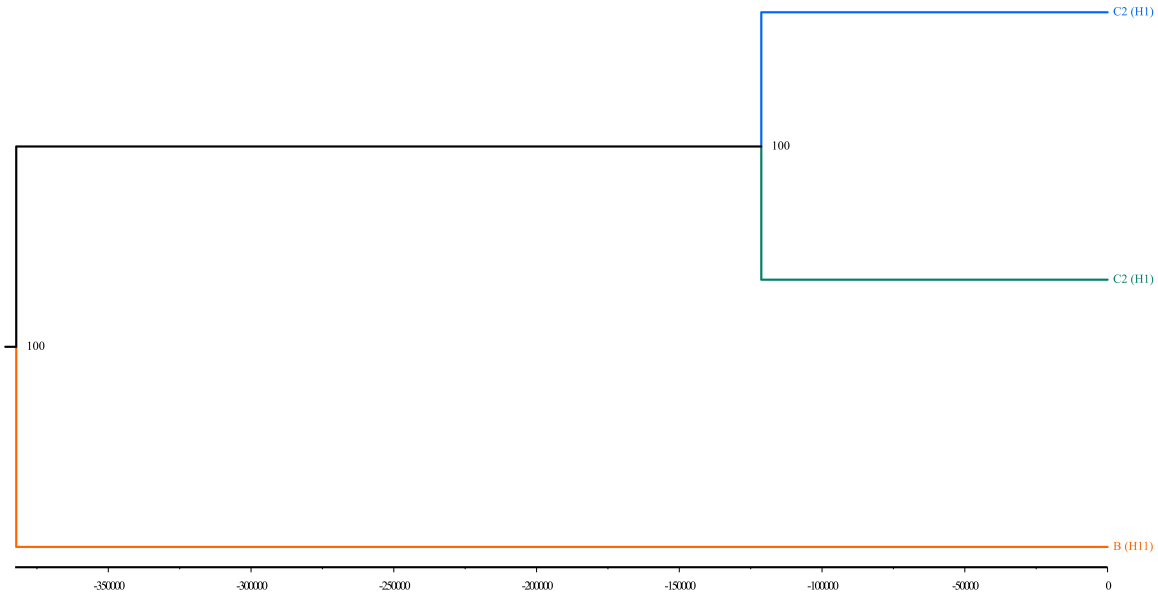


Figure 3.14. The divergence tree of the most common haplotypes of three haplogroups of *M. capaccinii* obtained using a strict molecular-clock with a rate of 4.8% divergence per million years. The units on the reverse scale axis are years.

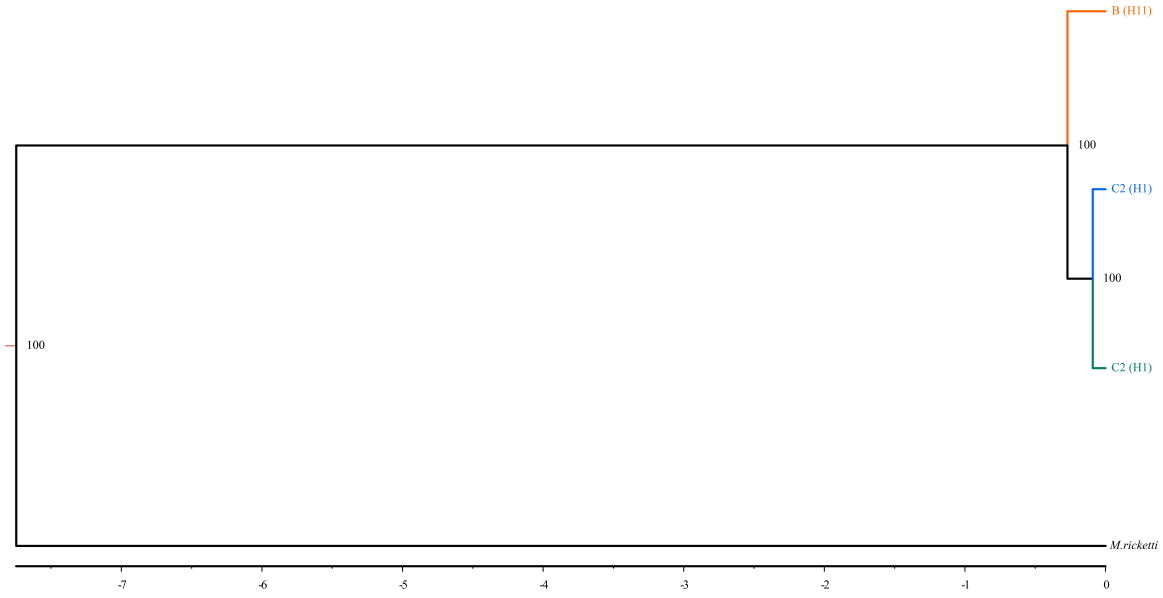


Figure 3.15. The divergence tree of the most common haplotypes of three haplogroups of *M. capaccinii* obtained using an uncorrelated relaxed clock and *M. ricketti* as an outgroup. The units on the reverse scale axis are million years.

The divergence times estimated when the 17 haplotypes were analyzed together to construct a phylogenetic tree via Bayesian inference were also very similar (Figure 3.10). The divergence time of clades B and C was calculated as 356,600 years (95% HPD Interval = 235,550 – 481,900 years). The divergence of C1 and C2 was determined as 125,840 years (95% HPD Interval = 69,245 – 194,070 years).

The F_{ST} values and their significance, as well as the average number of pairwise differences and haplotype distances reflect the clear separation between three haplogroups (Figures 3.16 -3.19).

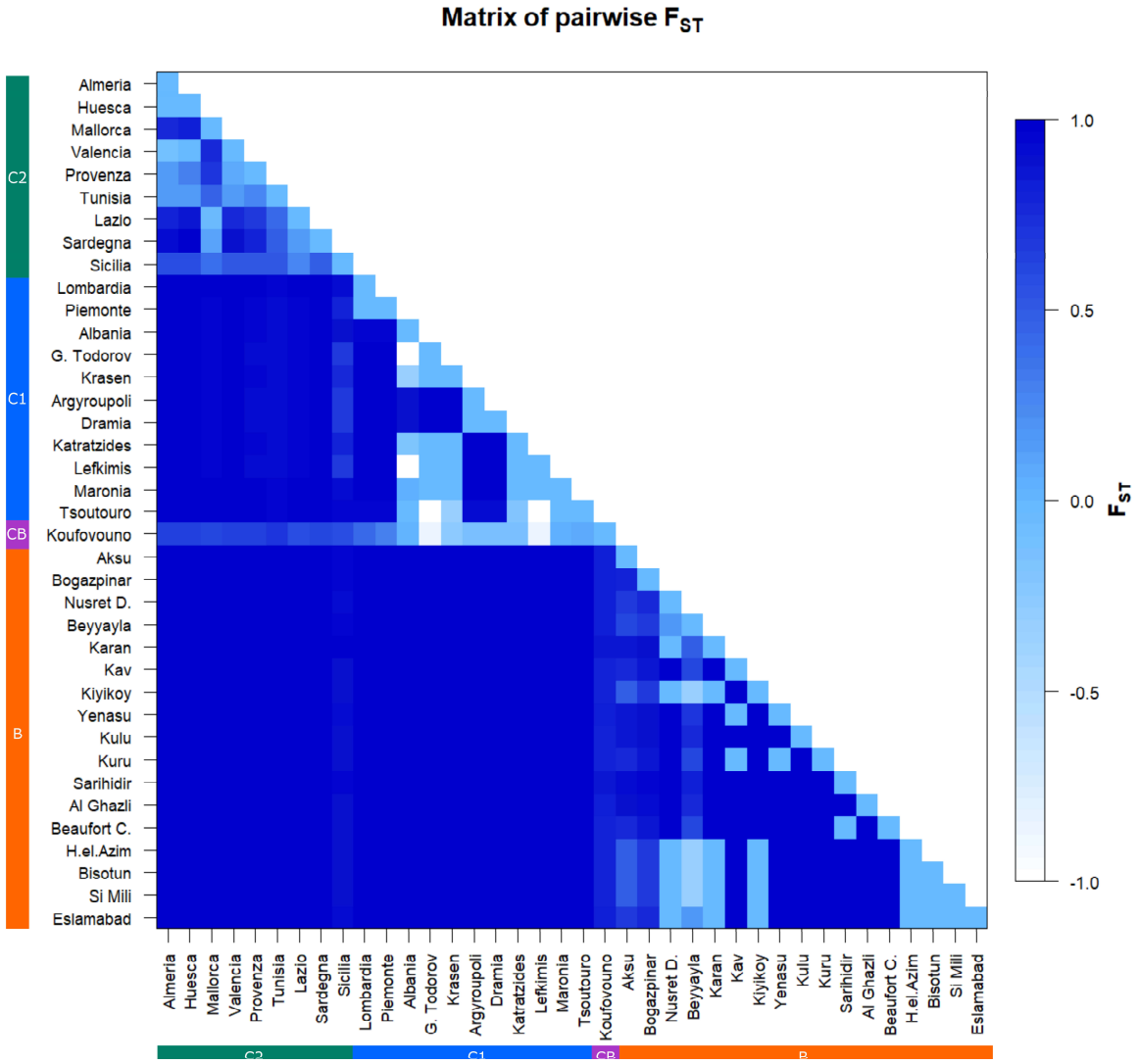


Figure 3.16. F_{ST} values for 38 populations and three haplogroups of *M. capaccinii*. The color codes of the haplogroups correspond to those on Figure 1.

When the mismatch distributions were plotted for a constant-size model and a model of expansion, the range expansion model fitted better than constant size population model for haplogroup B and slightly better for haplogroup C2, whereas the mismatch distributions and raggedness index values showed no signs of a population expansion for haplogroup C1 (see Discussion) (Figure 3.20, Table 3.4). Bayesian skyline plots, on the other hand, indicate onset of population expansions to around 2,500 years, 2,000 years and 1,500 years before present (BP) for haplogroups B, C1 and C2, respectively (Figure 3.21).

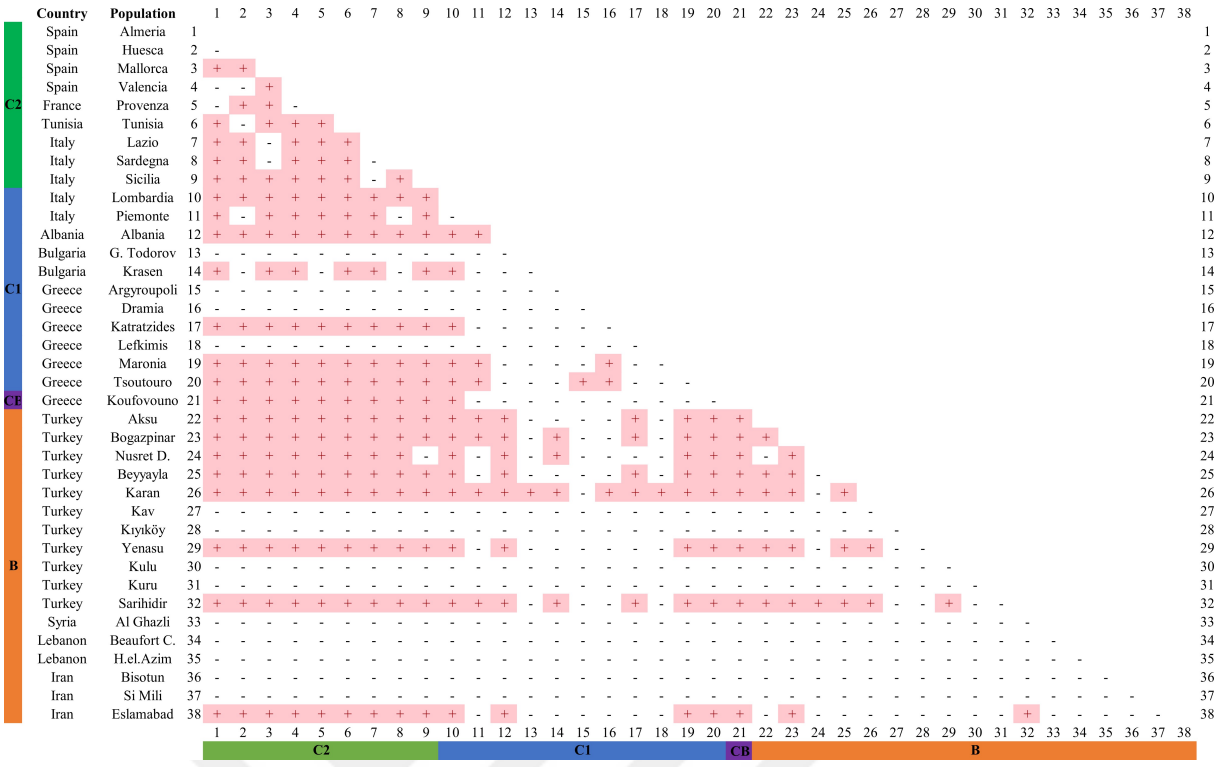


Figure 3.17. The significance of the F_{ST} values. The color codes of the haplogroups correspond to those on Figure 1.

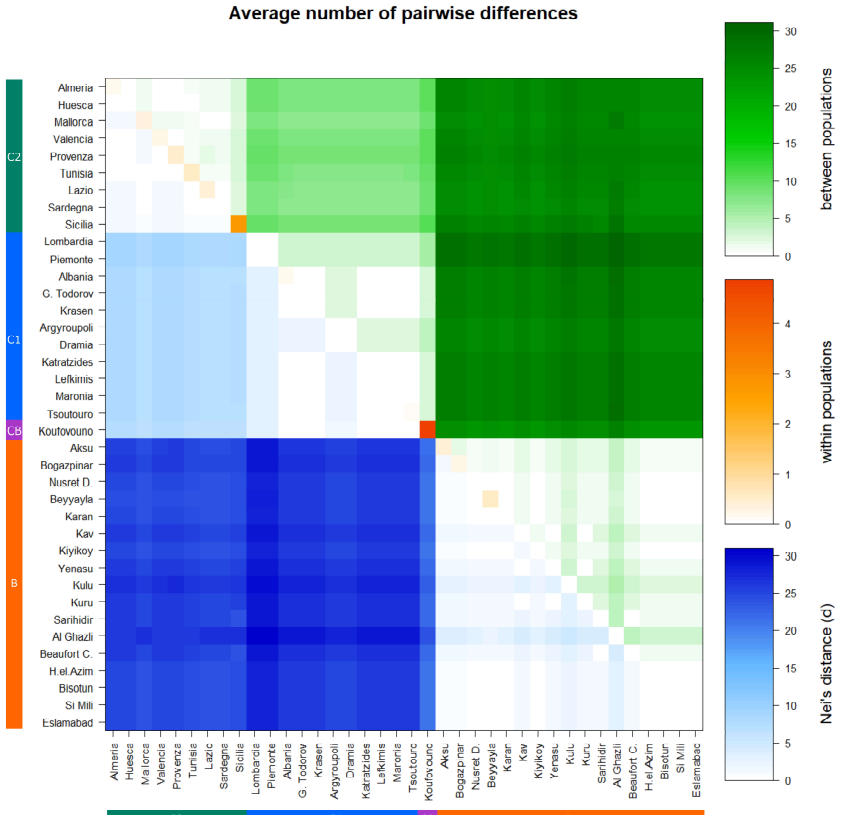


Figure 3.18. Nei's distance and average number of pairwise differences within and between 38 populations of *M. capaccinii*.

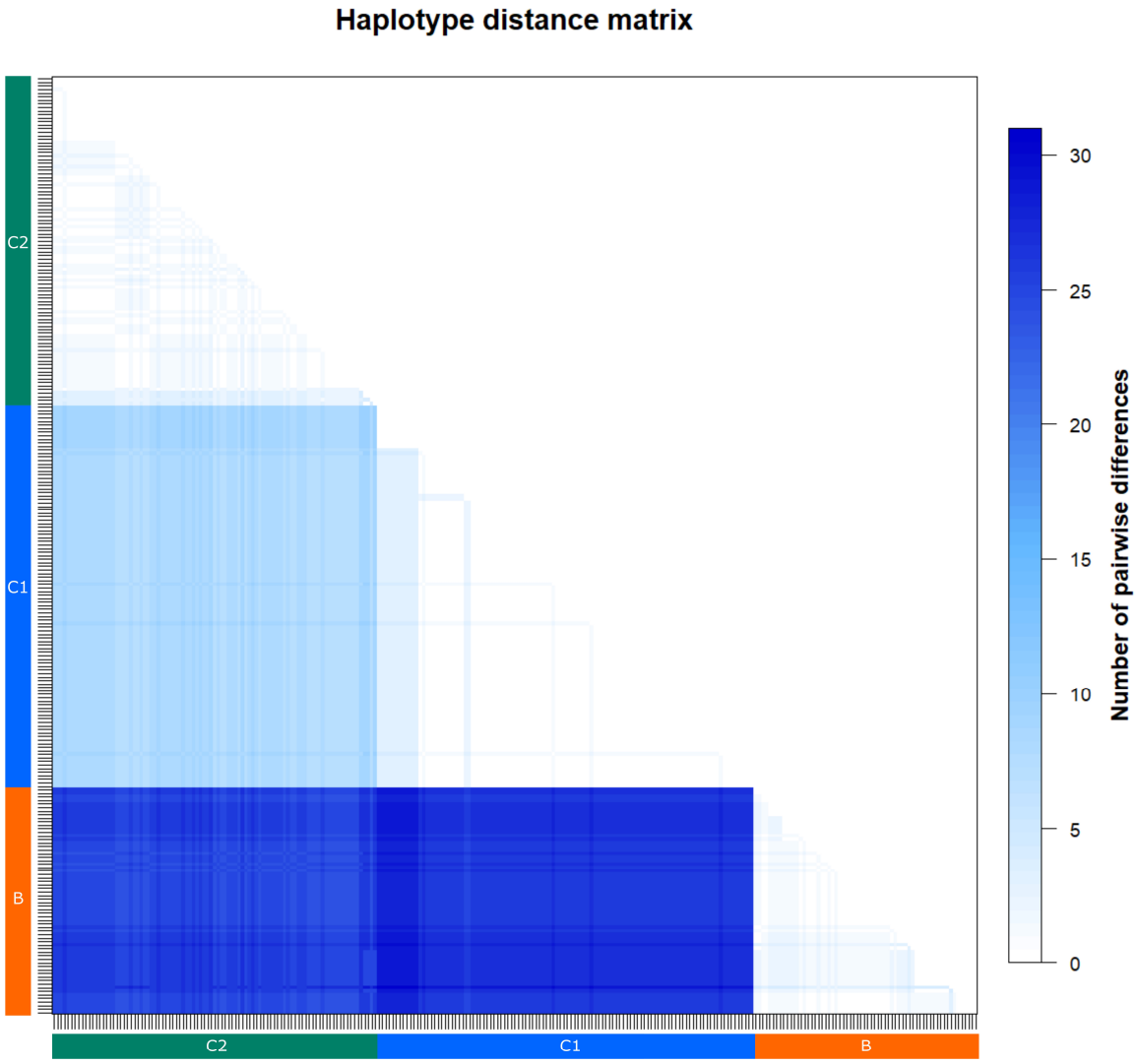


Figure 3.19. The haplotype distance matrix of 265 *M. capaccinii* samples.

When AMOVA was used to compare haplogroups B and C (which consisted of haplogroups C1 and C2), the global F_{ST} was 0.87 and was significantly greater than 0 ($P < 0.001$). In this comparison, the percentage of differentiation, which can be attributed to among group differences was 86.78%, whereas within groups variation was only 13.22%. When the haplogroup C was divided as C1 and C2, and three haplogroups were analyzed together, the F_{ST} was 0.95 ($P < 0.001$). This time, among group differences constituted 95.26% of all variation, whereas the within group variations constituted only 4.74%. Additionally, the Mantel test for the populations carrying haplogroup C1 haplotypes detected a significant IBD effect in the corresponding geographical area (Mantel test $R^2 = 0.48$, $P < 0.01$; $R^2 = 0.45$, when Italian populations are excluded), whereas no significant IBD effect was detected for haplogroup B and haplogroup C2 populations in the east and in the west, respectively.

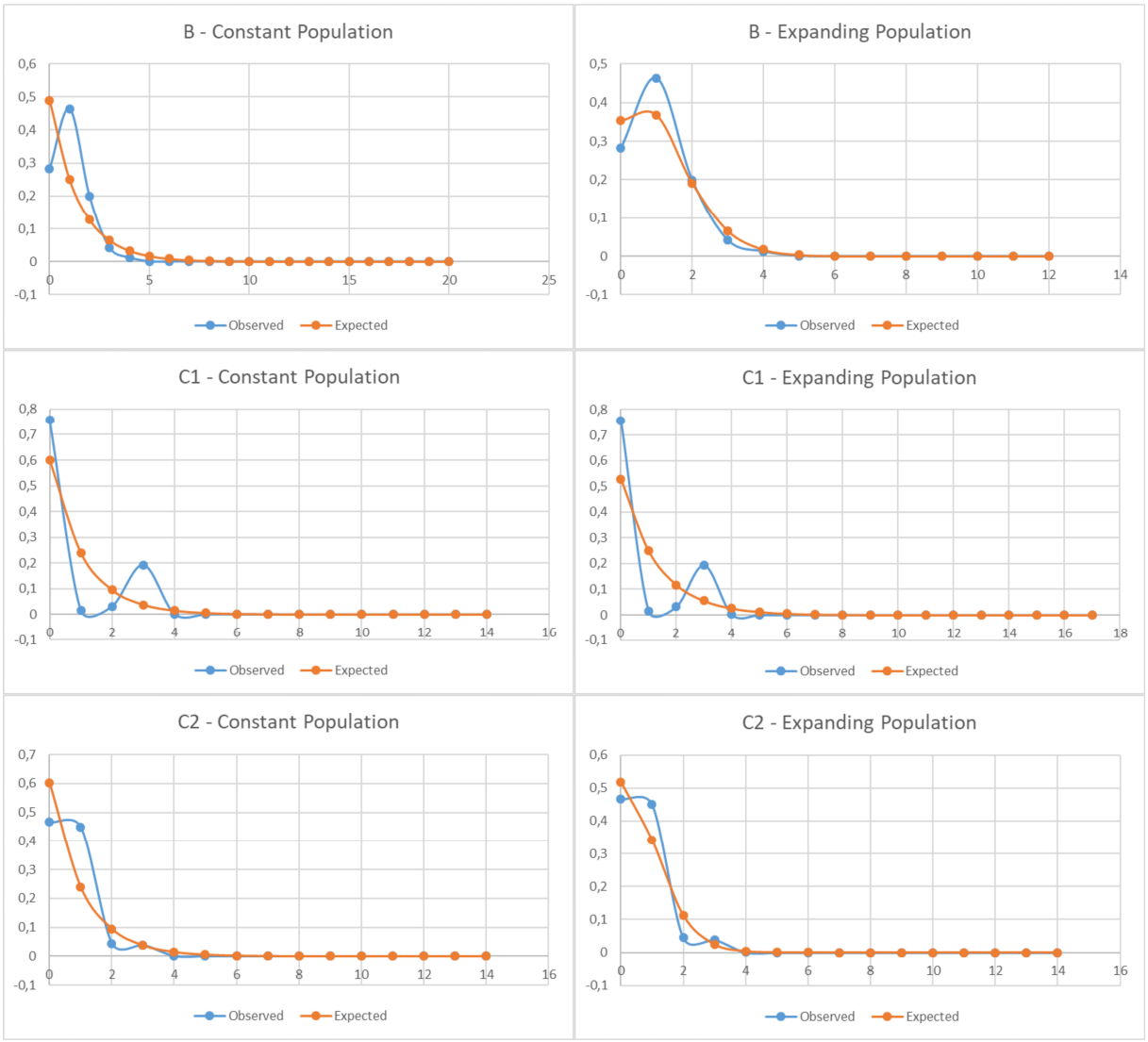


Figure 3.20. The observed and expected mismatch distributions in constant size and expanding population models for *M. capaccinii* haplogroups B, C1 and C2. The x-axis shows the number of pairwise differences between cyt-b haplotypes, and the y-axis represents the frequency of these differences.

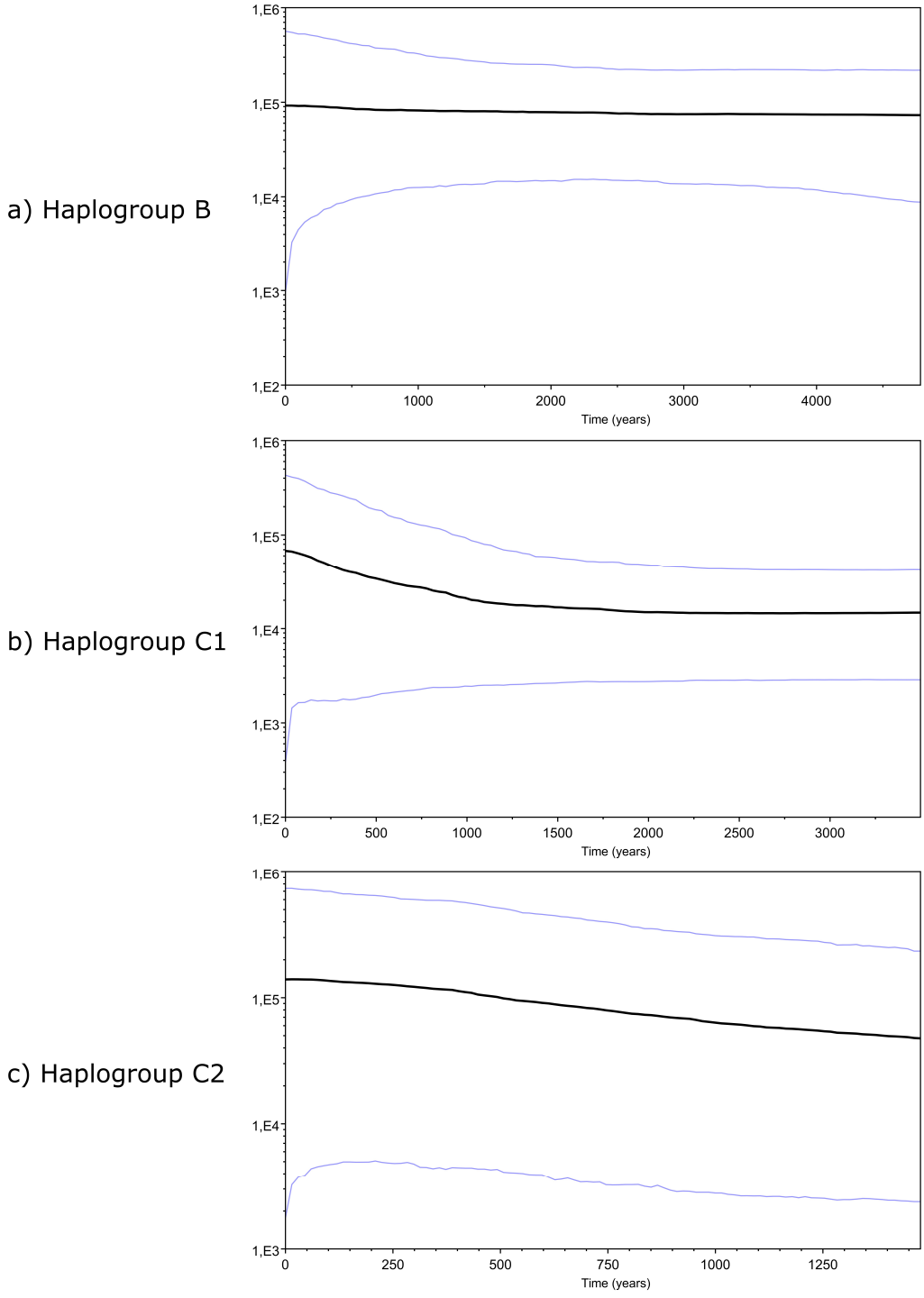


Figure 3.21. Bayesian skyline plots for *M. capaccinii* haplogroups (a) B, (b) C1 and (c) C2.

Comparison of three rooted trees depicting three possible scenarios regarding the phylogeographic history and ancestral relationships of these three haplogroups with ABC gave strong support for scenario 3, in which haplogroup B was the most ancestral one. Posterior probabilities

were high for both the logistic regression and direct estimation (for the logistic approach, P>0.98; for the direct approach, P>0.50) (Figure 3.22).

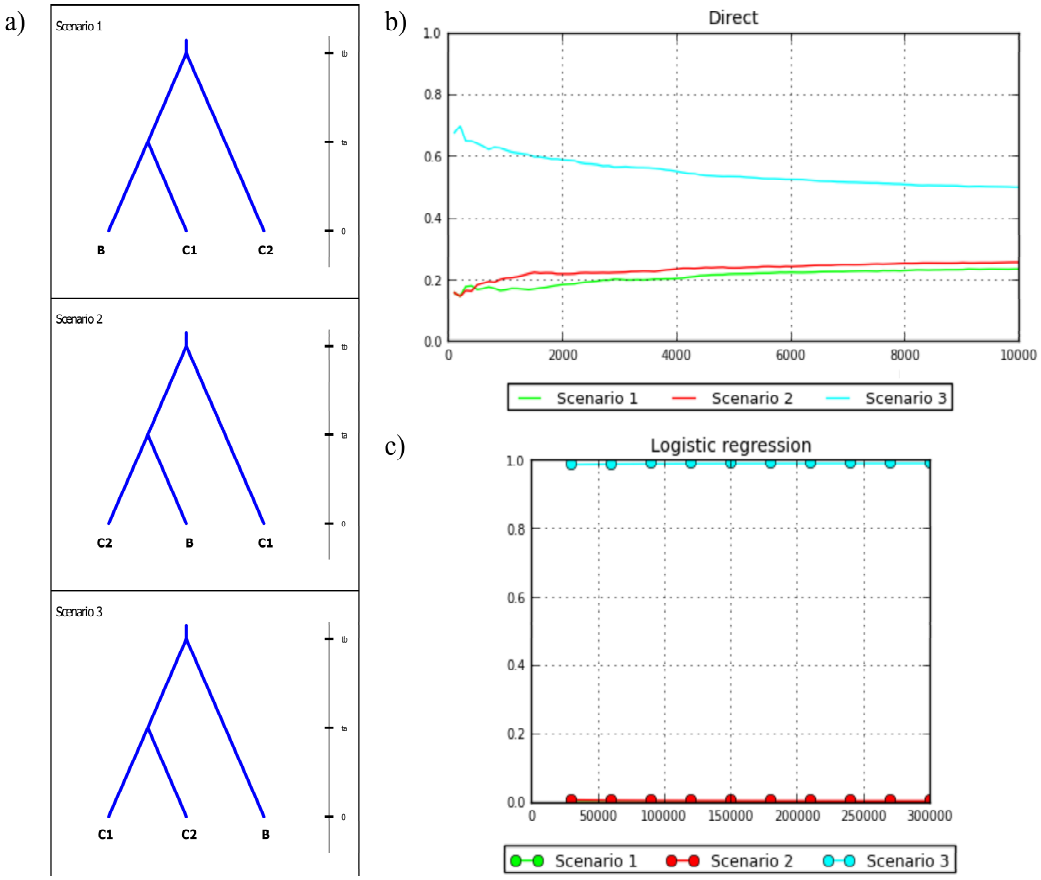


Figure 3.22. An ABC analysis of all 273 *M. capaccinii* individuals genotyped at cyt-b. a) All three possible scenarios (=rooted trees) regarding the ancestral relationship of haplogroups B, C1 and C2 were compared. The probabilities predicted by (b) direct estimation and (c) logistic regression are shown.

3.2.2. Modelling the present, past and future distributions of *M. capaccinii*

The predictive abilities of the models were very high and there was no overfitting of the occurrence data (the average test AUC = 0.959 ± 0.020 for Haplogroup B, AUC = 0.958 ± 0.010 for Haplogroup C1, AUC = 0.981 ± 0.006 for Haplogroup C2). The predicted distribution was in agreement with the known current distribution of *M. capaccinii* in the western Palearctic (Koopman, 1994), which covers southern Europe, Tunisia and Algeria in northern Africa, southern Turkey, Lebanon and Syria, spanning from Spain in the west to Iran and south-western Asia in the east. The model predictions suggested a high probability of distribution for haplogroup B in Thrace, southern Turkey, Lebanon, northern Syria and Iraq and southwestern Iran (Figures 3.23, 3.38a, 3.40a). The predicted distribution of haplogroup C1, on the other hand, covered Thrace and southeastern Europe, extending into northern Italy in the west, where it is apparently limited by the Alps and Apennines (Figures 3.24, 3.38b, 3.40b). Finally, haplogroup C2 seems to be distributed in southwestern Europe, extending from parts of Italy to the south of the Apennines into the eastern regions of Spain (Figures 3.25, 3.38c, 3.40c).

The LGM predictions using CCSM and MIROC GCMs were similar for haplogroup B, suggesting a distribution around southern Turkey, Cyprus and western Syria and Lebanon only (Figures 3.26, 3.27, 3.38a). Stable areas for haplogroup B, where it could survive in both glacial and interglacial periods covered a much narrow area consisting of southern Turkey, parts of northern Iraq and Syria and the eastern coasts of the Levantine Sea (Figure 3.40a). On the other hand, the predictions of two GCMs for haplogroup C1 differed a little: both models predicted possible presence to the north of the Adriatic Sea; yet the CCSM prediction put the highest probability of survival in Thrace, whereas MIROC suggested no presence in Thrace and instead suggested survival around coasts of northern Adriatic corresponding to Italy and Balkans today (Figures 3.28, 3.29, 3.38b). Stable areas for this haplogroup in its current range consisted only of southwestern parts of the Balkan Peninsula and northern Italy (Figure 3.40b). For the haplogroup C2, again both models predicted similar distributions in eastern Spain and on Balearic Islands, where CCSM assigned the highest probability to the Balearics, whereas MIROC suggested mainland Spain to be more suitable for the species during that period (Figure 3.30, 3.31, 3.38c). Stable areas in its current range were parts of eastern Iberia (Figure 3.40c).

Both models foresaw northward movement of suitable habitats for all haplogroups when projected into 2070 (Figure 3.39). For haplogroup B, the CCSM suggested relatively little change,

the most important difference from the current distribution being the increase in suitable areas in southern Ukraine and Russia, and the decreased suitability in the Middle East (Figure 3.32, 3.39a). These possible changes were more pronounced when MIROC was used for the analysis (Figure 3.33, 3.39a). Stable areas, where haplotypes belonging to this haplogroup may be encountered today as well as in 2070 are found in western Anatolia and in the eastern coasts of the Levantine Sea (Figure 3.40a). The predictions of the GCMs for haplogroup C1 were very similar, both suggesting a northward expansion into northern Europe and Ukraine, extending into Germany, the Netherlands, and even Scandinavia and the United Kingdom (Figures 3.34, 3.35, 3.39b). Stable areas for haplogroup C1 cover an area spanning western parts of the Balkan Peninsula to northern Italy (Figure 3.40b). Neither model suggested a significant change for the suitability of the southern European areas for *M. capaccinii*, where haplogroup C2 is encountered currently (Figures 3.36, 3.37, 3.39c). Stable areas for this haplogroup cover most of southern Italy and eastern parts of Iberia (Figure 3.40c).

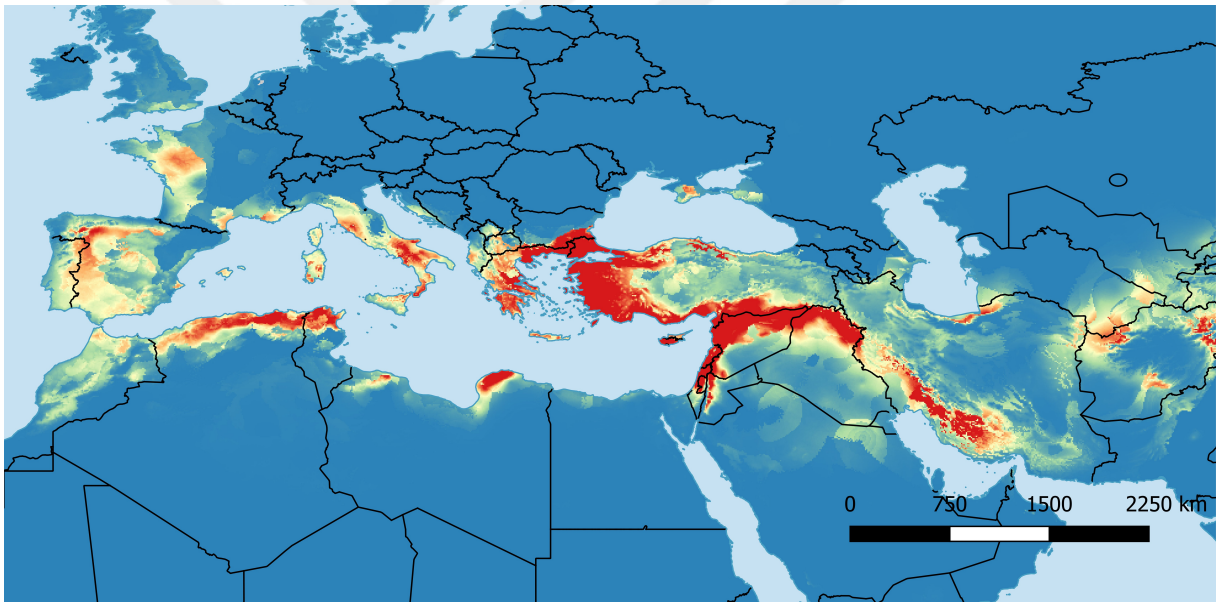


Figure 3.23. The predicted current distribution of haplogroup B.

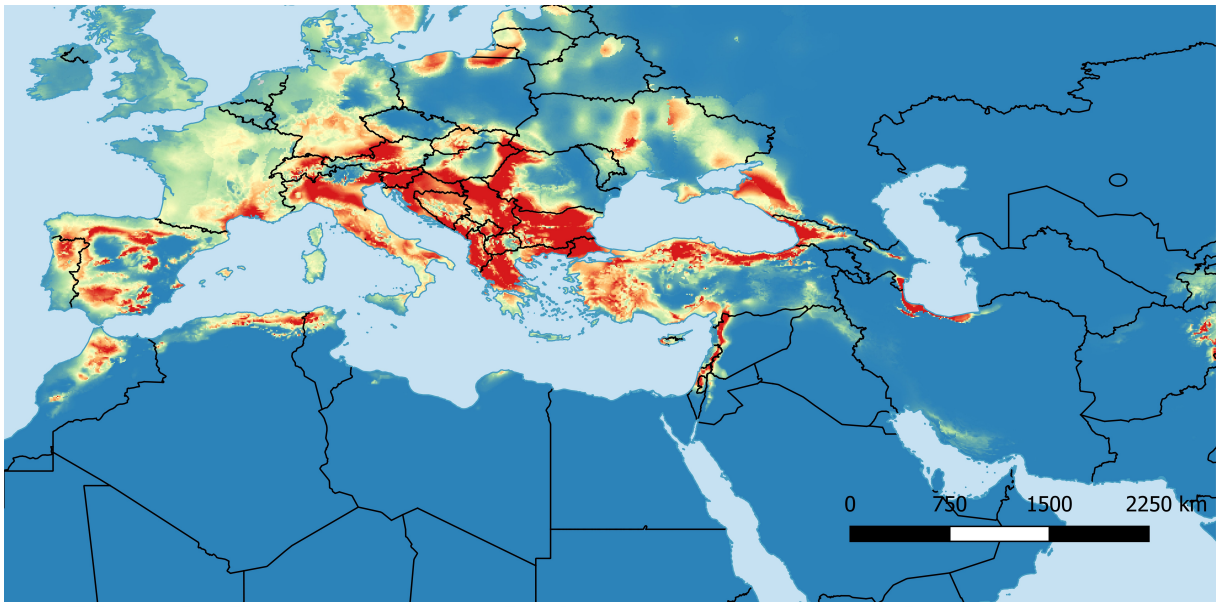


Figure 3.24. The predicted current distribution of haplogroup C1.

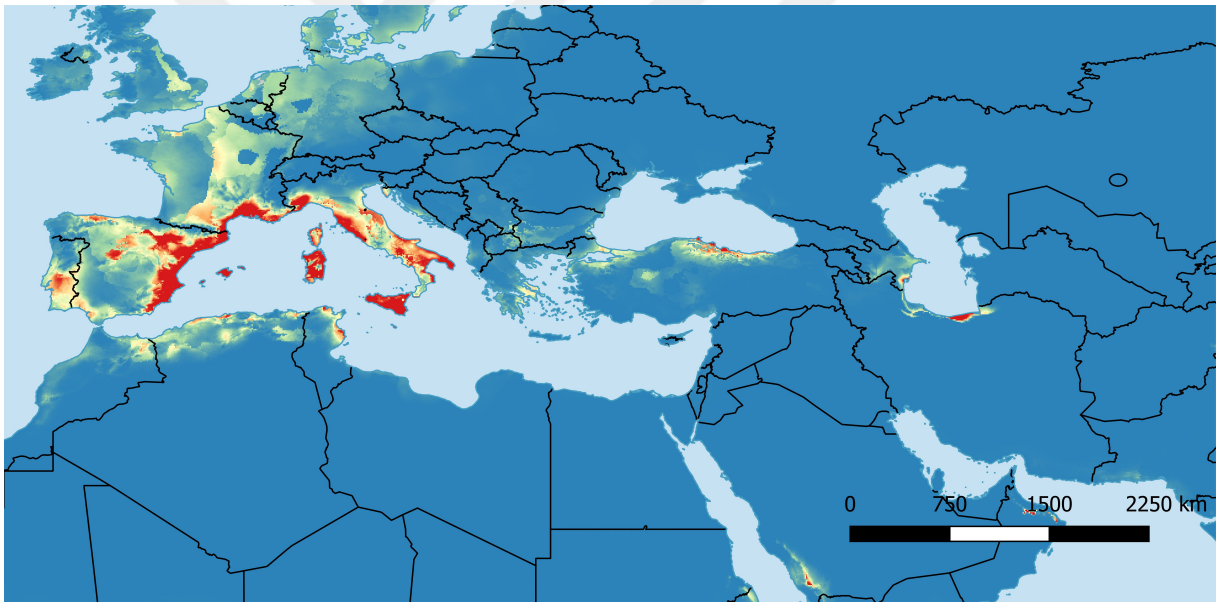


Figure 3.25. The predicted current distribution of haplogroup C2.

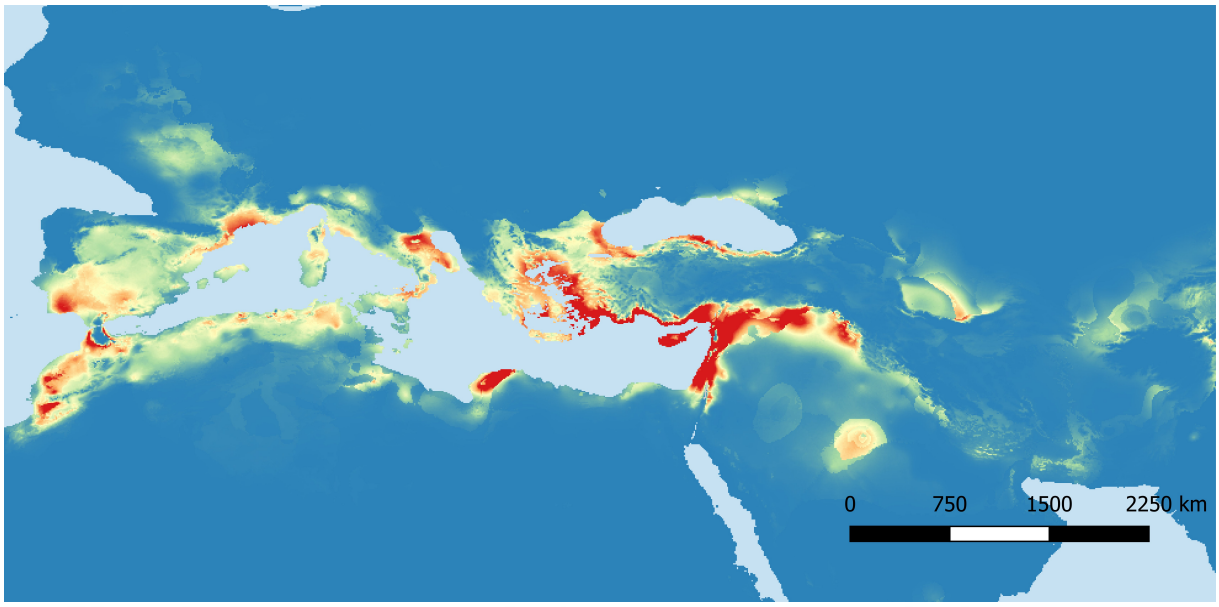


Figure 3.26. The predicted distribution of haplogroup B during the LGM according to the Community Climate System Model (CCSM) general circulation model (GCM).

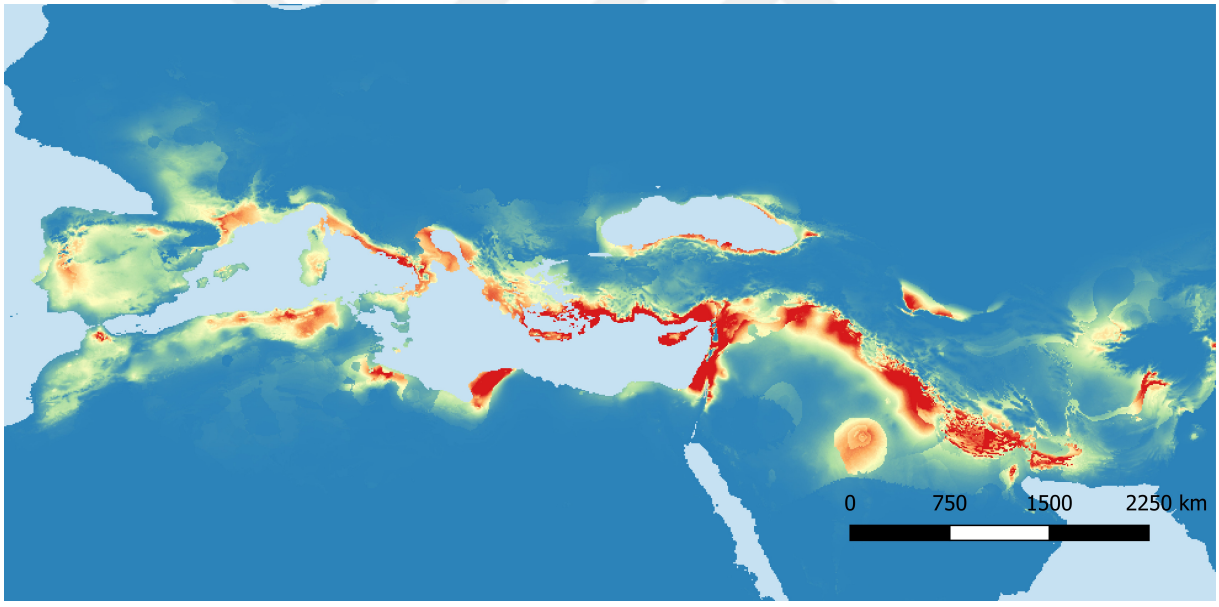


Figure 3.27. The predicted distribution of haplogroup B during the LGM according to the Model for Interdisciplinary Research on Climate Earth System Model (MIROC) GCM.

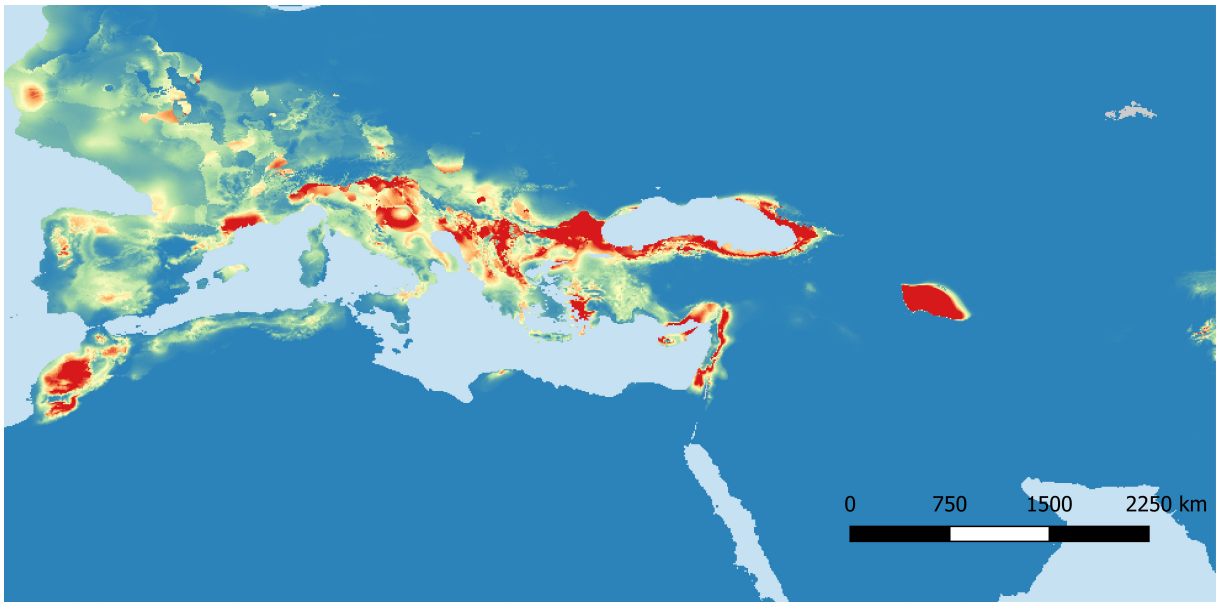


Figure 3.28. The predicted distribution of haplogroup C1 during the LGM according to the CCSM GCM.

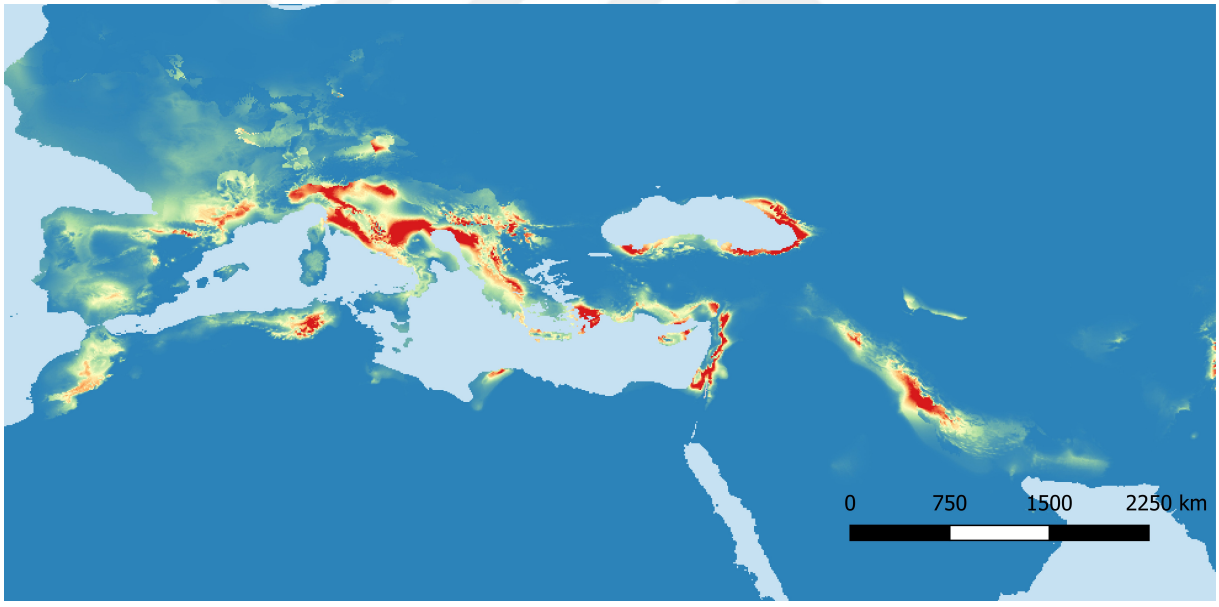


Figure 3.29. The predicted distribution of haplogroup C1 during the LGM according to the MIROC GCM.

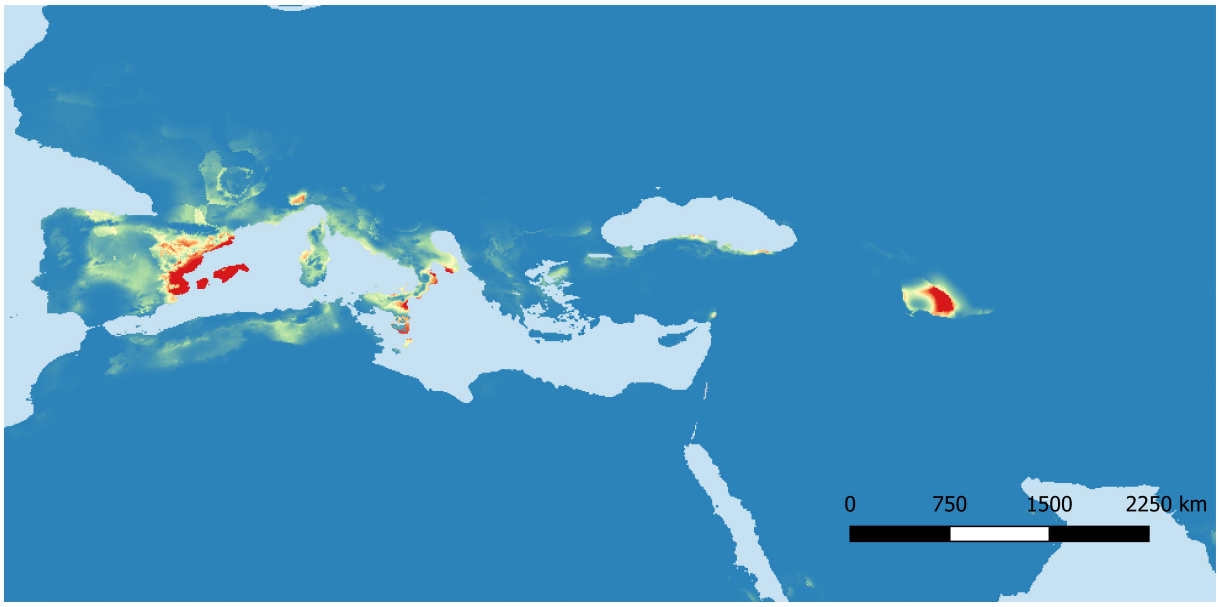


Figure 3.30. The predicted distribution of haplogroup C2 during the LGM according to the CCSM GCM.



Figure 3.31. The predicted distribution of haplogroup C2 during the LGM according to the MIROC GCM.

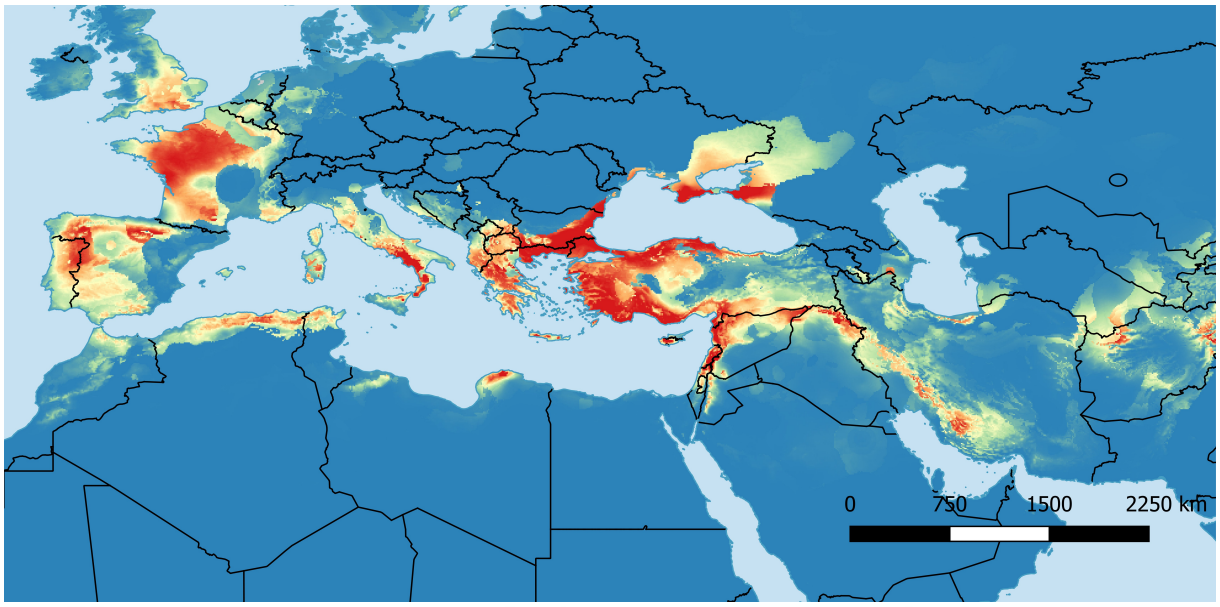


Figure 3.32. The predicted distribution of haplogroup B in 2070 according to the CCSM GCM.

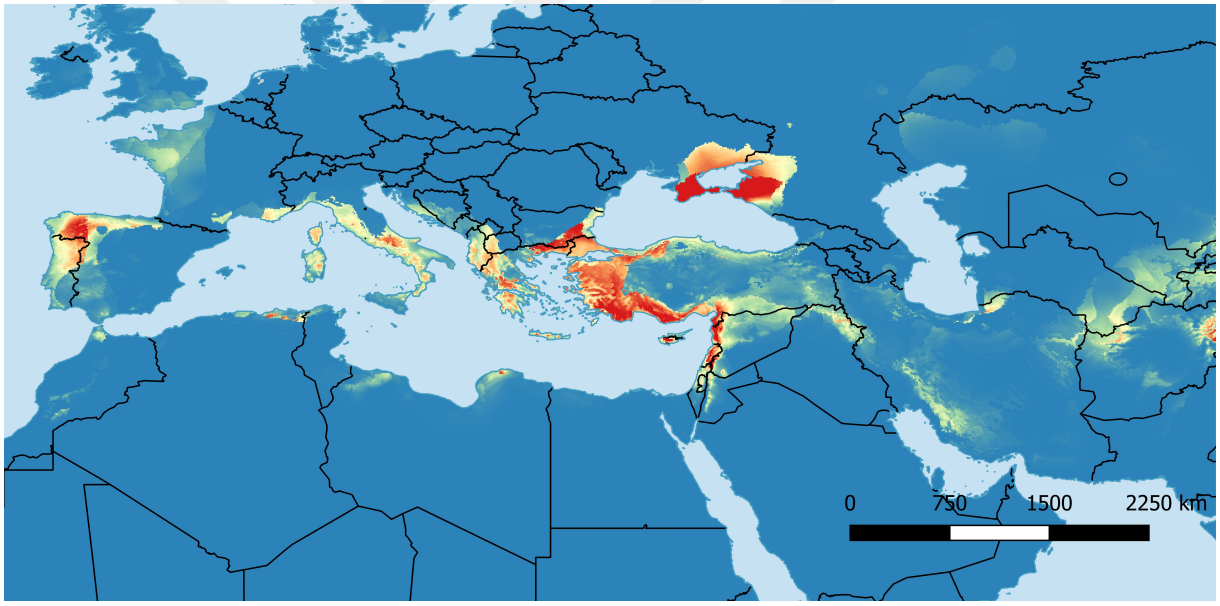


Figure 3.33. The predicted distribution of haplogroup B in 2070 according to the MIROC GCM.

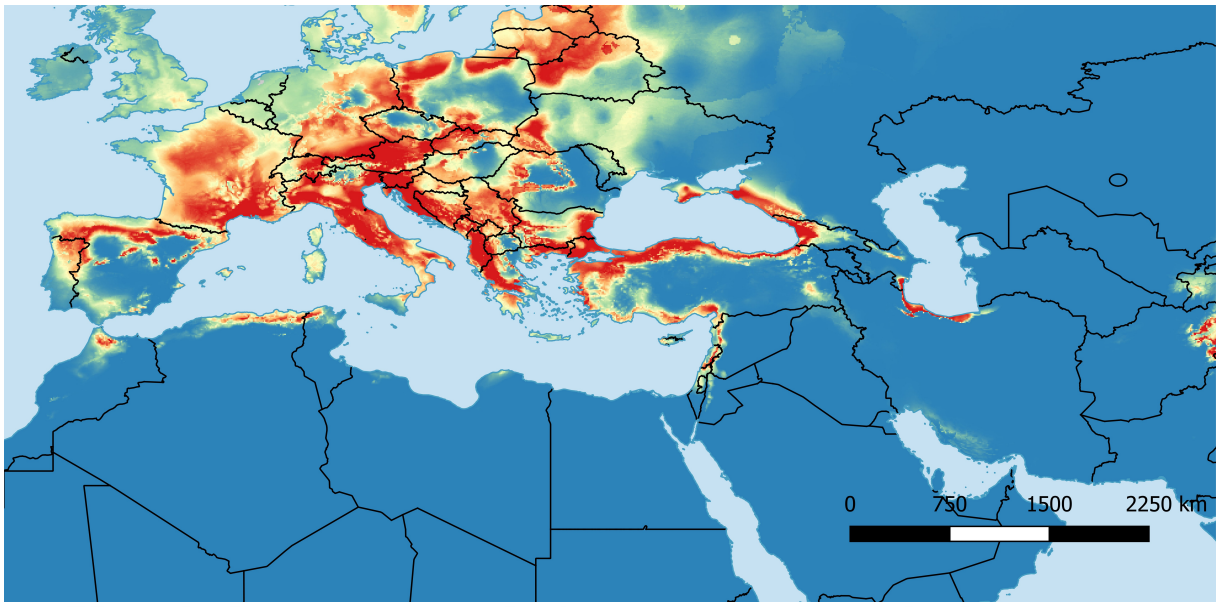


Figure 3.34. The predicted distribution of haplogroup C1 in 2070 according to the CCSM GCM.

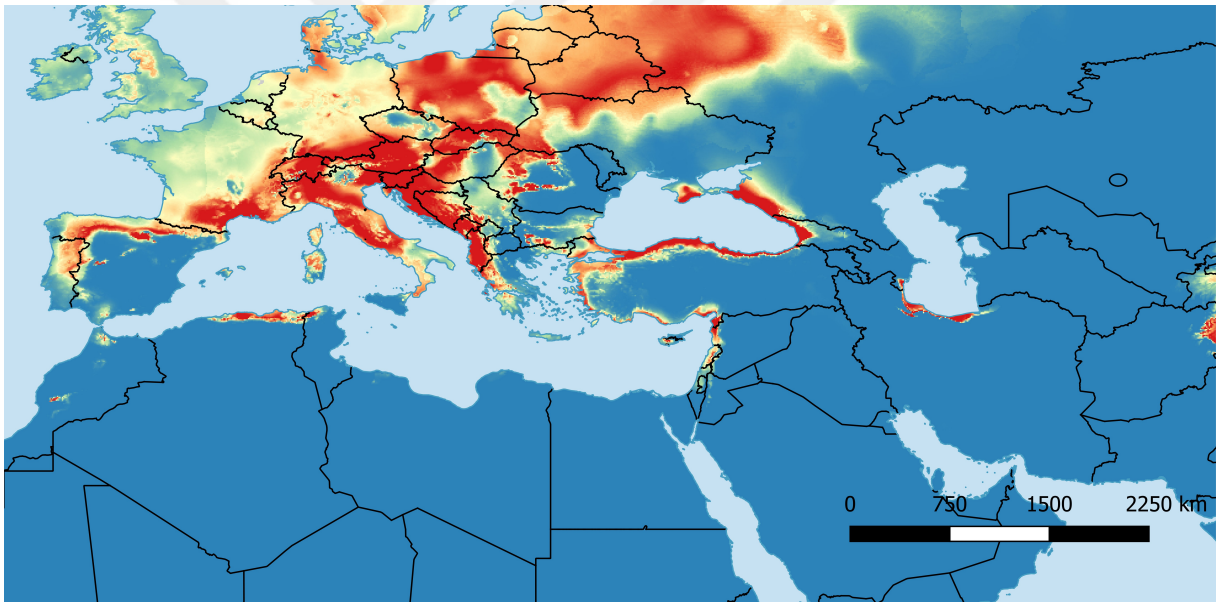


Figure 3.35. The predicted distribution of haplogroup C1 in 2070 according to the MIROC GCM.

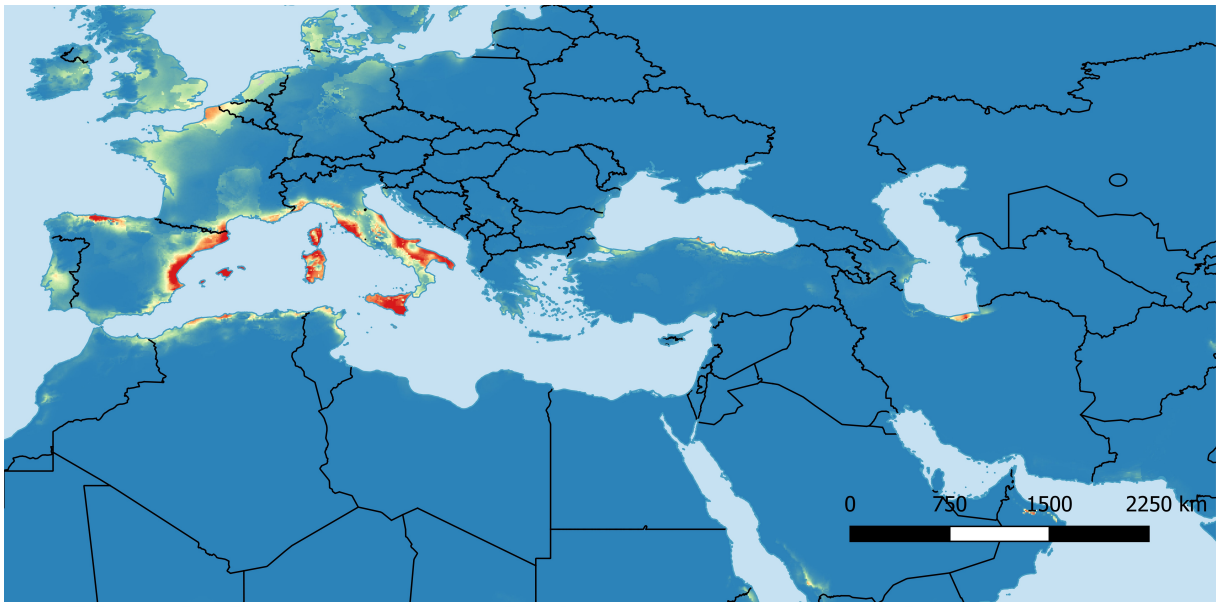


Figure 3.36. The predicted distribution of haplogroup C2 in 2070 according to the CCSM GCM.

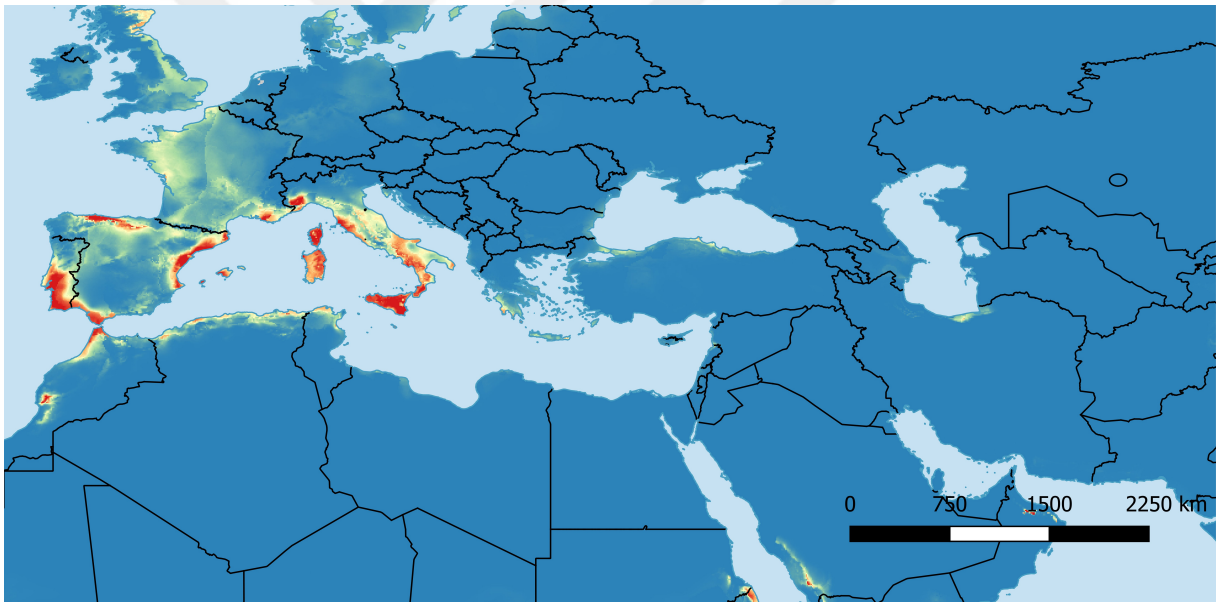


Figure 3.37. The predicted distribution of haplogroup C2 in 2070 according to the MIROC GCM.

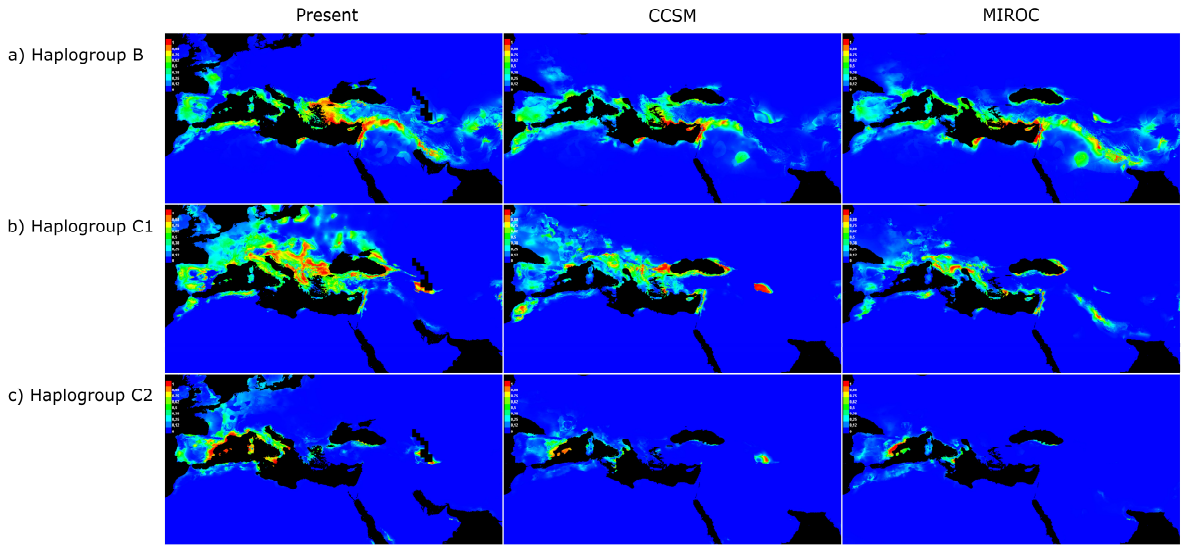


Figure 3.38. Three Maxent models for the potential distribution of three haplogroups of *M. capaccinii*. Maps in the first panel are calculated using bioclimatic variables for the current climatic conditions. Maps of suitable regions in the last glacial maximum (LGM) are obtained using the Model for Interdisciplinary Research on Climate (MIROC) or the Community Climate System Model (CCSM) for (a) haplogroup B, (b) haplogroup C1, and (c) haplogroup C2.

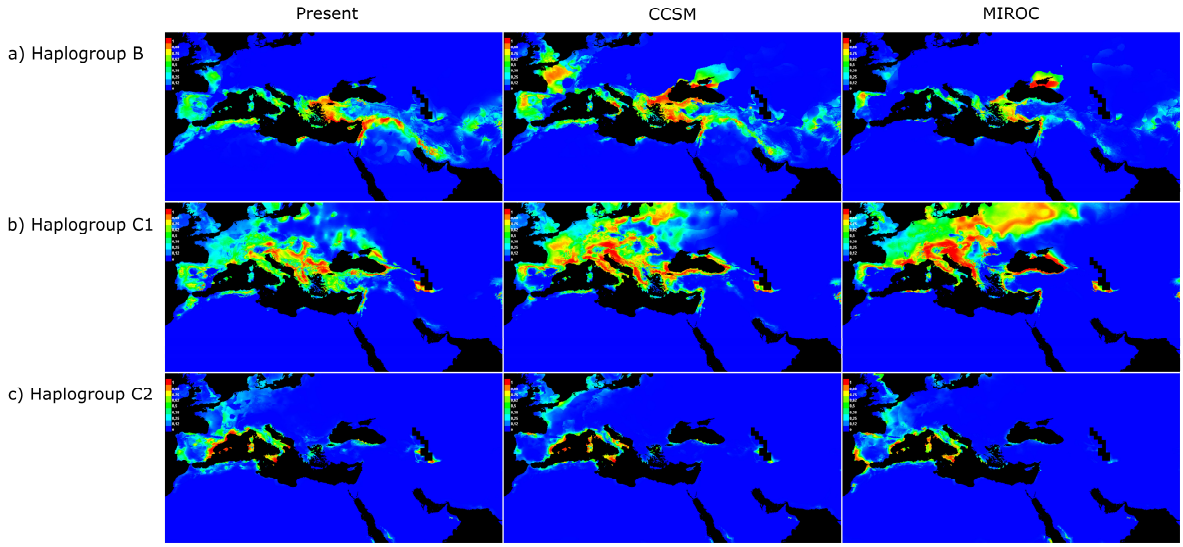


Figure 3.39. Three Maxent models for the potential distribution of three haplogroups of *M. capaccinii*. Maps in the first panel are calculated using bioclimatic variables for the current climatic conditions. Maps of suitable regions in 2070 are obtained using the Model for Interdisciplinary Research on Climate (MIROC) or the Community Climate System Model (CCSM) for (a) haplogroup B, (b) haplogroup C1, and (c) haplogroup C2.

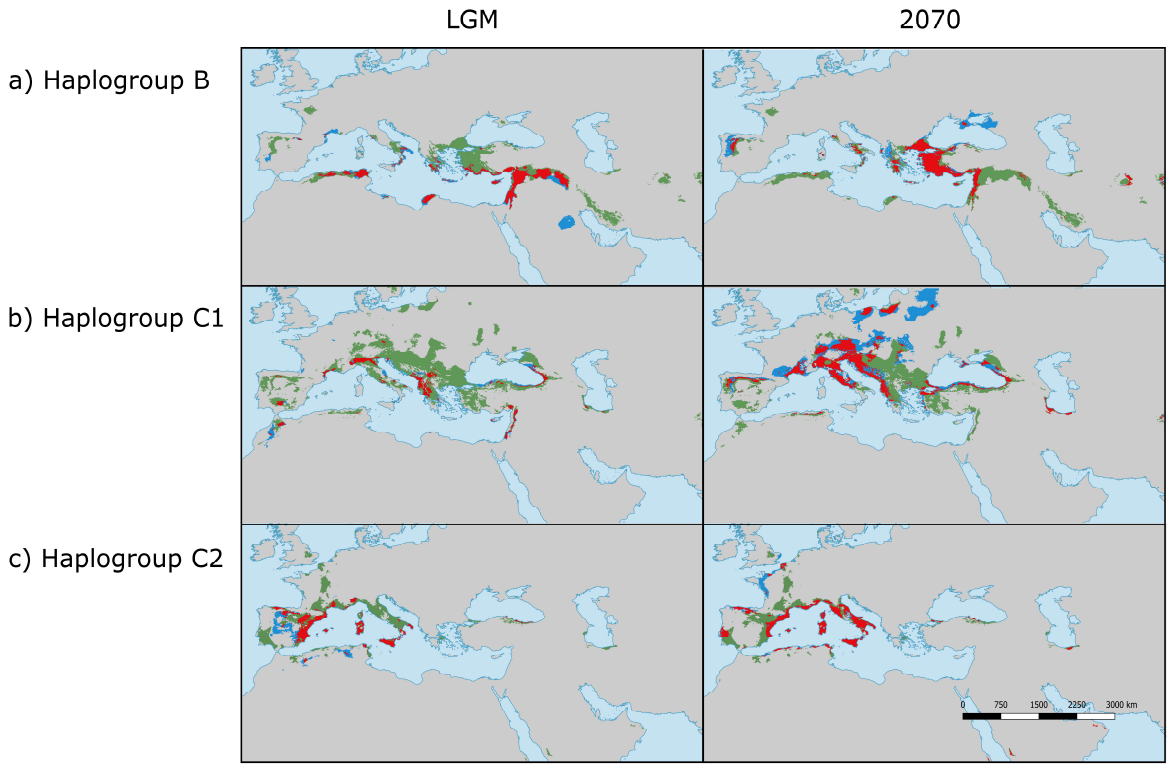


Figure 3.40. A reclassified map of the potential distribution of *M. capaccinii*. The green areas indicate areas where suitable conditions exist in the present alone, whereas blue areas stand for areas where suitable conditions existed in the LGM (or will exist in 2070) alone. The red areas are stable regions where suitable areas are predicted for both the present and the LGM (or 2070).

The response curves of the haplogroups to the eight bioclimatic variables and the results of the jackknife tests are given in Figures A2 – A7 in Appendix C.

4. DISCUSSION

4.1. *Miniopterus schreibersii*

4.1.1. Social Structure of *Miniopterus schreibersii*: A Continental Perspective

Although many studies use both nuclear and mitochondrial DNA data to evaluate the social structure and phylogeography of various species, including mammals, studies that investigate the social structure, sex-biased philopatry and dispersal of animals at a continental-scale using nuDNA and mtDNA markers are relatively rare. One example is the study on a bird, Kentish plover *Charadrius alexandrinus*, by Küpper et al. (2012), where the authors detected no significant structure or IBD across Eurasia, yet the mtDNA was less structured compared to autosomal microsatellites, indicating female-biased gene flow for the species. In another study, Caparroz et al. (2009) investigated population differentiation in Blue-and-yellow Macaw (*Ara ararauna*) throughout its geographical range in Brazil, using mitochondrial markers and six microsatellite loci. The mtDNA analyses revealed two major genetically differentiated populations, whereas no structuring was detected using nuclear markers. The authors concluded that there was male-biased dispersal and female philopatry in this bird species. Yet another bird, in which cytonuclear discordance was ascertained at a continental scale is the common eider (*Somateria mollissima*) (Tiedemann et al., 2004). Again, male-mediated gene flow was suggested, due to a much less pronounced differentiation among populations at nuclear loci, contrasting the significant differentiation detected at a mitochondrial locus.

There are also a few large geographic scale studies on mammals, which, albeit not specifically on their social structure, revealed discordance between mitochondrial and nuclear markers (Toews & Brelsford, 2012). Some of these studies were conducted over an area that covered >95% of the geographic extent of the species in question, such as the white-tailed deer and mule deer (Cathey et al., 1998), mountain and European hares (Melo-Ferreira et al., 2009), European goat (Ropiquet & Hassanin, 2006) and European common shrew (Yannic et al., 2010). In another study on polar, brown and black bears, Bidon et al. (2014) used samples from whole distribution areas, spanning Asia, Europe and North America for brown bears and Alaska, Greenland and Northern Russian islands for polar bears, in which Y-chromosomal markers were analyzed, and the results were

compared with those for mtDNA markers. The authors found weaker structuring of the patriline than the matriline, again indicating male-biased dispersal. In their study on African savannah elephants, using samples from 11 localities in eastern, western and southern Africa, Nyakaana et al. (2002) encountered genetic structuring of both mtDNA and microsatellites, yet the population differentiation deduced from nuDNA was much lower. The authors stated that this was probably due to female philopatry and matrilineal social structure of the elephants. A similar result was found in the study of Engelhaupt et al. (2009) on sperm whales in the Gulf of Mexico, western North Atlantic, Mediterranean Sea and North Sea. They detected significant differentiation of the mtDNA, but microsatellites showed relatively low differentiation of the Mediterranean populations.

Several studies regarding the phylogeography and/or dispersal strategies of bats have also been carried out, which mainly made use of cytonuclear discordance for their deductions (Petit *et al.*, 1999b, 2001; Dool *et al.*, 2013, 2016b; Fornůsková *et al.*, 2014; Kerth *et al.*, 2002). In the study of Petit *et al.* (2001) on the noctule bat (*Nyctalus noctula*), a 10-fold difference between the F_{ST} values of mtDNA and nuDNA markers was detected. They concluded that random male dispersal is consistent with the observed population structure, and using multilocus population differentiation tests was necessary to draw this conclusion with higher confidence. Similarly, Kerth *et al.* (2002) was able to detect absolute female philopatry and very strong male dispersal in Bechstein's bat (*Myotis bechsteinii*) using nuclear and mitochondrial markers together. Male-biased dispersal was also detected for common pipistrelle bats (*Pipistrellus pipistrellus*), after comparing microsatellites with mtDNA markers (Fornůsková *et al.*, 2014).

As for this study, the results based on nuclear microsatellite markers suggest an absence of structuring among populations and among the defined geographical regions of *M. schreibersii* at the large scale of the study, which covers the whole range of the species. All analyses revealed a more or less uniform distribution of nuDNA alleles at the 10 loci analyzed, without any significant differentiation between different populations. Most *M. schreibersii* samples were part of the same cluster when K was set to two in TESS and STRUCTURE analyses. The F_{ST} values were also relatively low for most comparisons of *M. schreibersii* populations, and there was no indication of IBD for the nuclear markers analyzed, both results pointing to a lack of structuring among populations.

Female philopatry and male-biased dispersal is the norm in bats as it is for many mammal species (Greenwood, 1980; Lawson Handley & Perrin, 2007; Arnold, 2007; Vonhof et al., 2008; Piaggio et

al., 2009). In this study, the observation that the maternally inherited mtDNA shows more geographic structuring than the autosomal microsatellite markers among populations of *M. schreibersii* provides further genetic support for male-biased gene flow previously reported for the species (Palmeirim et al., 1995; Ramos Pereira et al., 2009; Furman et al., 2010b). Ramos Pereira et al. (2009) detected more pronounced structuring of the mtDNA compared to that of microsatellites across all nursing colonies in Portugal due to female philopatry and male-mediated gene flow. Furman et al. (2010b) found concordant results in southern Anatolia: moderate structuring in the mitochondrial control region, but only a weak differentiation at the microsatellite level, again indicating male-biased dispersal. In this current study, the same pattern is encountered once again, this time at a continental scale, essentially spanning the whole distribution area of the species.

4.1.2. Anatolia as the Single Refugium for *Miniopterus schreibersii*

Out of the 10 loci analyzed, only one, namely CH37, had a significant F_{IS} value for Anatolia (F_{IS}=0.66, P<0.05), which is probably the cause of detection of a heterozygote deficit in this region by the bottleneck analysis. However, no other locus had a significant F_{IS} value, thus the region-specific overall F_{IS} was also not significant and this region can be considered to be in HWE. Regarding the regions that are in fact in HWE, Anatolia, which also has the highest mtDNA diversity along with Lebanon (Bilgin et al., 2016), has the highest values for most of the descriptive statistics for nuDNA, including the number of alleles, the number of effective alleles and Shannon's Information Index, implying a relatively high variation in this region, which may in turn be interpreted as providing support for the fifth paradigm. Moreover, the diversity statistics found by rarefaction methods, namely allelic richness and private allelic richness, are also highest for Anatolia among regions that are in HWE.

When the regions that are not in HWE (i.e., Slovakia, Thrace & Balkans and Iberia) are also considered, Anatolia still has the highest private allelic richness, and comes second to only Slovakia when it comes to allelic richness and number of effective alleles. Slovakia is actually the region with the highest genetic diversity for several descriptive statistics, including allelic richness determined by rarefaction. However, this finding probably stems from the fact that one of the two populations that constitute it, namely Drienovská jaskyňa, is the only individual population not in HWE. No heterozygote deficiency or excess was detected for Slovakia as a whole, or for the two populations of Slovakia, including the Drienovská jaskyňa population, which seems to stem from the fact that in this population, there is an excess of some heterozygotes at some loci (e.g., MM2, F_{IS}=-0.79, p<0.05) and

a deficit of others (CHD2 and MSC2, F_{IS}=0.19, $p<0.05$ for both), potentially cancelling each other out. Drienovská jaskyňa is a winter colony (Uhrinova et al., 2016) and this may also account for its high genetic diversity, as bats from several nurseries can gather and interact in hibernation roosts (Ramos Pereira et al., 2009; Petit & Mayer, 2000).

The expected heterozygosity (H_E) is another statistic that is higher in other regions compared to Anatolia, yet the values are similar (ranging between 0.417-0.489, when N>6). Furthermore, in relatively low-diversity populations (e.g., H_E<0.7-0.8; Pruett & Winker, 2008) this estimator deviates more for small samples sizes (N<20-30) and rarefaction methods are more reliable when diversities of populations of such small and varying sample sizes are compared (Pruett & Winker, 2008). The other two regions that are not in HWE (Iberia and the Thrace & Balkans) are two large distribution areas of the species that consist of several populations, all of which are in HWE. However, both the significant F_{IS} values and bottleneck analysis indicate a heterozygote deficit for these broad regions as a whole, which are consequently not in HWE. This deficit may stem from the presence of null alleles; alternatively, it may be the result of a recent population expansion (Cornuet & Luikart, 1997; Rooney et al., 1999). It may also be due to a Wahlund effect, in which a deviation from HWE arises, because individuals from different populations with different allele frequencies are assessed together (Wahlund, 1928; Johnson & Black, 1984). Yet to determine which among the potential reasons is the actual one may be difficult. Dakin & Avise (2004) assumed that it was relatively easy to distinguish between these two causes of heterozygosity deficit and deviation from HWE, i.e. null alleles and Wahlund effect, because if genetic factors such as a cryptic population structure (resulting in a Wahlund effect) or inbreeding are the main reasons, their effects should be similar across all loci, unlike null alleles that are expected to affect a single locus. However, Wahlund effect can also be accompanied by inter-locus variance, especially if the sample sizes are small and polymorphism levels differ among loci; thus distinguishing between the presence of null alleles and Wahlund effect is in fact not necessarily an easy task (Dharmarajan et al., 2013). Regarding the three regions that are not in HWE according to the results of this study, Thrace & Balkans showed signs of a heterozygosity deficit for five out of ten loci, Slovakia for four loci and Iberia for only two loci, with values varying between 0.05 and 1 (P<0.05) (Table A1 in Appendix A). It seems like there is no genetic factor affecting all loci in a parallel manner, hence we suggest that the most probable reason of heterozygosity deficit is the presence of null alleles. Additionally, the general lack of significant F_{ST} and relatively low F_{ST} values (as low as 0.015) suggest the presence of null alleles or a recent population expansion as a more likely explanation than a Wahlund effect. When the test proposed by Waples (2015) was used to distinguish between the possible causes of the deviations from the HWE,

no strong correlation was found between the F_{ST} and F_{IS} values for the 10 loci ($R^2 = 0.21$), once again indicating that the heterozygote deficiency is not caused by a Wahlund effect.

High values of estimators indicating a relatively high genetic diversity in western regions such as Iberia and North Africa (including Morocco), which contrasts with previous mtDNA results, may also be caused by males being less philopatric than females. These regions, especially Morocco (with a single mtDNA haplotype detected for *M. schreibersii*) at the western most part of the distribution area of the species show relatively low diversity at mtDNA (Bilgin et al., 2016). As in the case of genetic differentiation and structuring among populations, sex-biased dispersal and philopatry might explain the different results for the genetic diversity of different types of markers. There are other studies in which similar diversities were calculated for different populations of a given species along its whole geographical distribution area, whereas data obtained by using another type of marker told another story, providing support for specific post-glacial expansion scenarios. For example, in the continental scale study of Küpper et al. (2012) on Kentish plover, which exhibits male philopatry and high female breeding dispersal, there was a significant difference between the allelic richness of inland and mainland populations as measured by microsatellites. On the other hand, there was no significant difference between the genetic diversities of these breeding locations based on mtDNA. Similarly, in the study of Keeney et al. (2005) on blacktip shark (*Carcharhinus limbatus*) in the northwestern Atlantic Ocean, Gulf of Mexico, and Caribbean Sea, the similarity of genetic diversities of different populations depended on the marker used. The northwestern Atlantic nurseries had lower haplotype and nucleotide diversities, consistent with their previous finding of an Atlantic bottleneck and a postglacial range expansion from the Gulf (Keeney et al., 2003). However, nuclear diversities of the northwestern Atlantic and the Gulf are similar and the authors suggest that male dispersal might have replenished the nuclear diversity faster than mitochondrial diversity, which may also be the case for western *M. schreibersii* populations. Another possible contributor to the relatively high nuDNA diversity of *M. schreibersii* in Iberia and North Africa may be its hybridization with its sister species *M. maghrebensis* found in North Africa, which was recently elevated to species level (Puechmaille et al., 2014). This possibility should be checked in further studies by analyzing *M. maghrebensis* and *M. schreibersii* samples together and using more loci, considering that more than 40 informative markers are needed to identify hybrids accurately (Putman & Carbone, 2014; Fitzpatrick, 2012).

Finally, ABC analysis did not provide statistically strong support for any particular phylogenetic scenario. However, Anatolia was the most basal region in all three most probable scenarios, when only the mtDNA is used. The weakness of the support might be due to relatively low mutation rate

of this marker (Bolfikova et al., 2013). Another possible reason is the low number of informative nucleotide positions, rendering the mitochondrial signal weak (Bolfikova et al., 2013). When microsatellite data is also used, the most probable scenarios, which again do not have strong support, seem to be random, without exhibiting a phylogeographically meaningful pattern. This outcome is not surprising, because of the abovementioned similarity of the different regions based on F_{ST} values, due to male dispersal. In addition, the assessment of all regions that are not in HWE and that have relatively high values for descriptive statistics (e.g., Ne, He and I) alongside Anatolia, without the integration of a rarefaction method might have also diminished the already weak signal from the mtDNA sequences.

4.2. *Myotis capaccinii*

4.2.1. Phylogeography of *Myotis capaccinii*

A species distribution model (SDM) was used to obtain past and future projections and investigate the phylogeographic history, as well as the possible phylogeographic changes of *M. capaccinii* in the near future that may arise due to climate change (Knowles et al., 2007; Cordellier & Pfenninger, 2009; Stanton et al., 2012). SDMs were combined with genetic approaches to study bats in several other studies (Flanders et al., 2011; Rebelo et al., 2010, 2012). In this study, classical population genetic methods and SDMs were used together to explain the taxonomy and phylogeographic history of *M. capaccinii*. All analyses revealed a clear separation of three haplogroups, namely haplogroups B, C1 and C2. The separation of haplogroups B and C was already detected in the study of Bilgin et al. (2008b). This study confirmed a further separation of haplogroup C into haplogroups C1 and C2, which was previously detected in the study by Viglino (2012). As in that study, the haplogroup C1 is encountered in Thrace, Balkans and Northern Italy, and haplogroup C2 is found in Iberia and southern Italy. The separation of these haplogroups in Italy is probably due to the presence of the Apennine Mountains, which act as a geographical barrier that restricts the movement of the bats. In fact, the Apennines, along with the Alps seem to be the main cause of the divergence of these two haplogroups in Europe. When compared with the study by Viglino (2012), the analysis of a higher number of samples in this study, and especially the inclusion of samples from southern France resulted in a better detection of the borders separating these haplogroups. The most important difference in this respect is the designation of the French samples as haplogroup C2 individuals, which were assumed to belong to haplogroup C1 (that was called haplogroup A therein) in Viglino's study (Viglino, 2012).

Among the three haplogroups, haplogroup B has the highest haplotype and nucleotide diversities, suggesting it to be the most ancestral among the three. On the other hand, haplogroups C2 has higher values for these statistics compared to haplogroup C1, meaning that the decreasing pattern in genetic diversity from east to west that was encountered in the mtDNA of *Miniopterus schreibersii* was not observed in *Myotis capaccinii*, indicating a relatively long history of separate refugial populations for the three haplogroups.

The phylogenetic trees produced using neighbor-joining and maximum likelihood methods are very similar, both detecting the presence of the three haplogroups as three separate clades, suggesting an older divergence of clade B from the C clades, compared to that between clades C1 and C2. Using Bayesian inference, these divergence times were estimated to be about 350,000 years BP for the divergence of B and C, and 130,000 years BP for the divergence of C1 and C2. Both of these divergence times seem to coincide with glacial periods and especially glacial maxima (Figure 4.1, taken from Cheng et al., 2009). More specifically, the divergence of haplogroups C1 and C2 took place during the Riss glaciation in and around the Alps, whereas the older divergence of B and C haplogroups occurred during the Mindel and Elster glaciations, in the Alps and northern Europe, respectively (Habbe, 2007; Litt et al., 2007). In fact, according to these estimations, haplogroups B and C seem to have differentiated during at least three major glacial maxima (starting with the glacial maximum of Mindel), and their corresponding glaciation periods, and haplogroups C1 and C2 during at least two major glacial maxima (starting with the Riss glaciation), when they became isolated from other haplogroups in their refugial populations.

Pockets of glacial refugia, where animal and plant species have been isolated and differentiated during the LGM have been proposed for Iberian, Italian and Balkan peninsulas and southwestern and eastern Anatolia (Hewitt, 1999; Veith et al., 2003; Schmitt, 2007). The “refugia within refugia” hypothesis suggests that such pockets, or parts of major refugial peninsulas are suitable for the persistence of species and populations (Bilgin, 2011; Bilgin et al., 2016; Canestrelli et al., 2007; Gómez & Lunt, 2007; Medail & Diadema, 2009). The phylogeography of several bat species including *Nyctalus noctula* (Petit et al., 1999a), *Myotis myotis* (Ruedi & Castella, 2003), *Rhinolophus ferrumequinum* (Rossiter et al., 2007), *Rhinolophus hipposideros* (Dool et al., 2013), *Plecotus austriacus* (Razgour et al., 2013) and the *Myotis nattereri* complex (Puechmaille et al., 2012; Salicini et al., 2013) also supports the existence of these glacial refugia. The dating of the mitochondrial splits for *Myotis capaccinii* to these periods of the Pleistocene is yet another case, where glacial refugia are shown to promote genetic diversity and differentiation.

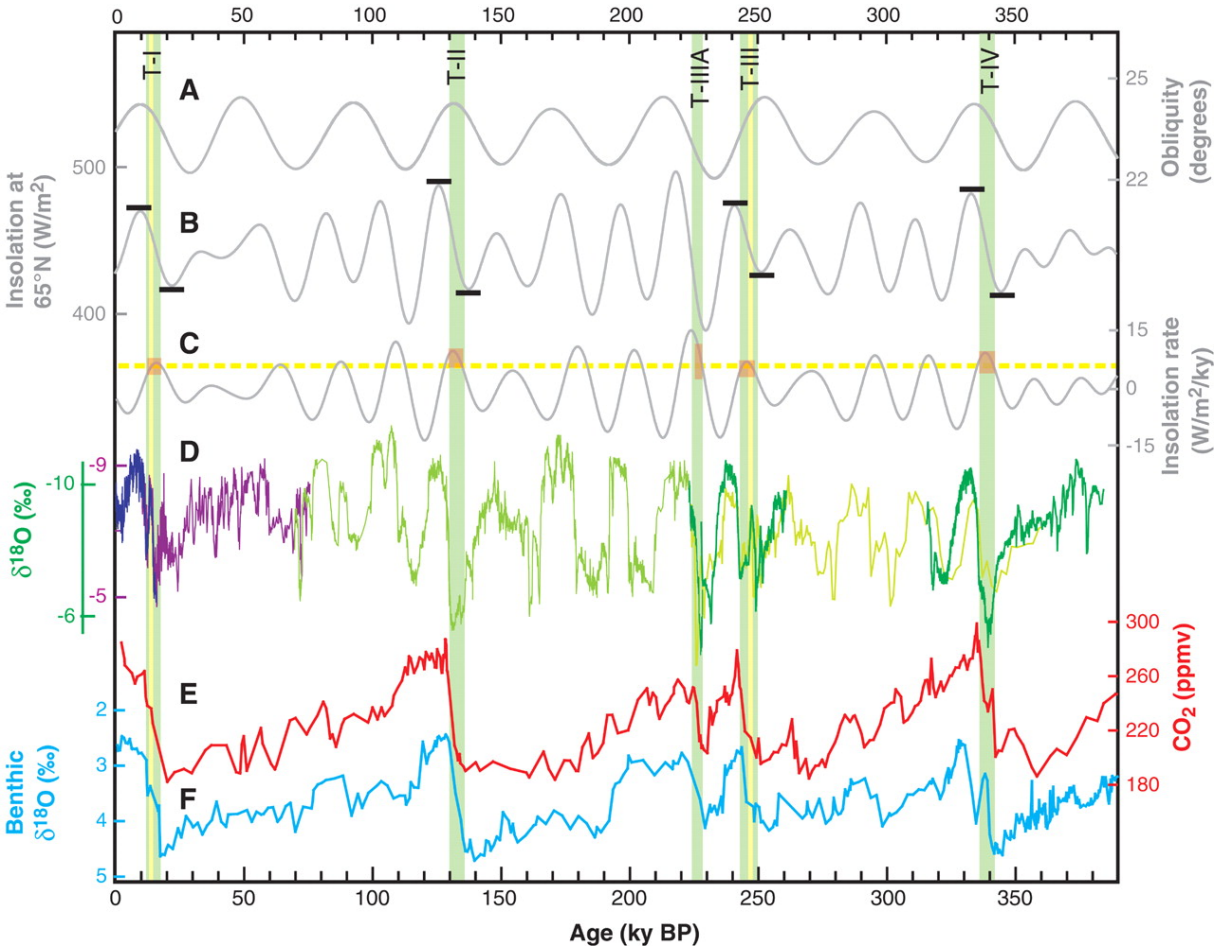


Figure 4.1. Ice age terminations. The green columns represent the time of termination for each glacial period, whereas the periods just before them (on their right), where the peaks are at minimum are the glacial maxima just before the termination of that period.

The SDM analyses for the three haplogroups suggest several areas as suitable refugia for the LGM. East of the Levantine Sea, which was suggested as the primary refugium of *Miniopterus schreibersii*, is again among the stable areas, where *Myotis capaccinii* might have survived during the LGM and is still encountered today. The suitable areas for the three haplogroups included some areas, where individuals carrying haplotypes of that haplogroup do not actually occur. This of course stems from the fact that in those areas the bioclimatic variables may be so suitable for individuals of that haplogroup the area is designated as a potentially suitable area, even though there is no occurrence data for that haplogroup. This lack of occurrence is in turn probably caused by the existence of geographical barriers (e.g., the Alps and the Apennines) and IBD. The absence of haplogroup C1 individuals from predicted suitable areas in western and northern Anatolia may stem from several factors. First, the Bosphorus Strait, the Sea of Marmara and the Strait of Çanakkale

(Dardanelles) may act as a geographical barrier rendering the movement of *Myotis capaccinii* more difficult. Yet the existence of haplogroup B individuals on both sites of these potential barriers weakens this possibility. Another, more probable reason is the relatively more recent expansion of haplogroup C1 populations compared to the expansion of haplogroup B. In fact, the combined effect of this relatively short expansion period and the presence of the abovementioned barriers hindering the movement of the bats may be the reason of the absence of haplogroup C1 in Anatolia, whereas haplogroup B individuals are known to occur as far as Greece and Bulgaria (Bilgin et al., 2008). Still, B individuals also do not occur in all predicted areas, such as in southern Greece, which may be due to the significant and strong IBD effect detected in the Anatolian-Balkan suture zone, where haplogroup C1 populations reside, and due to the presence of the Aegean Sea, a significant geographical barrier in this zone (Bilgin et al., 2011). On the other hand, the limited extend of the actual stable areas that species are able to inhabit both during the LGM and at the present is in accordance with the "refugia within refugia" hypothesis.

The divergence of haplogroups C1 and C2, as well as the genetic similarity of individuals from Iberia, France, southern Italy and North Africa can be explained by the geographical changes that took place at the estimated time of divergence of these haplogroups, as well as the existence of obvious geographical barriers (i.e., the Alps and the Apennines). During that time (circa 130,000 BP) the levels of the Mediterranean Sea were significantly lowered and the Tyrrhenian Sea was much narrower so that there was substantial genetic exchange between populations of Spain, Balearic Islands, North Africa, Sardinia and the Apennine Peninsula (Shackleton et al., 1984). The sharing of haplotype 2 among populations of Tunisia, Mallorca, Sardegna and Lazio is an example of this exchange. It is also possible that Tunisia was colonized relatively recently, directly through the Strait of Sicily (rather than through Gibraltar), as haplotype 1 is encountered both in Sicily and in Tunisia. Even though Sicily and Tunisia are separated by a distance of about 140 km, there is evidence of high levels of genetic similarity among Sicilian and Tunisian populations in other taxa (Husemann et al., 2014; Stöck et al., 2008; Cosson et al., 2005; Habel et al., 2009, 2010, 2011). There are some variations to this pattern as well, for instance, there is evidence that the butterfly *Melanargia galathea* colonized Italy through the Strait of Sicily, and then recolonized Tunisia back from Sicily (Habel et al., 2011). Further discussion about the connectivity of the Iberian and Apennine peninsulas can be found in section 4.3.

Even though in this study the SDM analyses were carried out mainly to obtain empirical results regarding the possible distribution of the haplogroups, still the effect of the final variables used in the

models can be interpreted mechanistically. Bioclimatic predictors that are associated with extreme cold events seem to have a drastic effect on the distribution of the species. Especially the importance of variables such as the precipitation of the coldest and wettest quarters, annual precipitation and annual temperature change suggest that *Myotis capaccinii* avoids arid areas and colder temperatures, similar to another Mediterranean bat species, *Miniopterus schreibersii* (Bilgin et al., 2016). Actually, from an ecological perspective, this outcome makes more sense for *Myotis capaccinii* than it does for *Miniopterus schreibersii*, considering the specific habitat requirements of *Myotis capaccinii*. Unlike *Miniopterus schreibersii*, *Myotis capaccinii* is a specialist, hunting in aquatic environments by trawling insects and fish from surface waters (Aizpurua et al., 2013, Gardiner et al., 2014). This type of prey is known to be in low abundance in areas with extreme temperatures and dry climates (Ramos Pereira et al., 2002), so the unsuitability of these areas for *Myotis capaccinii* makes sense from a feeding ecology perspective, as well.

The maximum entropy modelling technique used for the SDM part of the study has some shortcomings. For instance, a high number of predictors compared to the number of occurrences can result in the overfitting of the models (Breiner et al., 2015). However, this issue was addressed in this study by using a high number of occurrence data, and reducing the number of predictors to eight. Another potential problem is obtaining conflicting results when more than one GCM is used (Bilgin et al, 2016; Varela et al., 2015). Yet in spite of all the differences of the two GCMs, the predictions obtained by using them separately were fairly similar. Overlapping these predictions resulted in more reliable, albeit conservative predictions.

Both the comparison of the LGM models with the present distribution area and Bayesian Skyline plots suggest a relatively recent, post-glacial expansion for all three haplogroups in the Holocene. The final range of the expansions for the haplogroups were dated to start around 2,500 years BP, 2,000 years BP and 1,500 years BP for the haplogroups B, C1, and C2, respectively. Thus, the final expansions have started long after the end of Pleistocene, when the climate was warmer and the deglaciation of continental ice sheets had long been completed (Walker, 1995). When the mismatch distributions are analyzed, however, the fitness of the constant-size model and the range expansion model were similar, with the exception of haplogroup B, for which the expansion model fits the data better. For haplogroup C1, there is an excess of three pairwise differences in the observed data, which stems from the three nucleotide difference of the most common haplotypes of this haplogroup, namely haplotypes 6 and 7, of which haplotype 6 is only encountered in northern Italy, and haplotype 7 only in the Thrace & Balkans region. This, in turn, was possibly caused by the presence of a large area

between these two locations that was not sampled in this study. Fu's F_S and Tajima's D statistics are negative for all haplogroups, indicating population expansion, yet none of these values are significant at the $P<0.05$ level. However, when all samples and haplogroups are assessed together, a significantly positive Tajima's D (but not F_S) value is found, indicating a decreasing population size or balancing selection (Tajima, 1989). The only other significant statistic among all detected values was Fu's and Li's D value for the Koufovouno population, which was also significantly positive, indicating an excess of parsimony informative sites, which in turn probably stems from the fact this population carries haplotypes from different haplogroups.

4.2.2. Taxonomy and conservation of *Myotis capaccinii*

The star-like haplotype network of haplogroup B also indicates a recent population expansion. The most common haplotype of this haplogroup, haplotype 11, is also the haplotype with the largest number of connections or descendant nodes among all 17 haplotypes, indicating it may be the most ancestral haplotype with the highest outgroup probability. Additionally, haplogroup B has the highest haplotype and nucleotide diversities among three haplogroups, and Anatolia and Middle East, two regions that only inhabit individuals carrying haplotypes of haplogroup B, are the two regions with the highest haplotype and nucleotide diversities. Furthermore, the ancestral state of haplogroup B is also supported by the ABC analysis. The large genetic break between haplogroups B and the C, as well as the lack of a gradually decreasing pattern of genetic diversity out of Anatolia, supports the hypothesis that the C haplogroups were derived from haplogroup B a long time ago and differentiated during several glacial and interglacial periods. Hence there is no indication of Anatolian and Middle Eastern populations populating Europe recently, as was the case for *M. schreibersii* (Bilgin et al., 2016).

Individuals from haplogroups B and C1 were found in the same cave in Koufovouno in this study. (Actually, one bat with a haplogroup B haplotype was captured in Koufovouno first and then recaptured in the proximally located roost of Tsoutouro). Moreover, in the study of Bilgin et al. (2008), two individuals from clade B were found in a cave in Bulgaria. The probability of individuals carrying these largely differentiated mtDNA living in the same caves in sympatry raises the possibility of them being members of different species. In any case, the division of haplogroups B and C1 and the geographical position of their distribution areas is in accordance with the proposal of *M. c. bureschi* (Heinrich, 1936) being (at least) a subspecies of *M. capaccinii* (DeBlase, 1980; Koopman, 1994; Kumerloeve, 1975). Whether it is a separate, cryptic species requires further

analyses and especially the analysis of nuDNA markers. If further studies show that haplogroup B individuals carry morphologic characters of *M. c. bureschi* and that there is sufficient differentiation in the nuDNA level between *M. c. capaccinii* and *M. c. bureschi* individuals, it will be possible to elevate *M. c. bureschi* to species level, as it is found to be living in sympatry with C1 individuals that are already distinct in terms of their mtDNA. Finally, the existence of two differentiated C haplogroups, namely haplogroups C1 and C2, raises the possibility of another subspecific designation between populations of the Balkan Peninsula and those of Iberia, southern Italy, and North Africa, as their populations are allopatric and there is significant differentiation between their mtDNA, which might be reflected in morphology and nuclear DNA. In this respect, analysis of their morphology and nuclear DNA may make it possible to suggest a new, western subspecies, which corresponds to haplogroup C2.

From a conservation perspective, it should be noted that the size of a population is one of the most significant considerations in conservation (Shaffer, 1981). This concern about the viability of bat populations was emphasized before for several bat species, including the common bent-wing bat, *M. schreibersii*, western big-eared bat, *Corynorhinus townsendii*, the little brown myotis, *Myotis lucifugus*, and the New Zealand long-tailed bat, *Chalinolobus tuberculatus* (Furman et al., 2010b; Bilgin et al., 2012; Puechmaille et al., 2014; Pryde et al., 2005; Smith et al., 2008; Frick et al., 2010). Thus the probability of discovering new cryptic species within populations of *M. capaccinii* in suture and hybrid zones, which will drastically reduce its population size, may result in further aggravation of its conservation status, which is already Vulnerable under the IUCN classification (Hutson et al., 2008b).

4.2.3. Future SDM Projections

The SDM analysis suggest a serious threat for the future conservation of *Myotis capaccinii*, because of the predicted climate change and the resulting loss of suitable habitats for the species, which will potentially force its populations move rapidly north, and culminate in a reduction in its current range. For Mediterranean bat species such as *Myotis capaccinii*, the predicted suitability for the future depends significantly on the scenario and the general circulation model (GCM) chosen for the SDM analysis (Rebelo et al., 2010; Varela et al., 2015). Yet, even though there are differences between the future predictions obtained using the two GCMs, namely the Community Climate System Model (CCSM4.0) and the Model for Interdisciplinary Research on Climate Earth System Model (MIROC-ESM) (Gent et al., 2011; Watanebe et al., 2011), both models suggest significant loss of

suitable areas for the species by 2070. When analyzed separately, it was seen that MIROC predicts a larger loss of suitable areas for haplogroup B compared to CCSM, but the predicted suitability of (both lost and gained) habitats for haplogroups C1 and C2 were very similar. When the outcomes of both models were overlapped and compared with the predicted present distribution of the species, however, it was seen that the areas that will be potentially lost by far exceed the areas that will be gained for all three haplogroups.

The variables chosen for the analysis suggest that the most important factor affecting the distribution of *M. capaccinii* is the quantity of water (precipitation etc.), which was previously detected for Mediterranean bats including *M. capaccinii* (Rebelo et al., 2010). Also another factor, annual temperature change, which turned out to be one of the most important variables of the models, was previously shown to be strongly correlated with species richness of European bats (Ulrich et al., 2007).

The relatively narrow overlap of the predicted and current ranges of a species indicate an increased risk of extinction, as forced shifts in distribution that are caused by climate change put extra pressure on animal and plant species (Root et al., 2003). When significant changes occur in a habitat, where a population is currently adapted to, the population might have to move to more suitable areas or go extinct (Thomas et al., 2004; Hijmans & Graham, 2006). *M. capaccinii* may be especially vulnerable to such changes as it is a species requiring a specific type of habitat, like water bodies and an accompanying vegetation with a height not exceeding 50m (Viglino, 2012). Some bats are already affected by climatic change, e.g. *Pipistrellus kuhlii* has shifted its range towards north, probably because of increased temperatures (Sachanowicz et al., 2006), and there is also evidence for phenological changes, e.g. the birth period of *Myotis myotis* has already shifted six months in southern Spain (Ibanez, 1997). Considering the relatively small climatic changes experienced so far, and the predictions of the IPCC, the distribution of many animal and plant species, including bats, are prone to much more drastic changes in the near future (Root et al., 2003). Furthermore, anthropogenic influences and resulting habitat change may limit the distribution range of many species even further (Moreira & Russo, 2007). Even though bats are able to fly and thus migrate more effectively compared to most other mammal species, a potential decrease in the number of potential roosts may limit their dispersal significantly as well (Scheel et al., 1996; Rodrigues et al., 2003; Russo et al., 2004). In addition, the suitability of the roosts may change because of a changing climate, affecting the survival rate of the bats in roosts (Racey et al., 1987; Webb et al., 1995). Consequently, the predicted northward movement, especially the narrow overlap of current and future ranges of *M.*

capaccinii is a cause for conservation concern for this vulnerable bat species, which already has relatively few colonies due to its specific habitat requirements.

4.3. A comparison of postglacial expansion patterns of *Miniopterus schreibersii* and *Myotis capaccinii* from an phylogeographic and ecological perspective

The postglacial expansion patterns of other bat species from the genus *Myotis* were studied before (Puechmaille et al., 2012; Salicini et al., 2013; Ruedi & Castella, 2003; Ruedi et al., 2008). One of these, *Myotis nattereri* was originally considered a single species, yet it turned out that it was actually a species complex consisting of four cryptic species, namely *Myotis nattereri* s.s., *Myotis escalerai*, *Myotis* sp. B and *Myotis* sp. A. (Puechmaille et al., 2012; Salicini et al., 2013). *Myotis nattereri* s.s. apparently recolonized Europe from the Balkan Peninsula, and since the northward movement of its three cryptic species was blocked by the Alps and Pyrenees, the expansion pattern of the *Myotis nattereri* species complex most likely fits the grasshopper paradigm (Salicini et al., 2013). However, the connectivity between the populations of *Myotis* sp. A. in Iberia and Italy complicates the issue, rendering the special case of the *Myotis nattereri* complex unique and different from the paradigmatic model species (Salicini et al., 2013). On the other hand, the connectivity of *Myotis capaccinii* haplogroup C2 populations in Italy and in Iberia is very similar to the connectivity of *Myotis* sp. A. populations.

In fact, connectivity between Iberian and Italian populations was detected for several bat species (Bogdanowicz et al., 2015), although the direction of the genetic exchange depends on individual species (Salicini et al., 2013; Hulva et al., 2010). For instance, Salicini et al. (2013) concluded that *Myotis* sp. A. most likely colonized Iberia from Italy, whereas Hulva et al. (2010) suggest an expansion from Maghreb to Italy for *Pipistrellus pipistrellus*. Other species that have expanded from Iberia and Maghreb to Italy include *Myotis punicus* and *Hypsugo savii* (Bogdanowicz et al., 2015). The possible direction of expansion and colonization for *Myotis capaccinii* might be through Italy, because it hosts four haplotypes of haplogroup C2, two of which also occur in Iberia and North Africa. In addition, the haplotype and nucleotide diversities are higher for Italy. Whatever the case, haplogroup C2 of *Myotis capaccinii* is clearly separated from haplogroup C1. If haplogroup B really specifies a cryptic, sister species of *Myotis capaccinii* s.s., which in fact can be named as *M. bureschi*, C1 and C2 may be seen as the only two mitochondrial lineages of *Myotis capaccinii* s.s. In this case, the bear or the butterfly paradigm may explain the postglacial expansion of *Myotis capaccinii* s.s.,

depending on whether the source of haplogroup C2 was Iberia or Italy. Besides, the northward expansion of haplogroup C2 and its presence in central Europe should be investigated, even though this haplogroup was detected in France in this study. On the other hand, if all three haplogroups of *Myotis capaccinii* are assessed together, the postglacial expansion pattern of the species does not fit any of the five paradigms. Still, it is possible that there are actually other species or species complexes that followed a similar pattern of glacial differentiation and subsequent expansions, which were not detected, because Anatolia might not have been sampled sufficiently (Bilgin, 2011).

The postglacial expansion routes of refugial populations can be deduced with data on their genetic relationships, ancestral distribution patterns, fossil data, and geographic landscapes, among others. The expansion of *Miniopterus schreibersii* after the LGM seems to have followed a pattern that fits a fifth paradigm of postglacial colonization (Bilgin et al., 2016). The possible routes of dispersal for this species in this period can be hypothesized as in Figure 4.2. On the other hand, the possible, again highly hypothetical, routes of expansion for *Myotis capaccinii*, are given in Figure 4.3.

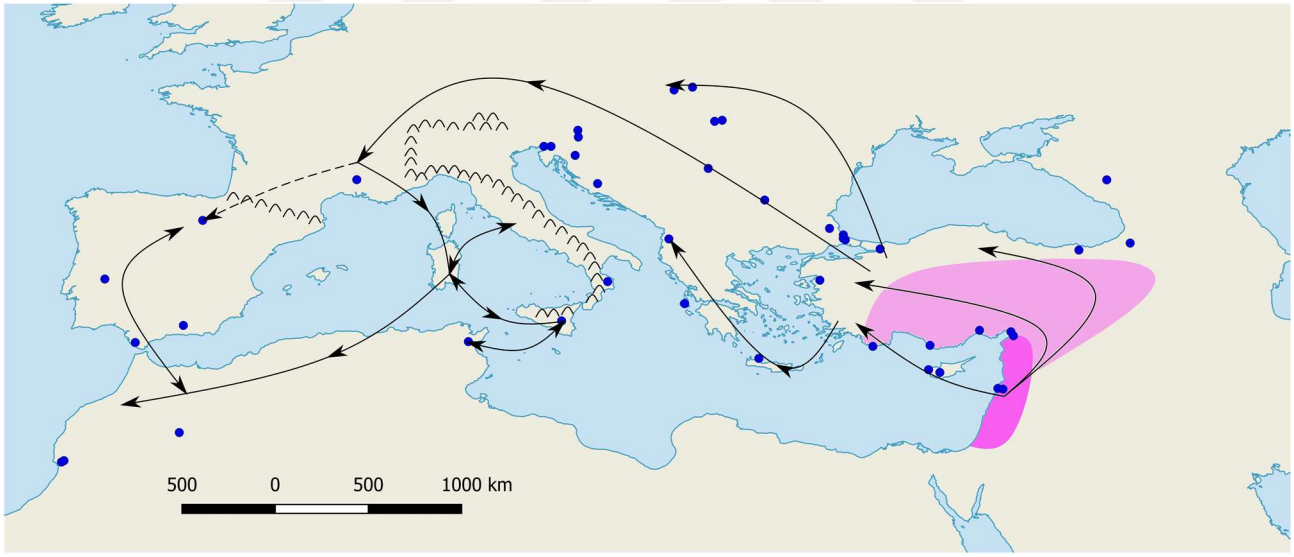


Figure 4.2. The potential postglacial expansion routes of *Miniopterus screibersii* after the LGM. The possible location of glacial refugia were drawn according to the phylogeographic hypothesis suggested in Bilgin et al. (2016). The dark pink area represents the east of the Levantine Sea, the primary refugium for *M. schreibersii* during the LGM. Part of Anatolia, which might have acted as a secondary refugium is shown in light pink. Bidirectional arrows were used, where the direction of expansion was not certain.

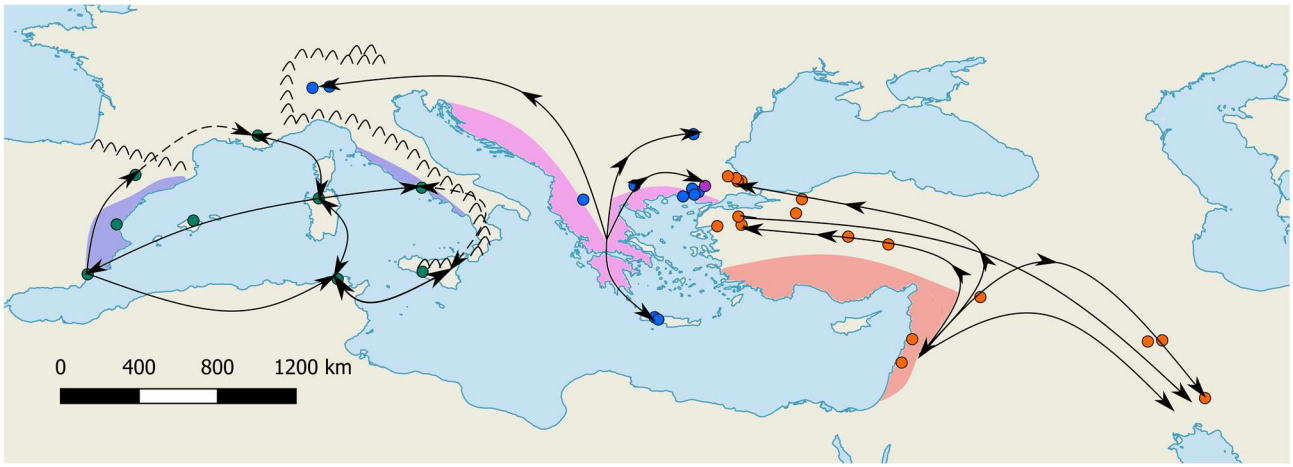


Figure 4.3. The potential postglacial expansion routes of *Myotis capaccinii* after the LGM. The possible location of glacial refugia were drawn according to the MAXENT predictions. The coloring of the potential refugia is random, whereas the color codes of the sample haplogroups correspond to those in Table 3.4. As mentioned in the text, the primary refugia for the *M. capaccinii* haplogroup C2 might have been Italy or Iberia, even though Italy is more likely to be so, and that is why both are shown as potential candidates using bidirectional arrows.

One of the most important reasons of the significant difference between the expansion patterns of these two species of bats is the hypothesized extinction of *Miniopterus schreibersii* in all three major European refugia, namely the Iberian, Italian (Apennine) and Balkan Peninsulas, during the LGM (Bilgin et al., 2016). After the LGM, *Miniopterus schreibersii* was able to recolonize its whole, current, Mediterranean distribution area, including southern Europe and North Africa from Anatolia, thanks to its high flight capacity and being a generalist species able to prey on most insect orders. The mode of expansion of *Miniopterus schreibersii* implies that its ancestral populations were able to relocate effectively and pass through the Pyrenees, Apennines, and even the Alps. Other bat species, such as *Myotis myotis* and *Myotis bechsteinii* are known to have been able to migrate through Pyrenees, even though surpassing the Alps seems to be much more difficult (Ruedi et al., 2008; Bogdanowicz et al., 2009). Gibraltar and Strait of Sicily are also known to be colonization routes for bats, and other species (Hulva et al., 2010; Husemann et al., 2014; Stöck et al., 2008; Cosson et al., 2005; Habel et al., 2009, 2010, 2011). The relation between low levels of phylogeographic structuring and long-range, aerial hawking is observed in many bat species, including *Miniopterus schreibersii* (Bogdanowicz et al., 2015). For instance, *Nyctalus noctula*, which is another generalist, aerial hawker, and one of the bats with the longest migrations on record (Petit et al., 1999a; Norberg& Rayner, 1987), emerged from only one main glacial refugia, similar to *Miniopterus schreibersii*.

Myotis capaccinii, on the other hand, has not been able to migrate through the Alps or Apennines, after the LGM. France and Iberia, however, seem to be connected; most probably through Pyrenees, but also possibly through Italy and North Africa, although this is a considerably longer route. In any case, three lineages of *Myotis capaccinii* are clearly separated with strong structuring at the mitochondrial level. *Myotis capaccinii* is a specialist species, with specific, narrow habitat requirements and a lower flight capacity compared to that of *Miniopterus schreibersii*. This difference could be the main reason for the different patterns of genetic structuring in these two species.

It can be hypothesized that the extinction of the populations of *Miniopterus schreibersii* in Europe during the LGM might have resulted from competitive exclusion. In this case, the generalist feeding strategy of *Miniopterus schreibersii* might have turned into a disadvantage. That is, as the number of extant insect species dropped significantly during the LGM, the competition of generalist bats that feed on them, which survived in the narrow refugial areas might have gotten more intense, resulting in the elimination of some of these bats. Of course investigating this suggestion necessitates the determination of candidate rival species that had overlapping niches with that of *Miniopterus schreibersii*. This in turn, will require the thorough examination of fossil data of the bats in question as well as the common prey species in their diet. In fact, a similar suggestion regarding the competitive exclusion of *Miniopterus schreibersii* from its European habitat was already made in the study of Bilgin et al. (2016), where the presence of *Miniopterus maghrebensis* in Iberia was suggested to be the potential reason of the extinction of its sister species *Miniopterus schreibersii* in this region, which isone of the three main refugial peninsulas in Europe. A specialist such as *Myotis capaccinii*, on the other hand, was able to survive in at least two of the main European glacial refugia (i.e., the Balkans and Iberia and/or Italy), not only during the LGM, but during several consecutive glacial maxima. This might have been due to its highly specialized diet, as it was already feeding on prey not many other bat species depended on, so that the lack of competition could have enabled *Myotis capaccinii* to survive in the case where not all of its prey species went extinct in these areas. Consequently, this highly specialized adaptation of the species to a certain type of niche, which along with its relatively low mobility restricts its dispersal capability, might have ironically enabled it to survive more successfully during extreme climatic conditions, compared to generalist species such as *Miniopterus schreibersii* that have much higher mobility and flexibility of their habitat requirements.

5. CONCLUSION

5.1. *Miniopterus schreibersii*

Male-biased gene flow and female philopatry were detected at a continental scale in *Miniopterus schreibersii.* Some additional support was found for the oak gallwasp paradigm of postglacial expansion patterns, based on nuclear markers as well, which using mtDNA results stated that Anatolia was the primary refugium during the LGM, previously. Among the phylogeographic regions that consist of several populations and that are in HWE (thus established) for nuDNA markers, Anatolia seems to be the most diverse. Yet male-mediated dispersal seems to have diminished the difference between the diversities of these regions and populations, as there was no significant differentiation in nuDNA between regions, even though it was detected in mtDNA. Still, further studies taking the sex of the individuals into account (information that we did not have in this study) will be helpful to investigate the patterns of dispersal of different genetic markers more thoroughly. Additionally, more samples from North Africa and Eastern Anatolia should be analyzed to reveal the expansion patterns of these populations in greater detail. Specifically, potential hybridizations of three sister species of *Miniopterus*, that is hybridization of *M. schreibersii* and *M. pallidus* around southeast Anatolia and hybridization of *M. schreibersii* and *M. maghrebensis* in and around North Africa should be studied, as these can significantly affect the genetic structure and diversity of all three species.

5.2. *Myotis capaccinii*

All analyses revealed the existence of three haplogroups of *Myotis capaccinii*, namely haplogroups B, C1, and C2, where the distribution area of haplogroup B coincides with that of the previously proposed subspecies *M. c. bureschi* (Heinrich 1936). Among these three haplogroups, haplogroup B seems to be the most ancestral one, its divergence from the other haplogroups dating back to around 350,000 years BP. This date, as well as the estimated divergence time of 130,000 years BP of haplogroups C1 and C2, correspond to the maxima of Mindel and Riss glaciations, indicating the isolation and differentiation of these haplogroups during several glacial periods. The fact that individuals from haplogroups B and C1 are found sympatrically in the same cave suggests that *M. c. bureschi* might in fact be a separate species. Similarly, the C1 – C2 divergence indicates another subspecies in the western most part of the distribution area of *M. capaccinii*. However, further

analysis, especially investigation of nuclear markers is required in order to be able to make subspecific and specific recommendations.

SDM analysis for the future suggests that the changes of potentially suitable areas for *M. capaccinii*, which may be caused by climate change, will put greater pressure on survival of the species in the near future. The current conservation status of the species, which is already Vulnerable (Hutson et al., 2008b) may suffer changes. Especially its western populations, representing haplogroup C2, which are already threatened (Guillén, 1999) may be more prone to such changes and could face an enhanced risk of extinction. Consequently, the implementation of environmental measures that will mitigate climate change and effective habitat management are of utmost importance for the preservation of this and other bat species, as is the case for numerous other animals and plants.

5.3. *Miniopterus schreibersii* & *Myotis capaccinii* comparison: How does biology affect phylogeography and conservation?

The postglacial expansion pattern of *Myotis capaccinii* seems to be significantly different from that of *Miniopterus schreibersii*. This pattern may be a species-specific case, an indication of the presence of a yet undetected paradigm of postglacial expansions or it may even fit one of the existing paradigms depending on the possible new specific designations of *Myotis capaccinii* haplogroups. In any case, the difference of the way of recolonization between the two species apparently stems from their ecological and morphological differences. The generalist diet and relatively high flight capacity of *Miniopterus schreibersii* seems to have enabled it to recolonize an entire continent quickly and efficiently, whereas *Myotis capaccinii*, being a specialist with specific habitat requirements and a lower mobility, has three clearly separated genetic lineages that inhabit three corresponding geographical areas. These factors probably contributed significantly to its poorer conservation status, when compared to *Miniopterus schreibersii*. Furthermore, the presence of three distinct genetic clusters that might correspond to up to three species or subspecies may potentially decrease its population size further, worsening the conservation status of these individual species/subspecies even further. This observation once again reveals how critical it is to take action for the conservation of this vulnerable species. However, it should also be noted that *Miniopterus schreibersii*, which is listed as a Near Threatened species by the IUCN, is not in the clear as well, especially as its several cryptic species have been recently defined, effectively decreasing its total population size.

REFERENCES

Adams, R. A., Hayes, M. A., 2008. Water availability and successful lactation by bats as related to climate change in arid regions of western North America. Journal of Animal Ecology, 77, 115–1121.

Aihartza, J., Almenar, D., Salsamendi, E., Goiti, U., Garin, I., 2008. Fishing behaviour in the long-fingered bat *Myotis capaccinii* (Bonaparte, 1837): an experimental approach. Acta Chiropterologica, 10, 287-301.

Aizpurua, O., Garin, I., Alberdi, A., Salsamendi, E., Baagoe, H., Aihartza, J., 2013. Fishing long-fingered bats (*Myotis capaccinii*) prey regularly upon exotic fish. PLoS ONE, 8, e80163.

Albayrak, I., Aşan, N., 2002. Taxonomic status and karyotype of *Myotis capaccinii* (Bonaparte, 1837) from Turkey (Chiroptera: Vespertilionidae). Mammalian Science, 66, 63–70.

Alberdi, A., Gilbert, M. T. P., Razgour, O., Aizpurua, O., Aihartza, J., Garin, I., 2015. Contrasting population-level responses to Pleistocene climatic oscillations in an alpine bat revealed by complete mitochondrial genomes and evolutionary history inference. Journal of Biogeography, 42, 1689–1700.

Amengual, B., López-Roig, M., Serra-Cobo, J., 2007. First record of seasonal over sea migration of *Miniopterus schreibersii* and *Myotis capaccinii* between Balearic Islands (Spain). Acta Chiropterologica, 9, 319–322.

Amos, W., Hoffman, J. I., Frodsham, A., Zhang, L., Best, S., Hill, A. V. S., 2007. Automated binning of microsatellite alleles: Problems and solutions. Molecular Ecology Notes, 7, 10–14.

Appleton, B. R., McKenzie, J. A., Christidis, L., 2004. Molecular systematic and biogeography of the bent-wing bat complex *Miniopterus schreibersii* (Kuhl, 1817) (Chiroptera: Vespertilionidae). Molecular Phylogenetics and Evolution, 31, 431–439.

Appleton, B., 2008. *Miniopterus oceanensis*. In IUCN 2012. IUCN Red List of Threatened Species. Version 2012.1. http://www.iucnredlist.org (accessed October 2012).

Araujo, M. M., 2002. Biodiversity Hotspots and Zones of Ecological Transition. Conservation Biology, 16, 1662–1663.

Arnold, B. D., 2007. Population structure and sex-biased dispersal in the forest dwelling vespertilionid bat, *Myotis septentrionalis*. American Midland Naturalist, 157, 374–384.

Ashley, M. V., Willson, M. F., Pergams, O. R. V., O'Dowd, D. J., Gende, S. M., Brown, J. S., 2003. Evolutionarily enlightened management. Biological Conservation, 111, 115–123.

Astakhov, V., 2004. Middle Pleistocene glaciations of the Russian North. Quaternary Science Reviews, 23, 1285–1311.

Avise, J. C., Arnold, J., Ball, R. M., Bermingham, E., Lamb, T., Neigel, J.E., Reeb, C.A., Saunders, N.C., 1987. Intraspecific phylogeography: The mitochondrial DNA bridge between population genetics and systematics. Annual Review of Ecology and Systematics, 18, 489–522.

Avise, J. C., 1989. Gene trees and organismal histories: A phylogenetic approach to population biology. Evolution, 43, 1192–1208.

Avise, J. C., 1994. Molecular Markers, Natural History, and Evolution, Chapman & Hall, London.

Avise, J. C., 1998. The history and purview of phylogeography: a personal reflection. Molecular Ecology, 7, 371–379.

Avise, J. C., 2000. Phylogeography: The history and formation of species, Harvard University Press, Cambridge, MA.

Avise, J. C., 2009. Phylogeography: retrospect and prospect. Journal of Biogeography, 36, 3–15.

Balint, M., Domisch, S., Engelhardt, C. H. M., Haase, P., Lehrian, S., Sauer, J., Theissinger, K., Pauls, S. U., Nowak, C., 2011. Cryptic biodiversity loss linked to global climate change. Nature Climate Change, 1, 313–318.

Bangert, R. K., Turek, R., Martinsen, G. D., Wimp, G. M., Bailey, J. K., Whitham, T. G., 2005. Benefits of Conservation of Plant Genetic Diversity to Arthropod Diversity. Conservation Biology, 19, 379–390.

Bellard, C., Bertelsmeier, C., Leadley, P., Thuiller, W., Courchamp, F., 2012. Impacts of climate change on the future of biodiversity. Ecology Letters, 15, 365–377.

Bellinger, M. R., Johnson, J. A., Toepfer, J., Dunn, P., 2003. Loss of Genetic Variation in Greater Prairie Chickens Following a Population Bottleneck in Wisconsin, U.S.A. Conservation Biology, 17, 717–724.

Benton, M. J., Twitchett, R. J., 2003. How to kill (almost) all life: the end-Permian extinction event. Trends in Ecology & Evolution, 18, 358–365.

Bidon, T., Janke, A., Fain, S. R., Eiken, H. G., Hagen, S. B., Saarma, U., Hallström, B.M., Lecomte, N., Hailer, F., 2014. Brown and polar bear Y chromosomes reveal extensive male-biased gene flow within brother lineages. Molecular Biology and Evolution, 31, 1353–1363.

Bilgin, R., Karataş, A., Çoraman, E., Pandurski, I., Papadatou, E., Morales, J. C., 2006. Molecular taxonomy and phylogeography of *Miniopterus schreibersii* (Kuhl, 1817) (Chiroptera: Vespertilionidae), in the Eurasian transition. Biological Journal of the Linnean Society, 87, 577–582.

Bilgin, R., Karataş, A., Çoraman, E., Disotell, T., Morales, J. C., 2008a. Regionally and climatically restricted patterns of distribution of genetic diversity in a migratory bat species, *Miniopterus schreibersii* (Chiroptera: Vespertilionidae). BMC Evolutionary Biology, 8, 209.

Bilgin, R., Karataş, A., Çoraman, E., Morales, J. C., 2008b. The mitochondrial and nuclear genetic structure of *Myotis capaccinii* (Chiroptera: Vespertilionidae) in the Eurasian transition, and its taxonomic implications. Zoologica Scripta, 37, 253–262.

Bilgin, R., Furman, A., Çoraman, E., Karataş, A., 2008c. Phylogeography of the Mediterranean horseshoe bat, *Rhinolophus euryale* (Chiroptera: Rhinolophidae), in southeastern Europe and Anatolia. Acta Chiropterologica, 10, 41–49.

Bilgin, R., 2011. Back to the Suture: The Distribution of Intraspecific Genetic Diversity in and Around Anatolia. International Journal of Molecular Sciences, 12, 4080–4103.

Bilgin, R., Gürün, K., Maracı, Ö, Furman, A., Hulva, P., Çoraman, E., Lucan, R. K., Bartonicka, T., Horacek, I., 2012. Syntopic occurrence in Turkey supports separate species status for *Miniopterus schreibersii schreibersii* and M *schreibersii pallidus* (Mammalia: Chiroptera). Acta Chiropterologica, 14, 279–289.

Bilgin, R., Gürün, K., Rebelo, H., Puechmaille, S. J., Maracı, Ö, Presetnik, P., Benda, P., Hulva, P., Ibanez, C., Hamidovic, D., Fressel, N., Horaček, I., Karataş, A., Karataş, A., Allegrini, B., Georgiakakis, P., Gazaryan, S., Nagy, Z., Abi-Said, M., Lučan, R. K., Bartonička, T., Nicolaou, H., Scaravelli, D., Karapandža, B., Uhrin, M., Paunović, M., Juste, J., 2016. Circum-Mediterranean phylogeography of a bat coupled with past environmental niche modelling: a new paradigm for the recolonization of Europe? Molecular Phylogenetics and Evolution, 99, 323–336

Bogdanowicz, W., Van Den Bussche, R. A., Gajewska, M., Postawa, T., Harutyunyan, M., 2009. Ancient and contemporary DNA sheds light on the history of mouse-eared bats in Europe and the Caucasus. Acta Chiropterologica, 11, 289–305.

Bogdanowicz, W., Hulva, P., Bolfíkova, B. C., Bus, M. M., Rychlicka, E., Sztencel-Jablonka, A., Cistrone, L., Russo, D., 2015. Cryptic diversity of Italian bats and the role of the Apennine refugium in the phylogeography of the western Palaearctic. Zoological Journal of the Linnean Society, 174, 635–648.

Bohmann, K., Monadjem, A., Lehmkuhl Noer, C., Rasmussen, M., Zeale, M. R. K., Clare, E. L., Jones, G., Willerslev, E., Gilbert, M. T. P., 2011. Molecular diet analysis of two African free-tailed bats (Molossidae) using high-throughput sequencing. PLoS ONE, 6, 21441.

Bolfíková, B., Konečneý, A., Pfäffle, M., Skuballa, J., Hulva, P., 2013. Population biology of establishment in New Zealand hedgehogs inferred from genetic and historical data: conflict or compromise? Molecular Biology, 22, 3709–3720.

Bond, J. E., Beamer, D. A., Lamb, T., Hedin, M., 2006. Combining genetic and geospatial analyses to infer population extinction in mygalomorph spiders endemic to the Los Angeles region. Animal Conservation, 9, 145–157.

Bouzat, J. L., Cheng, H., Lewin, H. A., Westemeier, R. L., Brawn, J. D., Paige, K. N., 1998. Genetic evaluation of a demographic bottleneckin the Greater Prairie Chicken. Conservation Biology, 12, 836–843.

Brito, P. H., 2007. Contrasting patterns of mitochondrial and microsatellite genetic structure among Western European populations of tawny owls (*Strix aluco*). Molecular Ecology, 16, 3423–3437.

Brown, J. H., Lomolino, M. V., 1998. Biogeography, Second Ed., Sinauer Associates, Sunderland, MA, USA, 691.

Buckley, T. R., Marske, K., Attanayake, D., 2010. Phylogeography and ecological niche modelling of the New Zealand stick insect *Clitarchus hookeri* (White) support survival in multiple coastal refugia. Journal of Biogeography, 37, 682–695.

Buisson, L., Thuiller, W., Casajus, N., Lek, S., Grenouillet, G., 2010. Uncertainty in ensemble forecasting of species distribution. Global Change Biology, 16, 1145–1157.

Burland, T. M., Entwistle, A. C., Racey, P. A., 2006. Social and population structure in the brown long-eared bat *Plecotus auritus* In Zubaid, A., MacCracken, G. F., Kunz, T. H. (Eds), Functional and evolutionary ecology of bats, Oxford University Press, New York, 185–198.

Canestrelli, D., Cimmaruta, R., Nascetti, G., 2007. Phylogeography and historical demography of the Italian treefrog, *Hyla intermedia*, reveals multiple refugia, population expansions and secondary contacts within peninsular Italy. Molecular Ecology, 16, 4808–4821.

Caparroz, R., Miyaki, C. Y., Baker, A. J., 2009. Contrasting phylogeographic patterns in mitochondrial DNA and microsatellites: evidence of female philopatry and male-biased gene flow among regional populations of the blue-and-yellow macaw (Psittaciformes: *Ara ararauna*) in Brazil. The Auk, 126, 359–370.

Carroll, R., Augspurger, C., Dobson, A., Franklin, J., Orians, G., Reid, W., Tracy, R., Wilcove, D., Wilson, J., 1996. Strengthening the Use of Science in Achieving the Goals of the Endangered Species Act: An Assessment by the Ecological Society of America. Ecological Applications, 6, 1–11.

Cathey, J. C., Bickham, J. W., Patton, J. C., 1998. Introgressive hybridization and nonconcordant evolutionary history of maternal and paternal lineages in North American deer. Evolution, 52, 1224–1229.

Caye, K., Deist, T. M., Martins, H., Michel, O., François, O., 2016. TESS3: Fast inference of spatial population structure and genome scans for selection. Molecular Ecology Resources, 16, 540–548.

Chan, L. M., Brown, J. L., Yoder, A. D., 2011. Integrating statistical genetic and geospatial methods brings new power to phylogeography. Molecular Phylogenetics and Evolution, 59, 523–537.

Cheng, H., Edwards, R. L., Broecker, W. S., Denton, G. H., Kong, X. G., Wang, Y. J., Zhang, R., Wang, X. F., 2009. Ice age terminations. Science, 326, 248–252.

Cheviron, Z. A., Brumfield, R. T., 2009. Migration-selection balance and local adaptation of mitochondrial haplotypes in rufous collared sparrows (*Zonotrichia capensis*) along an elevational gradient. Evolution, 63, 1593–1605.

Clare, E. L, Fraser, E. E., Braid, H. E., Fenton, M. B., Hebert, P. D. N., 2009. Species on the menu of a generalist predator, the eastern red bat (*Lasiurus borealis*): Using a molecular approach to detect arthropod prey. Molecular Ecology, 18, 2532–2542.

Clement, M., Posada, D., Crandall, K. A., 2000. TCS: A computer program to estimate gene genealogies. Molecular Ecology, 9, 1657–1659.

Corbet, G. B., 1978. The Mammals of the Palearctic Region: A Taxonomic Review, British Museum and Cornell University Press, Ithaca, New York.

Cordellier, M., Pfenninger, M., 2009. Inferring the past to predict the future: climate modelling predictions and phylogeography for the freshwater gastropod *Radix balthica* (Pulmonata, Basommatophora). Molecular Ecology, 18, 534–544.

Cornuet, J. M., Luikart, G., 1997. Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. Genetics, 144, 2001–2014.

Cornuet, J. M., Pudlo, P., Veyssier, J., Dehne-Garcia, A., Gautier, M., Leblois, R., Marin, J. M., Estoup, A., 2014. DIYABC v2.0: a software to make approximate Bayesian computation inferences about population history using single nucleotide polymorphism, DNA sequence and microsatellite data. Bioinformatics, 30, 1187–1189.

Cosson, J. F., Hutterer, R., Libois, R., Sarà, M., Taberlet, P., Vogel, P., 2005. Phylogeographical footprints of the Strait of Gibraltar and Quaternary climatic fluctuations in the western Mediterranean: a case study with the greater white-toothed shrew, *Crocidura russula* (Mammalia: Soricidae). Molecular Ecology, 14, 1151–1162.

Dakin, E. E., Avise, J. C., 2004. Microsatellite null alleles in parentage analysis. Heredity, 93, 504–509.

Dalmaris, E., Ramalho, C. E., Poot, P., Veneklaas, E. J., Byrne, M., 2015. A climate change context for the decline of a foundation tree species in south-western Australia: insights from phylogeography and species distribution modelling. Annals of Botany, 116, 941–952.

Davis, A. J., Jenkinson, L. S., Lawton, J. L., Shorrocks, B., Wood, S., 1998a. Making mistakes when predicting shifts in species range in response to global warming. Nature, 391, 783–786.

Davis, A. J., Lawton, J. L., Shorrocks, B., Jenkinson, L. S., 1998b. Individualistic species responses invalidate simple physiological models of community dynamics under global environmental change. Journal of Animal Ecology, 67, 600–612.

Davis, M. B., Shaw, R. G., 2001. Range shifts and adaptive responses to Quaternary climate change. Science, 292, 673–679.

DeBlase, A. T., 1980. The bats of Iran: Systematics, distribution, ecology. Fieldiana Zoology, 4, 1–424.

Dharmarajan, G., Beatty, W. S., Rhodes, O. E., 2013. Heterozygote deficiencies caused by a Wahlund effect: dispelling unfounded expectations. The Journal of Wildlife Management, 77, 226–234.

Di Salvo, I., Russo, D., Sara, M., 2009. Habitat preferences of bats in a rural area of Sicily determined by acoustic surveys. Hystrix, the Italian Journal of Mammalogy, 20, 137-146.

Diniz-Filho, J. A. F., Bini, L. M., Rangel, T. F., Loyola, R. D., Hof, C., Nogues-Bravo, D., Araujo, M. B., 2009. Partitioning and mapping uncertainties in ensembles of forecasts of species turnover under climate change. Ecography, 32, 897–906.

Dobson, A. P., Rodriguez, J. P., Roberts, W. M., Wilcove, D. S., 1997. Geographic Distribution of Endangered Species in the United States. Science, 275, 550–553.

Dool, S. E., Puechmaille, S. J., Dietz, C., Juste, J., Ibanez, C., Hulva, P., Roué, S. G., Petit, E. J., Jones, G., Russo, D., 2013. Phylogeography and postglacial recolonization of Europe by *Rhinolophus hipposideros*: evidence from multiple genetic markers. Molecular Ecology, 22, 4055–4070.

Dool, S. E., Puechmaille, S. J., Foley, N. M., Allegrini, B., Bastian, A., Mutumi, G. L., Maluleke, T. G., Odendaal, L. J., Teeling, E. C., Jacobs, D. S., 2016a. Nuclear introns outperform mitochondrial DNA in intra-specific phylogenetic reconstruction: lessons from horseshoe bats (Rhinolophidae: Chiroptera). Molecular Phylogenetics and Evolution, 97, 196–212.

Dool, S. E., Puechmaille, S. J., Kelleher, C., McAney, K., Teeling, E. C., 2016b. The effects of human-mediated habitat fragmentation on a sedentary woodland-associated species (*Rhinolophus hipposideros*) at its range margin. Acta Chiropterologica, 18, 377–393.

Drummond, A. J., Suchard, M. A., Xie, D., Rambaut, A., 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7. Molecular Biology and Evolution, 29, 1969–1973.

Earl, D. A., Vonholdt, B. M., 2012. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conservation Genetics Resources, 4, 359–361.

Ehrlich, P. R., 1961. Systematic Zoology, 10, 167.

Elith, J., Graham, C., Anderson, R., Dudik, M., Ferrier, S., Guisan, A., Hijmans, R., Huettmann, F., Leathwick, J., Lehmann, A., Li, J., Lohmann, L., Loiselle, B., Manion, G., Moritz, C., Nakamura, M., Nakazawa, Y., Overton, J., Peterson, A., Phillips, S., Richardson, K., Scachetti-Pereira, R., Schapire, R., Soberon, J.,Williams, S.,Wisz, M., Zimmermann, N., 2006. Novel methods improve prediction of species' distributions from occurrence data. Ecography, 29, 129–151.

Elith, J., Phillips, S. J., Hastie, T., Dudik, M., Chee, Y. E., Yates, C. J., 2011. A statistical explanation of Maxent for ecologists. Diversity and Distributions, 17, 43–57.

Ellerman, J. R., Morrison-Scott, T. C. S., 1966. Checklist of Palearctic and Indian Mammals, Second Ed., British Museum of Natural History, London.

Engelhaupt, D., Hoelzel, A., Nicholson, C., Frantzis, A., Mesnick, S., Gero, S., Whitehead, H., Rendell, L., Miller, P., Stefanis, R. De, Cañadas, A., Airoldi, S., Mignucci-Giannoni, A. A., 2009. Female philopatry in coastal basins and male dispersion across the North Atlantic in a highly mobile marine species, the sperm whale (*Physeter macrocephalus*). Molecular Ecology, 18, 4193–4205.

Ettling, J. A., Parker, P. G., 2017. Genetic Diversity and Population Structure of Armenian Vipers, *Montivipera raddei*, in Two Landscapes: Implications for Conservation. Herpetological Conservation and Biology, 12, 435–446.

Excoffier, L., Smouse, P. E., Quattro, J. M., 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes — application to human mitochondrial-DNA restriction data. Genetics, 131, 479–491.

Excoffier, L., Lischer, H. E., 2010. Arlequin suite ver 35: A new series of programs to perform population genetics analyses under Linux and Windows. Molecular Ecology Resources, 10, 564–567.

Evanno, G., Regnaut, S., Goudet, J., 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Molecular Ecology, 14, 2611–2620.

Fitzpatrick, B. M., 2012. Estimating ancestry and heterozygosity of hybrids using molecular markers. BMC Evolutionary Biology, 12, 131.

Flanders, J., Jones, G., Benda, P., Dietz, C., Zhang, S., Li, G., Sharifi, M., Rossiter, S. J., 2009. Phylogeography of the greater horseshoe bat, *Rhinolophus ferrumequinum*: contrasting results from mitochondrial and microsatellite data. Molecular Ecology, 18, 306–318.

Flanders, J., Wei, L., Rossiter, S. J., Zhang, S., 2011. Identifying the effects of the Pleistocene on the greater horseshoe bat, *Rhinolophus ferrumequinum*, in East Asia using ecological niche modelling and phylogenetic analyses. Journal of Biogeography, 38, 439–452.

Fleishman, E., Murphy, D. D., Brussard, P. F., 2000. A new method for selection of umbrella species for conservation planning. Ecological Applications, 10, 569–579.

Forester, B. R., DeChaine, E. G., Bunn, A. G., 2013. Integrating ensemble species distribution modelling and statistical phylogeography to inform projections of climate change impacts on species distributions. Diversity and Distributions, 19, 1480–1495.

Fornůsková, A., Petit, E. J., Bartonička, T., Kaňuch, P., Butet, A., Řehák, Z., Bryja, J., 2014. Strong matrilineal structure in common pipistrelle bats (*Pipistrellus pipistrellus*) is associated with variability in echolocation calls. Biological Journal of the Linnean Society, 113, 1115–1125.

Freeland, J. R., 2005. Molecular Ecology, Second Ed., John Wiley and Sons, Ltd, U.K.

Frick, W. F., Pollock, J. F., Hicks, A. C., Langwig, K. E., Reynolds, D. S., Turner, G. R., Butchkoski, C. M., Kunz, T. H., 2010. An emerging disease causes regional population collapse of a common North American bat species. Science, 329, 679–682.

Fritsche, F., Kaltz, O., 2000. Is the Prunella (Lamiaceae) hybrid zone structured by an environmental gradient? Evidence from a reciprocal transplant experiment. American Journal of Botany, 87, 995–1003.

Fu, Y. X., 1997. Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. Genetics, 147, 915–925.

Fu, Y. X., Li, W. H., 1993. Statistical tests of neutrality of mutations. Genetics, 133, 693–709.

Funk, D. J., Omland, K. E., 2003. Species-level paraphyly and polyphyly: frequency, causes, and consequences, with insights from animal mitochondrial DNA. Annual Review of Ecology Evolution and Systematics, 34, 397–423.

Furman, A., Özgül, A., 2002. Distribution of cave-dwelling bats and conservation status of underground habitats in the Istanbul area. Ecological Research, 17, 69–77.

Furman, A., Özgül, A., 2004. The distribution of cave-dwelling bats and conservation status of underground habitats in Northwestern Turkey. Biological Conservation, 120, 243–248.

Furman, A., Çoraman, E., Bilgin, R., Karataş, A., 2009. Molecular ecology and phylogeography of the bent-wing bat complex (*Miniopterus schreibersii*) (Chiroptera: Vespertilionidae) in Asia Minor and adjacent regions. Zoologica Scripta, 38, 129–141.

Furman, A., Öztunç, T., Çoraman, E., 2010a. On the phylogeny of *Miniopterus schreibersii schreibersii* and *Miniopterus schreibersii pallidus* from Asia Minor in reference to other *Miniopterus* taxa (Chiroptera: Vespertilionidae). Acta Chiropterologica, 12, 61–72.

Furman, A., Postawa, T., Öztunç, T., Çoraman, E., 2010b. Cryptic diversity of the bent-wing bat, *Miniopterus schreibersii* (Chiroptera: Vespertilionidae), in Asia Minor. BMC Evolutionary Biology, 10, 121.

Furman, A., Öztunç, T., Postawa, T., Çoraman, E., 2010c. Shallow genetic differentiation in *Miniopterus schreibersii* (Chiroptera: Vespertilionidae) indicates a relatively recent re-colonization of Europe from a single glacial refugium. Acta Chiropterologica, 12, 51–59.

Furman, A., Çoraman, E., Çelik, E. Y., Postawa, T., Bachanek, J., Ruedi, M., 2014. Cytonuclear discordance and the species status of *Myotis myotis* and *Myotis blythii* (Chiroptera). Zoologica Scripta, 549–561.

Galbreath, K. E., Hafner, D. J., Zamudio, K. R., 2009. When cold is better: climate-driven elevation shifts yield complex patterns of diversification and demography in an alpine specialist (American pika, *Ochotona princeps*). Evolution, 63, 2848–2863.

Gardiner, J. D., Altringham, J. D., Papadatou, E., Nudds, R. L., 2014. Excepting *Myotis capaccinii*, the wings' contribution to take-off performance does not correlate with foraging ecology in six species of insectivorous bat. Biology Open, 3, 1057–1062.

Gent, P. R., Danabasoglu, G., Donner, L. J., Holland, M. M., Hunke, E. C., Jayne, S. R., Lawrence, D. M., Neale, R. B., Rasch, P. J., Vertenstein, M., Worley, P. H., Yang, Z.-L., Zhang, M., 2011. The Community Climate System Model Version 4. Journal of Climate, 24, 4973–4991.

Gómez, A., Lunt, D. H. (Eds), 2007. Refugia Within Refugia: Patterns of Phylogeographic Concordance in the Iberian Peninsula, Springer, Dordrecht.

Goudet, J., 1995. FSTAT, Version 1.2. A program for IBM PC compatibles to calculate Weir and Cockerham's estimators of F-statistics. Journal of Heredity, 86, 485–486.

Goudet, J, 2001. FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3). Available from http://www2.unil.ch/popgen/softwares/fstat.htm. Updated from Goudet (1995).

Graham, R. W., Lundelius, E. L. Jr., Graham, M. A., Schroeder, E. K., Toomey, R. S. III., Anderson, E., Barnosky, A. D., Burns, J. A., Churcher, C. S., Grayson, D. K., Guthrie, R. D,. Harington, C. R., Jefferson, G. T., Martin, L. D., McDonald, H. G., Morlan, R. E., Semken, H. A. Jr., Webb, S. D., Werdelin, L., Wilson, M. C., 1996. Spatial Response of Mammals to Late Quaternary Environmental Fluctuations. Science, 272, 1601–1606.

Graur, D., Li, W. H., 2000. Fundamentals of molecular evolution, Second Ed., Sinauer Associates, Sunderland, MA, xiv, 481.

Greenwood, P. J., 1980. Mating systems, philopatry and dispersal in birds and mammals. Animal Behaviour, 28, 1140–1162.

Grinnell, J., 1917. The niche-relationships of the California Thrasher. Auk, 34, 427–433.

Guillén, A., 1999. *Myotis capaccinii* (Bonaparte, 1837). In Mitchell-Jones, A. J., Amori, G., Bogdanowicz, W., Kristufek, B., Rejinders, P. J. H., Spitzenberger, F., Stubbe, M., Thissen, J. B. M., Vohralik, V., Zima, J. (Eds), the Atlas of European Mammals, 106–107, Academic Press, London.

Guillot, G., Renaud, S., Ledevin, R., Michaux, J., Claude, J., 2012. A Unifying Model for the Analysis of Phenotypic, Genetic and Geographic Data. Systematic Biology, 61, 897–911.

Guindon, S., Gascuel, O., 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Systematic Biology, 52, 696–704.

Habbe, K. A., 2007. Stratigraphische Begriffe für das Süddeutsche Alpenvorland (in German), Eiszeitalter und Gegenwart (Quaternary Science Journal), 56, 66–83.

Habel, J. C., Schmitt, T., Müller, P., 2005. The fourth paradigm pattern of post-glacial range expansion of European terrestrial species: the phylogeography of the Marbled White butterfly (Satyrinae, Lepidoptera). Journal of Biogeography, 32, 1489–1497.

Habel, J. C., Dieker, P., Schmitt, T., 2009. Biogeographical connections between the Maghreb and the Mediterraenan peninsulas of southern Europe. Biological Journal of the Linnean Society, 98, 693–703.

Habel, J. C., Rödder, D., Scalercio, S., Meyer, M., Schmitt, T., 2010. Strong genetic cohesiveness between Italy and the Maghreb in four butterfly species. Biological Journal of the Linnean Society, 99, 818–830.

Habel, J. C., Lens, L., Rödder, D., Schmitt, T., 2011. From Africa to Europe and back: refugia and range shifts cause high genetic differentiation in the Marbled White butterfly *Melanargia galathea*. BMC Evolutionary Biology, 11, 215.

Hamann, A., Wang., T. L., 2006. Potential effects of climate change on ecosystem and tree species distribution in British Columbia. Ecology, 87, 2773–2786.

Han, B. Y., Hua, P. Y., Gu, X. M., Miller-Butterworth, C. M., Zhang, S. Y., 2008. Isolation and characterization of microsatellite loci in the western long-fingered bat, *Miniopterus magnater*. Molecular Ecology Notes, 8, 1445–1447.

Harpending, H. C., Sherry, S. T., Rogers, A. R., Stoneking, M., 1993. Genetic structure of ancient human populations. Current Anthropology, 34, 483-496.

Harrison, D. L., 1964. A new subspecies of Natterer's bat, *Myotis nattereri* Kuhl, 1818 (Mammalia: Chiroptera) from Israel. Zeitschrift für Säugetierkunde, 29, 58–60.

Hartl, D. L., Clark, G. C., 1997. Principles of Population Genetics, Sinauer Associates, Sunderland, MA.

Hersteinsson, P., MacDonald, D. W., 1992. Interspecific competition and the geographical distribution of Red and Arctic foxes *Vulpes vulpes* and *Alopex lagopus*. Oikos, 64, 505–515.

Hewitt, G., 1996. Some genetic consequences of ice ages, and their role, in divergence and speciation. Biological Journal of the Linnean Society, 58, 247–276.

Hewitt, G., 1999. Postglacial recolonization of European Biota. Biological Journal of the Linnean Society, 68, 87–112.

Hewitt, G. M., 2001. Speciation, hybrid zones and phylogeography – or seeing genes in space and time. Molecular Ecology, 10, 537–549.

Hewitt, G. M., 2004a. Genetic consequences of climatic oscillations in the Quaternary. Philosophical Transactions of the Royal Society of London Series B-Biological Sciences, 359, 183–195.

Hewitt, G. M., 2004b. The structure of biodiversity — insights from molecular phylogeography. Frontiers in Zoology, 1, 4.

Hijmans, R. J., Cameron, S. E., Parra, J. L., Jones, P. G., Jarvis, A., 2005. Very high resolution interpolated climate surfaces for global land areas. International Journal of Climatology, 25, 1965–1978.

Hirzel, A. H., Hausser, J., Chessel, D., Perrin, N., 2002. Ecological-niche factor analysis: how to compute habitat-suitability maps without absence data? Ecology, 83, 2027–2036.

Hof, C., Araujo, M. B., Jetz, W., Rahbek, C., 2011. Additive threats from pathogens, climate and land-use change for global amphibian diversity. Nature, 480, 516–519.

Holbrook, S. J., Schmitt, R. J., Stephens, J. S., 1997. Changes in an assemblage of temperate reef fishes associated with a climate shift. Ecological Applications, 7, 1299–1310.

Horacek, I., Hanak, V., Gaisler, J., 2002. Bats of Palearctic region: A taxonomic and biogeographic review. Proceedings of the 8th European Bat Research Symposium, Kraków, Poland, 23–27 August 1999, 1, 11–157.

Hughes, L., 2000. Biological consequences of global warming: is the signal already apparent? Trends in Ecology and Evolution, 15, 56–61.

Hulva, P., Fornuskova, A., Chudárková, A., Evin, A., Allegrini, B., Benda, P., Bryja, J., 2010. Mechanisms of radiation in a bat group from the genus *Pipistrellus* inferred by phylogeography, demography and population genetics. Molecular Ecology, 19, 5417–5431.

Huntley, B., 1999. Species distribution and environmental change: considerations from the site to the landscape scale. In Maltby, E., Holdgate, M., Acreman M., Weir A. (Eds), Ecosystem management: questions for science and society, 115–130, Royal Holloway Institute for Environmental Research, Virginia Water, UK.

Husemann, M., Schmitt, T., Zachos, F. E., Ulrich, W., Habel, J. C., 2014. Palaearctic biogeography revisited: evidence for the existence of a North African refugium for Western Palaearctic biota. Journal of Biogeography, 41, 81–94.

Hutchinson, G. E., 1957. Concluding remarks. Cold Spring Harbor Symposium on Quantitative Biology, Cold Spring Harbor, New York, 3-12 June 1957, 22, 415–457.

Hutson, A. M., Aulagnier, S., Benda, P., Karataş, A., Palmeirim, J., Paunović, M., 2008a. *Miniopterus schreibersii*. In: IUCN 2012. IUCN Red List of Threatened Species. Version 2012.1. http://www.iucnredlist.org (accessed October 2012).

Hutson, A. M., Spitzenberger, F., Aulagnier, S., Juste, J., Karataş, A., Palmeirim, J., Paunović, M., 2008b. *Myotis capaccinii*. In: IUCN 2012. IUCN Red List of Threatened Species. Version 2012.1. http://www.iucnredlist.org (accessed October 2012).

Ibanez, C., 1997. Winter reproduction in the greater mouseeared bat (*Myotis myotis*) in South Iberia. Journal of Zoology, 243, 836–840.

Ibanez, C., Garcia-Mudarra, J. L., Ruedi, M., Stadelmann, B., Juste, J., 2006. The Iberian contribution to cryptic diversity in European bats. Acta Chiropterologica, 8, 277–297.

Ingman, M., Kaessmann, H., Paabo, S., Gyllensten, U., 2000. Mitochondrial genome variation and the origin of modern humans. Nature, 408, 708–713.

IPCC, 2014: Climate Change 2014: Impacts, Adaptation, and Vulnerability. Part A: Global and Sectoral Aspects. In Field, C.B., Barros, V.R., Dokken, D.J., Mach, K.J., Mastrandrea, M.D., Bilir, T.E., Chatterjee, M., Ebi, K.L., Estrada, Y.O., Genova, R.C., Girma, B., Kissel, E.S., Levy, A.N., MacCracken, S., Mastrandrea, P.R., White, L.L. (Eds), Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, 1132.

IPCC, 2014: Climate Change 2014: Impacts, Adaptation, and Vulnerability. Part B: Regional Aspects. In Field, C.B., Barros, V.R., Dokken, D.J., Mach, K.J., Mastrandrea, M.D., Bilir, T.E., Chatterjee, M., Ebi, K.L., Estrada, Y.O., Genova, R.C., Girma, B., Kissel, E.S., Levy, A.N., MacCracken, S., Mastrandrea, P.R., White, L.L. (Eds), Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, 688.

Irwin, D. E., 2012. Local adaptation along smooth ecological gradients causes phylogeographic breaks and phenotypic clustering. The American Naturalist, 180, 35–49.

Jakob, S. S., Martinez-Meyer, E., Blattner, F. R., 2009. Phylogeographic analyses and paleodistribution modeling indicate pleistocene in situ survival of *Hordeum* species (Poaceae) in southern Patagonia without genetic or spatial restriction. Molecular Biology and Evolution, 26, 907–923.

Jakobsson, M., Rosenberg, N. A., 2007. CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. Bioinformatics, 23, 1801–1806.

Johnson, M. S., Black, R., 1984. The Wahlund effect and the geographical scale of variation in the intertidal limpet *Siphonaria* sp. Marine Biology, 79, 295–302.

Kalinowski, S. T., 2005. hp-rare 1.0: a computer program for performing rarefaction on measures of allelic richness. Molecular Ecology Notes, 5, 187–189.

Kasapidis, P., Suchentrunk, F., Magoulas, A., Kotoulas, G., 2005. The shaping of mitochondrial DNA phylogeographic patterns of the brown hare (*Lepus europaeus*) under the combined influence of late pleistocene climatic fluctuations and anthropogenic translocations. Molecular Phylogenetics and Evolution, 34, 55–66.

Keeney, D. B., Heupel, M. R., Hueter, R. E., Heist, E. J., 2005. Microsatellite and mitochondrial DNA analyses of the genetic structure of blacktip shark (*Carcharhinus limbatus*) nurseries in the northwestern Atlantic, Gulf of Mexico, and Caribbean Sea. Molecular Ecology, 14, 1911–1923.

Keim, P., Paige, K. N, Whitham, T. G, Lark, K. G., 1989. Genetic Analysis of an Interspecific Hybrid Swarm of Populus: Occurrence of Unidirectional Introgression. Genetics, 123, 557–565.

Kerth, G., Mayer, F., Petit, E., 2002. Extreme sex-biased dispersal in the communally breeding, nonmigratory Bechstein's bat (*Myotis bechsteinii*). Molecular Ecology, 11, 1491–1498.

Kerth, G., Petrov, B., Conti, A., Anastasov, D., Weishaar, M., Gazaryan, S., Jaquiéry, J., König, B., Perrin, N., Bruyndonckx, N., 2008. Communally breeding Bechstein's bats have a stable social system that is independent from the postglacial history and location of the populations. Molecular Ecology, 17, 2368–2381.

Kitching, I. J., Forey, P. L., Humphries, C. J., Williams, D., 2000. Cladistics: The Theory and Practice of Parsimony Analysis, Oxford University Press, New York.

Knowles, L. L., Maddison, W. P., 2002. Statistical phylogeography. Molecular Ecology, 11, 2623–2635.

Knowles, L. L., Carstens, B. C., Keat, M. L., 2007. Coupling genetic and ecological-niche models to examine how past population distributions contribute to divergence. Current Biology, 17, 940–946.

Koopman, K. F., 1994. Chiroptera: Systematics. In Handbook of Zoology, 8: Mammalia, Walter de Gruyter, New York.

Kumar, S., Stecher, G., Tamura, K., 2016. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0. Molecular Biology and Evolution, 33, 1870–1874.

Kumerloeve, H., 1975. Die saugetiere (Mammalia) der Turkei. Veröffentlichungen der Zoologischen Staatssammlung, 18, 69–158.

Küpper, C., Edwards, S. V., Kosztolányi, A., Alrashidi, M., Burke, T., Herrmann, P., Argüelles-Tico, A., Amat, J. A., Amezian, M., Rocha, A., Hötker, H., Ivanov, A., Chernicko, J., Székely, T., 2012. High gene flow on a continental scale in the polyandrous Kentish Plover *Charadrius alexandrines*. Molecular Ecology, 21, 5864–5879.

Landres, P. B., Verner, J., Thomas, J. W., 1988. Ecological uses of vertebrate indicator species: A critique. Conservation Biology, 2, 316–328.

Lawson Handley, L. J., Perrin, N., 2007. Advances in our understanding of mammalian sex-biased dispersal. Molecular Ecology, 16, 1559–1578.

Lawton, J. H., 1993. Range, population abundance and conservation. Trends in Ecology and Evolution, 8, 409–413.

Lawton, J. L., 2000. Concluding remarks: a review of some open questions. In Hutchings, M.J., John, E., Stewart, A. J. A. (Eds), Ecological consequences of heterogeneity, Cambridge University Press, Cambridge, 401–424.

Leadley, P., Pereira, H. M., Alkemade, R., Fernandez-Manjarres, J. F., Proenca, V., Scharlemann, J. P. W., Walpole, M. J., 2010. Biodiversity scenarios: projections of 21st century change in biodiversity and associated ecosystem services, Technical Series no. 50, Secretariat of the Convention on Biological Diversity, Montreal.

Leigh, J. W., Bryant, D., 2015. PopART: Full-feature software for haplotype network construction. Methods in Ecology and Evolution, 6, 1110–1116.

Levin, E., Barnea, A., Yovel, Y., Yom-Tov, Y., 2006. Have introduced fish initiated piscivory among the long-fingered bat? Mammalian Biology, 71, 139–143.

Librado, P., Rozas, J., 2009. DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. Bioinformatics, 25, 1451–1452.

Litt, T., Behre, K. E., Meyer, K. D., Stephan, H. J, Wansa, S., 2007. Stratigraphische Begriffe für das Quartär des norddeutschen Vereisungsgebietes. Eiszeitalter und Gegenwart (Quaternary Science Journal), 56, 7–65.

Lloyd, B. D., 2003. Intraspecific Phylogeny of the New Zealand short-tailed bat *Mystacina tuberculata* inferred from multiple mitochondrial gene sequences. Systematic Biology, 52, 460–476.

Maggs, C. A., Castilho, R., Foltz, D., Henzler, C., Jolly, M. T., 2008. Evaluating signatures of glacial refugia for North Atlantic benthic marine taxa. Ecology, 89, 108–122.

Martínez-Freiría, F., Sillero, N., Lizana, M., Brito. J. C., 2008. GIS-based niche models identify environmental correlates sustaining a contact zone between three species of European vipers. Diversity and Distributions, 14, 452–461.

Mayr, E., 1940. The American Naturalist, 74, 249.

McCarty, J. P., 2001. Ecological consequences of recent climate change. Conservation Biology, 15, 320–331.

Medail, F., Diadema, K., 2009. Glacial refugia influence plant diversity patterns in the Mediterranean Basin. Journal of Biogeography, 36, 1333–1345.

Melo-Ferreira, J., Alves, P. C., Freitas, H., Ferrand, N., Boursot, P., 2009. The genomic legacy from the extinct *Lepus timidus* to the three hare species of Iberia: contrast between mtDNA, sex chromosomes and autosomes. Molecular Ecology, 18, 2643–2658.

Medard, P., Guibert, E., 1990. Disparition d'un milieu et raréfaction d'une espèce en France: le murin de Capaccini, *Myotis capaccinii* (Bonaparte, 1837). Mammalia, 54, 297–300.

Menge, B. A., Berlow, E. L., Blanchette, C. A., Navarrette, S. A., Yamada, S. B., 1994. The keystone species concept: Variation in interaction strength in a rocky intertidal habitat. Ecological Monographs, 64, 249–286.

Merow, C., Smith, M. J., Silander, J. A., 2013. A practical guide to MaxEnt for modeling species' distributions: what it does, and why inputs and settings matter. Ecography, 36, 1058–1069.

Miller-Butterworth, C. M., Jacobs, D. S., Harley, E. H., 2002. Isolation and characterization of highly polymorphic microsatellite loci in Schreibers' long fingered bat, *Miniopterus schreibersii* (Chiroptera:Vespertilionidae). Molecular Ecology Notes, 2, 139–141.

Miller-Butterworth, C. M., Jacobs, D. S., Harley, E. H., 2003. Strong population substructure is correlated with morphology and ecology in a migratory bat. Nature, 424, 187–191.

Miller-Butterworth, C. M., Eick, G., Jacobs, D. S., Schoeman, M. C., Harley, E. H., 2005. Genetic and phenotypic differences between South African long-fingered bats, with a global Miniopterine phylogeny. Journal of Mammalogy, 86, 1121–1135.

Moore, W. S., 1995. Inferring phylogenies from mtDNA variation: mitochondrial-gene tree versus nuclear-gene trees. Evolution, 49, 718–726.

Moreira, F., Russo, D., 2007. Modelling the impact of agriculture abandonment and wildfires on vertebrate diversity in Mediterranean Europe. Landscape Ecology, 22, 1461–1476.

Myers, N., Mittermeier, R. A., Mittermeier, C. G., da Fonseca, G. A. B., Kent, J., 2000. Biodiversity hotspots for conservation priorities. Nature, 403, 853–858.

Nagy, M., Heckel, G., Voigt, C. C., Mayer, F., 2007. Female-biased dispersal and patrilocal kin groups in a mammal with resource-defence polygyny. Proceedings of the Royal Society, B, Biological Sciences, 274, 3019–3025.

Nagy, M., Günther, L., Knörnschild, M., Mayer, F., 2013. Female-biased dispersal in a bat with a female-defence mating strategy. Molecular Ecology, 22, 1733–1745.

Nei, M., Tajima, F., 1981. DNA polymorphism detectable by restriction endonucleases. Genetics, 97, 145–163.

Nietlisbach, P., Arora, N., Nater, A., Goossens, B., van Schaik, C. P., Krützen, M., 2012. Heavily male-biased long-distance dispersal of orang-utans (genus: *Pongo*), as revealed by Y-chromosomal and mitochondrial genetic markers. Molecular Ecology, 21, 3173–3186.

Nix, H., 1986. A biogeographic analysis of Australian elapid snakes. Atlas of elapid snakes of Australia, 7, 4-15.

Nogués-Bravo, D., 2009. Predicting the past distribution of species climatic niches. Global Ecology and Biogeography, 18, 521-553.

Norberg, U. M., Rayner, J. M., 1987. Ecological morphology and flight in bats (Mammalia: Chiroptera): wing adaptations, flight performance, foraging strategy and echolocation. Philosophical Transactions of the Royal Society of London, 316, 335–427.

Nyakaana, S., Arctander, P., 1999. Population genetic structure of the African elephant in Uganda based on variation at mitochondrial and nuclear loci: Evidence for male-biased gene flow. Molecular Ecology, 8, 1105–1115.

Nyakaana, S., Arctander, P., Siegismund, H. R., 2002. Population structure of the African savannah elephant inferred from mitochondrial control region sequences and nuclear microsatellite loci. Heredity, 89, 90–98.

O'Brien, S. J., Mayr, E., 1991. Bureaucratic mischief: recognizing endangered species and subspecies. Science, 251, 1187–1188.

Overpeck, J., Whitlock, C., Huntley, B., 2002. In Alverson, K., Bradley, R., Pedersen, T. (Eds), Paleoclimate, Global Change and the Future, 81–103, Springer, Berlin.

Paine, R. T., 1969. A note on trophic complexity and community stability. The American Naturalist, 103, 91–93.

Palmeirim, J. M., Rodrigues, L., 1995. Dispersal in colonial animals —the case of *Miniopterus schreibersii*. In Racey, P. A., Swift, S. M. (Eds), Ecology, evolution and behaviour of bats, 219–231, Oxford University Press, Oxford.

Parmesan, C., Ryrholm, N., Stefanescu, C., Hill, J. K., Thomas, C. D., Descimon, H., Huntley, B., Kaila, L., Kullberg, J., Tammaru, T., Tennent, W. J., Thomas, J. A., Warren, M., 1999. Poleward shifts in geographical ranges of butterfly species associated with regional warming. Nature, 399, 579–583.

Parmesan, C., Yohe, G., 2003. A globally coherent fingerprint of climate change impacts across natural systems. Nature, 421, 37–42.

Parmesan, C., Gaines, S., Gonzalez, L., Kaufman, D. M., Kingsolver, J., Townsend Peterson, A., Sagarin, R., 2005. Empirical perspectives on species borders: from traditional biogeography to global change. Oikos, 108, 58–75.

Parmesan, C., 2007. Influences of species, latitudes and methodologies on estimates of phenological response to global warming. Global Change Biology, 13, 1860–1872.

Parmesan, C., Duarte, C. M., Poloczanska, E., Richardson, A. J., Singer, M. C., 2011. Overstretching attribution. Nature Climate Change, 1, 2–4.

Pauls, S. U., Nowak, C., Bálint, M., Pfenninger, M., 2013. The impact of global climate change on genetic diversity within populations and species. Molecular Ecology, 22, 925–946.

Peakall, R., Smouse, P. E., 2012. GenAlEx 65: genetic analysis in Excel Population genetic software for teaching and research-an update. Bioinformatics, 28, 2537–2539.

Pearson, R. G., Dawson, T. P., 2003. Predicting the impacts of climate change on the distribution of species: are bioclimate envelope models useful? Global Ecology and Biogeography, 12, 361–371.

Pereira, H. M., Leadley, P. W., Proenca, V., Alkemade, R., Scharlemann, J. P. W., Fernandez-Manjarres, J. F., Araújo, M. J., Balvanera, P., Biggs, R., Cheung, W. W. L., Chini, L., Cooper, H. D., Gilman, E. L., Guénette, S., Hurtt, G. C., Huntington, H. P., Mace, G. M., Oberdorff, T., Revenga, C., Rodrigues, P., Scholes, R. J., Sumaila, U. R., Walpole, M., 2010. Scenarios for global biodiversity in the 21st century. Science, 330, 1496–1501.

Petit, E., Excoffier, L., Mayer, F., 1999a. No evidence of bottleneck in the postglacial recolonization of Europe by the noctule bat (*Nyctalus noctula*). Evolution, 53, 1247–1258.

Petit, E., Mayer, F., 1999b. Male dispersal in the noctule bat (*Nyctalus noctula*): where are the limits? Proceedings of the Royal Society of London, B, 266, 1717–1722.

Petit, E., Mayer, F., 2000. A population genetic analysis of migration: the case of the noctule bat (*Nyctalus noctula*). Molecular Ecology, 9, 683–690.

Petit, E., Balloux, F., Goudet, J., 2001. Sex-biased dispersal in a migratory bat: a characterization using sex-specific demographic parameters. Evolution, 55, 635–640.

Petit, R. J., El Mousadik, A., Pons, O., 1998. Identifying populations for conservation on the basis of genetic markers. Conservation Biology, 12, 844–855.

Petit, R. J., Aguinagalde, I., de Beaulieu, J. L., Bittkau, C., Brewer, S., Cheddadi, R., Ennos, R., Fineschi, S., Grivet, D., Lascoux, M., Mohanty, A., Müller-Starck, G., Demesure-Musch, B., Palme, A., Martin, J. P., Rendell, S., Vendramin, G. G., 2003. Glacial Refugia: Hotspots But Not Melting Pots of Genetic Diversity. Science, 300, 1561–1565.

Petit, R. J., Excoffier, L., 2009. Gene flow and species delimitation. Trends in Ecology and Evolution, 24, 386–393.

Piaggio, A. J., Navo, K. W., Stihler, C. W., 2009. Intraspecific comparison of population structure, genetic diversity, and dispersal among three subspecies of townsend's big-eared bats, *Corynorhinus townsendii townsendii*, *C. t. pallescens*, and the endangered *C. t. virginianus*. Conservation Genetics, 10, 143–159.

Piry, S., Luikart, G., Cornuet, J. M., 1999. BOTTLENECK: A Computer Program for Detecting Recent Reductions in the Effective Population Size Using Allele Frequency Data. The Journal of Heredity, 90, 4.

Posada, D., 2008. jModelTest: Phylogenetic Model Averaging. Molecular Biology and Evolution, 25, 1253–6.

Pounds, J. A., Crump, M. L., 1994. Amphibian declines and climate disturbance: the case of the golden toad and the harlequin frog. Conservation Biology, 8, 72–85.

Pounds, J. A., Fogden, M. L. P., Campbell, J. H., 1999. Biological response to climate change on a tropical mountain. Nature, 398, 611–615.

Prendergast, J. R., Quinn, R. M., Lawton, J. H., Eversham, B. C., Gibbons, D. W., 1993. Rare species, the coincidence of diversity hotspots and conservation strategies. Nature, 365, 335–337.

Prior, K. A., Gibbs, H. L., Weatherhead, P. J., 2003. Population genetic structure in the black rat snake: Implications for management. Conservation Biology, 11, 1147–1158.

Pritchard, J. K., Stephens, M., Donnelly, P., 2000. Inference of population structure using multilocus genotype data. Genetics, 155, 945–959.

Pruett, C. L., Winker, K., 2008. The effects of sample size on population genetic diversity estimates in song sparrows *Melospiza melodia*. Journal of Avian Biology, 39, 252–256.

Pryde, M. A., O'Donnell, C. F. J., Barker, R. J., 2005. Factors influencing survival and long-term population viability of New Zealand long-tailed bats *Chalinolobus tuberculatus*: implications for conservation. Biological Conservation, 126, 175–185.

Puechmaille, S. J., Ar Gouilh, M., Piyapan, P., Yokubol. M., Khin, M. M., Bates, P. J., Satasook, C., New, T., Bu, S. S. H., Mackie, I. J., Petit, E. J., Teeling, E. C., 2011. The evolution of sensory divergence in the context of limited gene flow in the bumblebee bat. Nature Communications, 2, 573.

Puechmaille, S. J., Allegrini, B., Boston, E. S., Dubourg-Savage, M. J., Evin, A., Knochel, A., Le Bris, Y., Lecoq, V., Lemaire, M., Rist, D., 2012. Genetic analyses reveal further cryptic lineages within the *Myotis nattereri* species complex. Zeitschrift für Säugetierkunde (Mammalian Biology), 77, 224–228.

Puechmaille, S. J., Allegrini, B., Benda, P., Gürün, K., Šrámek, J., Ibanez, C., Juste, J., Bilgin, R., 2014. A new species of the *Miniopterus schreibersii* species complex (Chiroptera: Miniopteridae) from the Maghreb Region, North Africa. Zootaxa, 3794, 108–124.

Putman, A. I., Carbone, I., 2014. Challenges in analysis and interpretation of microsatellite data for population genetic studies. Ecology and Evolution, 4, 4399–4428.

Quintero, I., Wiens, J. J., 2013. Rates of projected climate change dramatically exceed past rates of climate niche evolution among vertebrate species. Ecology Letters, 16, 1095–1103.

Racey, P. A., Speakman, J. R., 1987. The energy costs of pregnancy and lactation in heterothermic bats. Zoological Symposium, 107–125.

Racey, P. A., Speakman, J. R., Swift, S. M., 1987. Reproductive adaptations of heterothermic bats at the northern borders of their distribution. Suid-Afrikaanse Tydskrif Vir Wetenskap (South African Journal of Science), 83, 635–638.

Raes, N., Roos, M. C., Slik, J. W. F., van Loon, E. E., ter Steege, H., 2009. Botanical richness and endemicity patterns of Borneo derived from species distribution models. Ecography, 32, 180–192.

Rambaut, A., Suchard, M. A., Xie, D., Drummond, A. J., 2014. Tracer v1.6, Available from http://tree.bio.ed.ac.uk/software/trace

Ramos Pereira, M. J., Rebelo, H., Rainho, A., Palmeirim, J. M., 2002. Prey selection by *Myotis myotis*. Acta Chiropterologica, 2, 183–193.

Ramos Pereira, M. J., Salgueiro, P., Rodrigues, L., Coelho, M. M., Palmeirim, J. M., 2009. Population structure of a cave dwelling bat, *Miniopterus schreibersii*: Does it reflect history and social organization? Journal of Heredity, 100, 533–544.

Raven, P., 1977. Systematic Botany, 1, 284.

Razgour, O., Juste, J., Ibanez, C., Kiefer, A., Rebelo, H., Puechmaille, S. J., Arlettaz, R., Burke, T., Dawson, D. A., Beaumont, M., 2013. The shaping of genetic variation in edge-of-range populations under past and future climate change. Ecology Letters, 16, 1258–1266.

Rebelo, H., Tarroso, P., Jones, G., 2009. Predicted impact of climate change on European bats in relation to their biogeographic patterns. Global Change Biology, 16, 561–576.

Rebelo, H., Jones, G., 2010. Ground validation of presence-only modelling with rare species: a case study on barbastelles *Barbastella barbastellus* (Chiroptera: Vespertilionidae). Journal of Applied Ecology, 47, 410–420.

Rebelo, H., Froufe, E., Brito, J. C., Russo, D., Cistrone, L., Ferrand, N., Jones, G., 2012. Postglacial colonization of Europe by the barbastelle bat: agreement between molecular data and past predictive modelling. Molecular Ecology, 21, 2761–2774.

Reid, W. D., 1998. Biodiversity Hotspots. Trends in Ecology and Evolution, 13, 275–280.

Remington, C. L., 1968. Suture-zones of hybrid interaction between recently joined biotas. Evolutionary Biology, 2, 321–428.

Riahi, K., Rao, S., Krey, V., Cho, C., Chirkov, V., Fischer, G., Kindermann, G., Nakicenovic, N., Rafaj, P., 2011. RCP8.5—A scenario of comparatively high greenhouse gas emissions. Climatic Change, 109, 33–57.

Rice, W. R., 1989. Analyzing tables of statistical tests. Evolution, 43, 223–225.

Richards, C. L., Carstens, B. C., Knowles, L. L., 2007. Distribution modelling and statistical phylogeography: an integrative framework for generating and testing alternative biogeographical hypotheses. Journal of Biogeography, 34, 1833–1845.

Rissler, L. J., Hijmans, R. J., Graham, C. H., Moritz, C., Wake, D. B., 2006. Phylogeographic lineages and species comparisons in conservation analyses: a case study of California herpetofauna. American Naturalist, 167, 655–666.

Rodrigues, L., Zahn, A., Rainho, A., Palmeirim, J. M., 2003. Contrasting the roosting behaviour and phenology of an insectivorous bat (*Myotis myotis*) in its southern and northern distribution ranges. Mammalia, 67, 321–335.

Rodrigues, L., Ramos Pereira, M. J., Rainho, A., Palmeirim, J. M., 2010. Behavioural determinants of gene flow in the bat *Miniopterus schreibersii*. Behavioral Ecology and Sociobiology, 64, 835–843.

Rooney, A. P., Honeycutt, R. L., Davis, S. K., 1999. Evaluating a putative bottleneck in a population of bowhead whales from patterns of microsatellite diversity and genetic disequilibria. Journal of Molecular Evolution, 49, 682–690.

Root, T. L., Price, J. T., Hall, K. R., Schneider, S. H., Rosenzweig, C., Pounds, J. A., 2003. Fingerprints of global warming on wild animals and plants. Nature, 421, 57–60.

Root, T. L., MacMynowski, D. P., Mastrandrea, M. D., Schneider, S. H., 2005. Human-modified temperatures induce species changes: joint attribution. Proceedings of the National Academy of Sciences of the United States of America, 102, 7465–7469.

Ropiquet, A., Hassanin, A., 2006. Hybrid origin of the Pliocene ancestor of wild goats. Molecular Phylogenetics and Evolution, 41, 395–404.

Rosenberg, N. A., 2004. Distruct: a program for the graphical display of population structure. Molecular Ecology Notes, 4, 137–138.

Rossiter, S. J., Benda, P., Dietz, C., Zhang, S., Jones, G., 2007. Range wide phylogeography in the greater horseshoe bat inferred from microsatellites: Implications for population history, taxonomy and conservation. Molecular Ecology, 16, 4699–4714.

Rousset, F., 1997. Genetic Differentiation and Estimation of Gene Flow from F-Statistics Under Isolation by Distance. Genetics, 145, 1219–1228.

Rousset, F., 2008. Genepop'007: a complete reimplementation of the Genepop software for Windows and Linux. Molecular Ecology Resources, 8, 103–106.

Ruedi, M., Mayer, F., 2001. Molecular systematics of bats of the genus *Myotis* (Vespertilionidae) suggests deterministic ecomorphological convergences. Molecular Phylogenetics and Evolution, 21, 436–448.

Ruedi, M., Castella, V., 2003. Genetic consequences of the ice ages on nurseries of the bat *Myotis myotis*: a mitochondrial and nuclear survey. Molecular Ecology, 12, 1527–1540.

Ruedi, M., Walter, S., Fischer, M. C., Scaravelli, D., Excoffier, L., Heckel, G., 2008. Italy as a major Ice Age refuge area for the bat *Myotis myotis* (Chiroptera: Vespertilionidae) in Europe. Molecular Ecology, 17, 1801–1814.

Russo, D., Cistrone, L., Jones, G., Mazzolenis, S., 2004. Roost selection by barbastelle bats (*Barbastella barbastellus*, Chiroptera: Vespertilionidae) in beech woodlands of central Italy: consequences for conservation. Biological Conservation, 117, 73–81.

Sachanowicz, K., Wower, A., Bashta, A-T., 2006. Further range extension of *Pipistrellus kuhlii* (Kuhl, 1817) in central and eastern Europe. Acta Chiropterologica, 8, 543–548.

Sagarin, R., Barry, J. P., Gilman, S. E., Baxter, C. H., 1999. Climate-related change in an intertidal community over short and long time scales. Ecological Monographs, 69, 465–490.

Salicini, I., Ibanez, C., Juste, J., 2013. Deep differentiation between and within Mediterranean glacial refugia in a flying mammal, the *Myotis nattereri* bat complex. Journal of Biogeography, 40, 1182–1193.

Scheel, D., Vicent, T. L. S., Cameron, G. N., 1996. Global warming and the species richness of bats in Texas. Conservation Biology, 10, 452–464.

Schmitt, T., 2007. Molecular biogeography of Europe: Pleistocene cycles and postglacial trends. Frontiers in Zoology, 4, 11.

Scoble, J., Lowe, A. J., 2010. A case for incorporating phylogeography and landscape genetics into species distribution modelling approaches to improve climate adaptation and conservation planning. Diversity and Distributions, 16, 343–353.

Seeb, L. W., Crane, P. A., 1999. High Genetic Heterogeneity in Chum Salmon in Western Alaska, the Contact Zone between Northern and Southern Lineages. Transactions of the American Fisheries Society, 128, 58–87.

Shackleton, J. C., Vanandel, T. H., Runnels, C. N., 1984. Coastal paleogeography of the central and western Mediterranean during the last 125 000 years and its archaeological implications. Journal of Field Archaeology, 11, 307–314.

Shaffer, M. L., 1981. Minimum Population Sizes for Species Conservation. BioScience, 31, 131–134.

Shepard, D. B., Burbrink, F. T., 2008. Lineage diversification and historical demography of a sky island salamander, *Plethodon ouachitae*, from the Interior Highlands. Molecular Ecology, 17, 5315–5335.

Simberloff, D., 1998. Flagships, umbrellas and keystones: Is single-species management passé in the landscape era? Biological Conservation, 83, 247–257.

Simmons, N. B., 2005. Order Chiroptera. In Wilson, D. E., Reeder, D. M. (Eds), Mammal species of the World: A taxonomic and geographic reference, 312–529, John Hopkins University Press, Baltimore.

Smith, M. F., Patton, J. L., 1993. The diversification of south-American murid rodents: Evidence from mitochondrial DNA sequence data for the akodontine tribe. Biological Journal of the Linnean Society, 50, 149–177.

Smith, S. J., Leslie Jr, D. M., Hamilton, M. J., Lack, J. B., Van Den Bussche, R. A., 2008. Subspecific affinities and conservation genetics of western big-eared bats (*Corynorhinus townsendii pallescens*) at the edge of their distributional range. Journal of Mammalogy, 89, 799–814.

Smith, T. B., Kark, S., Schneider, C. J., Wayne, R. K., Moritz, C., 2001. Biodiversity hotspots and beyond: The need for preserving environmental transitions. Trends in Ecology and Evolution, 16, 431.

Spector, S., 2002. Biogeographic Crossroads as Priority Areas for Biodiversity Conservation. Conservation Biology, 16, 1480–1487.

Spitzenberger, F., von Helversen, O., 2001. *Myotis capaccinii* (Bonaparte, 1837) - Langfußfledermaus. In: Krapp, F., (Ed), Handbuch der Säugetiere Europas, Band 4: Fledertiere, Teil I: Chiroptera I, 281-302, Aula-Verlag, Wiebelsheim.

Stanton, J. C., Pearson, R. G., Horning, N., Ersts, P., Akçakaya H. R., 2012. Combining static and dynamic variables in species distribution models under climate change. Methods in Ecology and Evolution, 3, 349–357.

Stockwell, D. R. B., Noble, I. R., 1992. Induction of sets of rules from animal distribution data: a robust and informative method of analysis. Mathematics and Computers in Simulation, 33, 385–390.

Stöck, M., Sicilia, A., Belfiore, N. M., Buckley, D., Lo Brutto, S., Lo Valvo, M., Arculeo, M., 2008. Post-Messinian evolutionary relationships across the Sicilian channel: mitochondrial and nuclear markers link a new green toad from Sicily to African relatives. BMC Evolutionary Biology, 8, 56.

Swenson, N. G., Howard, D. J., 2005. Clustering of contact zones, hybrid zones, and phylogeographic breaks in North America. The American Naturalist, 166, 581–591.

Tajima, F., 1983. Evolutionary relationship of DNA sequences in finite populations. Genetics, 105, 437–460.

Tajima, F., 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics, 123, 585–595.

Taubmann, J., Theissinger, K., Feldheim, K. A., Haase, P., Graf, W., Johannesen, J., Laube, I., Pauls, S. U., 2011. Modelling range shifts and assessing genetic diversity distribution of the montane aquatic mayfly *Ameletus inopinatus* in Europe under climate change scenarios. Conservation Genetics, 12, 503–515.

Templeton, A., 1989. In Otte, D., Endler, J. A. (Eds), Speciation and Its Consequences, Sinauer, Sunderland, MA, 3-27.

Theissinger, K., Bálint, M., Haase, P., Johannesen, J. E. S., Laube, I., Pauls, S. U., 2011. Molecular data and species distribution models reveal the Pleistocene history of the mayfly *Ameletus inopinatus* (Ephemeroptera: Siphlonuridae). Freshwater Biology, 56, 2554–2566.

Thomas, C. D., Lennon, J. J., 1999. Birds extend their ranges northwards. Nature, 399, 213.

Thomas, C. D., Cameron, A., Green, R. E., Bakkenes, M., Beaumont, L. J., Collingham, Y. C., Erasmus, B. F. N., de Siqueira, M. F., Grainger, A., Hannah, L., Hughes, L., Huntley, B., van Jaarsveld, A. S., Midgley, G. F., Miles, L., Ortega-Huerta, M. A., Peterson, A. T., Phillips, O. L., Williams, S. E., 2004. Extinction risk from climate change. Nature, 427, 145–148.

Tian, L., Liang, B., Maeda, K., Metzner, W., Zhang, S., 2004. Molecular studies on the classification of *Miniopterus schreibersii* (Chiroptera: Vespertilionidae) inferred from mitochondrial cytochrome b sequences. Folia Zoologica, 53, 303–311.

Tiedemann, R., Paulus, K. B., Scheer, M., von Kistowski, K.G., Skírnisson, K., Bloch, D., Dam, M., 2004. Mitochondrial DNA and microsatellite variation in the eider duck (*Somateria mollissima*) indicate stepwise postglacial colonization of Europe and limited current long-distance dispersal. Molecular Ecology, 13, 1481–1494.

Toews, D. P. L., Brelsford, A., 2012. The biogeography of mitochondrial and nuclear discordance in animals. Molecular Ecology, 21, 3907–3930.

Ulrich, W., Sachanowicz, K., Michalak, M., 2007. Environmental correlates of species richness of European bats (Mammalia: Chiroptera). Acta Chiropterologica, 9, 347–360.

Van Oosterhout, C., Hutchinson, W. F., Wills, D. P., Shipley, P., 2004. MICROCHECKER: software for identifying and correcting genotyping errors in microsatellite data. Molecular Ecology Notes, 4, 535–538.

Veith, M., Schmidtler, J. F., Kosuch, J., Baran, I., Seitz, A., 2003. Palaeoclimatic changes explain Anatolian mountain frog evolution: A test for alternating vicariance and dispersal events. Molecular Ecology, 12, 185–199.

Viglino, A., 2012. Study of variability and genetic structure of European populations of *Myotis emarginatus* and *Myotis capaccinii* (Chiroptera, Vespertilionidae), Ph.D. Thesis, Bologna University.

Voigt, C. C., Lehmann, D., Greif, S., 2015. Stable isotope ratios of hydrogen separate mammals of aquatic and terrestrial food webs. Methods in Ecology and Evolution, 6, 1332–1340.

Vonhof, M. J., Strobeck, C., Fenton, M. B., 2008. Genetic variation and population structure in big brown bats (*Eptesicus fuscus*): is female dispersal important? Journal of Mammalogy, 89, 1411–1420.

Wahlund, S., 1928. Zusammensetzung von Population und Korrelationserscheinung vom Standpunkt der Vererbungslehre aus betrachtet. Hereditas, 11, 65–106.

Walker, M. J. C., 1995. Climatic changes in Europe during the last glacial/interglacial transition. Quaternary International, 28, 63–76.

Waltari, E., Hijmans, R. J., Peterson, A. T., Nyári, A. S., Perkins, S. L., Guralnick, R. P., 2007. Locating Pleistocene refugia: comparing phylogeographic and ecological niche model predictions. PLoS ONE, 2, 563.

Walter, R., 1995. Geologie von Mitteleuropa. E. Schweizerbartsche Verlagsbuchhandlung, Stuttgart.

Walter, R., Epperson, B. K., 2005. Geographic pattern of genetic diversity in *Pinus resinosa*: Contact zone between descendants of glacial refugia. American Journal of Botany, 92, 92–100.

Walther, G. R., Post, E., Convey, P., Menzel, A., Parmesan, C., Beebee, T. J. C., Fromentin, J. M., Hoegh-Guldberg, O., Bairlein, F., 2002. Ecological responses to recent climate change. Nature, 416, 389–395.

Waples, R. S., 2015. Testing for Hardy–Weinberg proportions: Have we lost the plot? Journal of Heredity, 106, 1–19.

Warren, D. L., Glor, R. E., Turelli, M., 2008. Environmental niche equivalency versus conservatism: quantitative approaches to niche evolution. Evolution, 62, 2868–2883.

Warren, D. L., Glor, R.E., Turelli, M., 2010. ENMTools: a toolbox for comparative studies of environmental niche models. Ecography, 33, 607–611.

Watanabe, S., Hajima, T., Sudo, K., Nagashima, T., Takemura, T., Okajima, H., Nozawa, T., Kawase, H., Abe, M., Yokohata, T., Ise, T., Sato, H., Kato, E., Takata, K., Emori, S., Kawamiya, M., 2011. MIROC-ESM 2010: Model description and basic results of CMIP5-20c3m experiments. Geoscientific Model Development, 4, 845–872.

Webb, P. I., Speakman, J. R., Racey, P. A., 1995. Evaporative water loss in two sympatric species of vespertilionid bat, *Plecotus auritus* and *Myotis daubentonii*: relation to foraging mode and implications for roost site selection. Journal of Zoology, 235, 269–278.

Whitaker, J. O., Karataş, A., 2009. Food and feeding habits of some bats from Turkey. Acta Chiropterologica, 11, 393–403.

Wielstra, B., Crnobrnja-Isailović, J., Litvinchuk, S. N., Reijnen, B. T., Skidmore, A. K., Sotiropoulos, K., Toxopeus, A. G., Tzankov, N., Vukov, T., Arntzen, J. W., 2013. Tracing glacial refugia of *Triturus* newts based on mitochondrial DNA phylogeography and species distribution modeling. Frontiers in Zoology, 10, 13.

Wood, R., Weyeneth, N., Appleton, B., 2011. Development and characterisation of 20 microsatellite loci isolated from the large bent-wing bat, *Miniopterus schreibersii* (Chiroptera: Miniopteridae) and their cross-taxa utility in the family Miniopteridae. Molecular Ecology Resources, 11, 675–685.

Woodward, F. I., 1987. Climate and plant distribution, Cambridge University Press, Cambridge.

Worthington Wilmer, J., Barratt, E., 1996. A non-lethal method of tissue sampling for genetic studies of chiropterans. Bat Research News, 37, 1–3.

Wright, S., 1943. Isolation by distance. Genetics, 28, 114–138.

Yang, D. S., Kenagy, G. J., 2009. Nuclear and mitochondrial DNA reveal contrasting evolutionary processes in populations of deer mice (*Peromyscus maniculatus*). Molecular Ecology, 18, 5115–5125.

Yannic, G., Dubey, S., Hausser, J., Basset, P., 2010. Additional data for nuclear DNA give new insights into the phylogenetic position of *Sorex granarius* within the *Sorex araneus* group. Molecular Phylogenetics and Evolution, 57, 1062–1071.

Zink, R. M., Barrowclough, G. F., 2008. Mitochondrial DNA under siege in avian phylogeography. Molecular Ecology, 17, 2107– 2121.

Zwickl, D. J., 2006. Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion, Ph.D. Thesis, The University of Texas at Austin.

APPENDIX A: A TABLE OF F_{IS} VALUES BETWEEN POPULATIONS OF *Miniopterus schreibersii*

Table A1. Genepop analysis results for the three regions that are not in HWE. *P* is the *P*-value of the null hypothesis of the HWE, SE is the standard error, F_{IS} is the inbreeding coefficient according to Weir & Cockerham (1984) and Steps is the number of switches for the Markov chain algorithm. The significant *P*-values ($P<0.05$) and their corresponding parameters are in bold.

Region	**Iberia**				**Slovakia**				**Thrace & Balkans**			
Locus	***P***	**SE**	**F_{IS}**	**Steps**	***P*-value**	**SE**	**F_{IS}**	**Steps**	***P*-value**	**SE**	**F_{IS}**	**Steps**
CHD2	0.085	0.010	0.323	5091	**0.033**	**0.007**	**0.134**	**4366**	0.491	0.029	0.052	4724
MSC4	0.539	0.013	0.060	15194	0.836	0.020	0.032	2542	**0.011**	**0.003**	**0.049**	**14251**
CH37	NA	NA	NA	NA	**0.034**	**0.003**	**0.318**	**4732**	1.000	0.000	-0.012	358
MSC2	**0.003**	**0.001**	**0.429**	**14388**	**0.016**	**0.003**	**0.258**	**8626**	0.295	0.015	0.101	5341
CHB12	0.103	0.007	0.109	12335	**0.024**	**0.003**	**0.219**	**15018**	**0.031**	**0.004**	**0.117**	**15961**
MM2	**0.020**	**0.004**	**0.400**	**7553**	0.125	0.002	-0.357	71694	0.144	0.011	-0.079	17277
MM18	0.124	0.013	0.142	7586	0.518	0.018	-0.012	10224	**0.002**	**0.001**	**0.103**	**6765**
MM23	0.406	0.022	-0.007	8046	0.795	0.011	-0.123	11965	0.061	0.009	0.077	19640
MM3	NA	NA	NA	NA	NA	NA	NA	NA	**0.003**	**0.000**	**1.000**	**314**
MM12	1.000	0.000	-0.016	1530	1.000	0.000	-0.059	10744	**0.001**	**0.001**	**0.086**	**2204**

APPENDIX B: 692 BP LONG mtDNA CYT-B SEQUENCES OF *Myotis capaccinii* INDIVIDUALS SEQUENCED IN THIS STUDY

H1

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H17

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APPENDIX C: SUPPLEMENTARY FIGURES FOR *MYOTIS CAPACCINII* ANALYSES

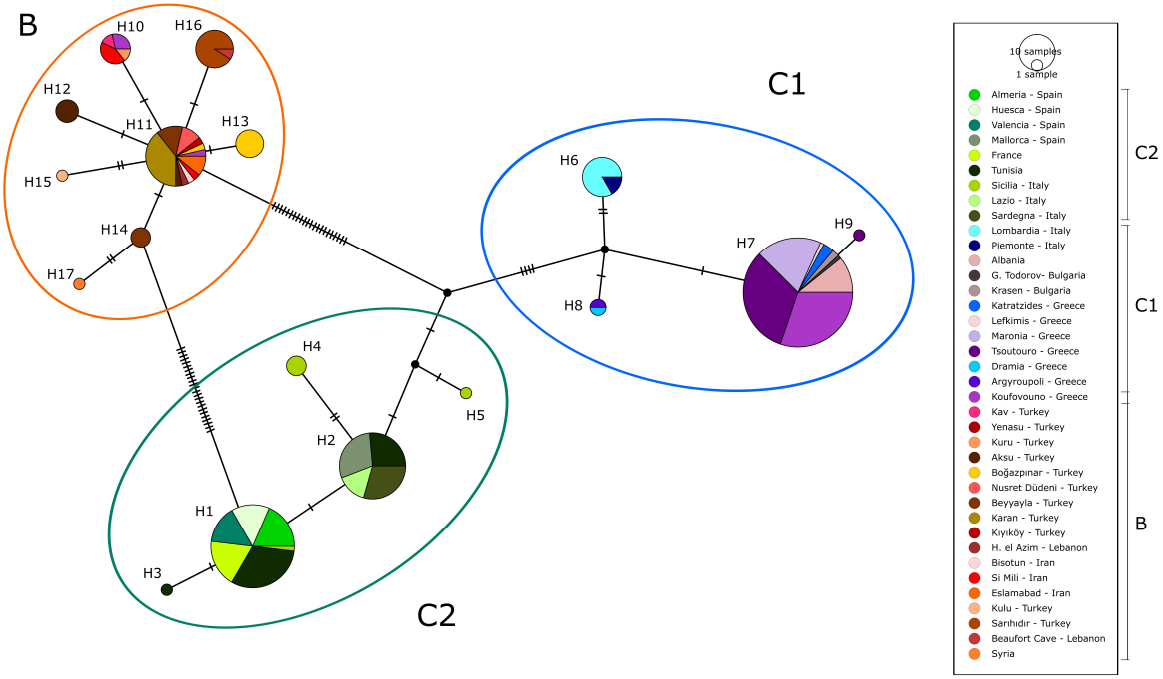


Figure A1. The haplotype network for 265 cyt-b sequences (692 bp) and their distribution based on 38 populations of *Myotis capaccinii*. The sizes of the circles are proportional to the frequency of the haplotypes they represent. The small dots stand for missing haplotypes.

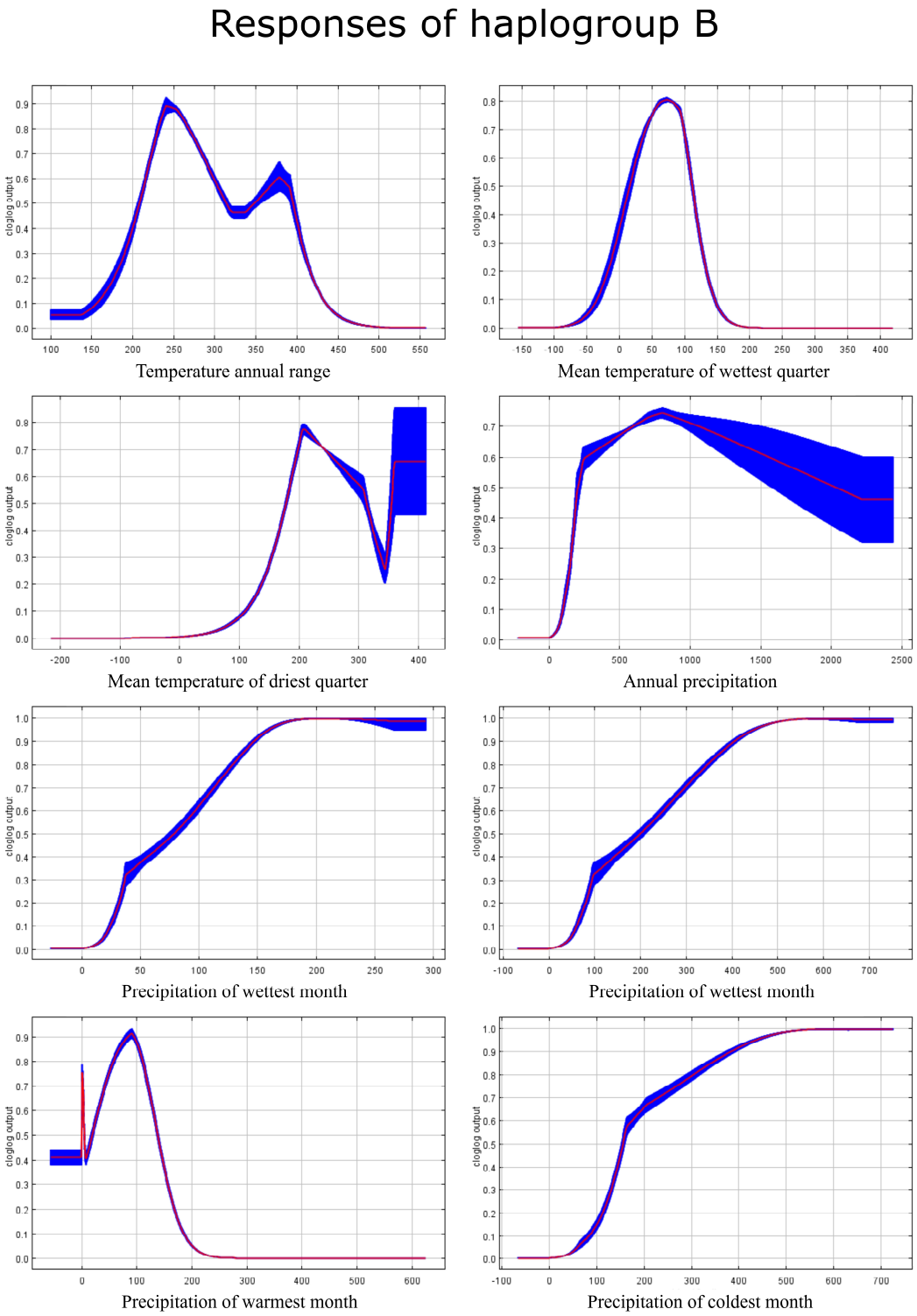


Figure A2. Response curves obtained in Maxent for the four environmental factors related to *Myotis capaccinii* haplogroup B presence. Blue bands indicate the standard deviation of the curves (see Methods).

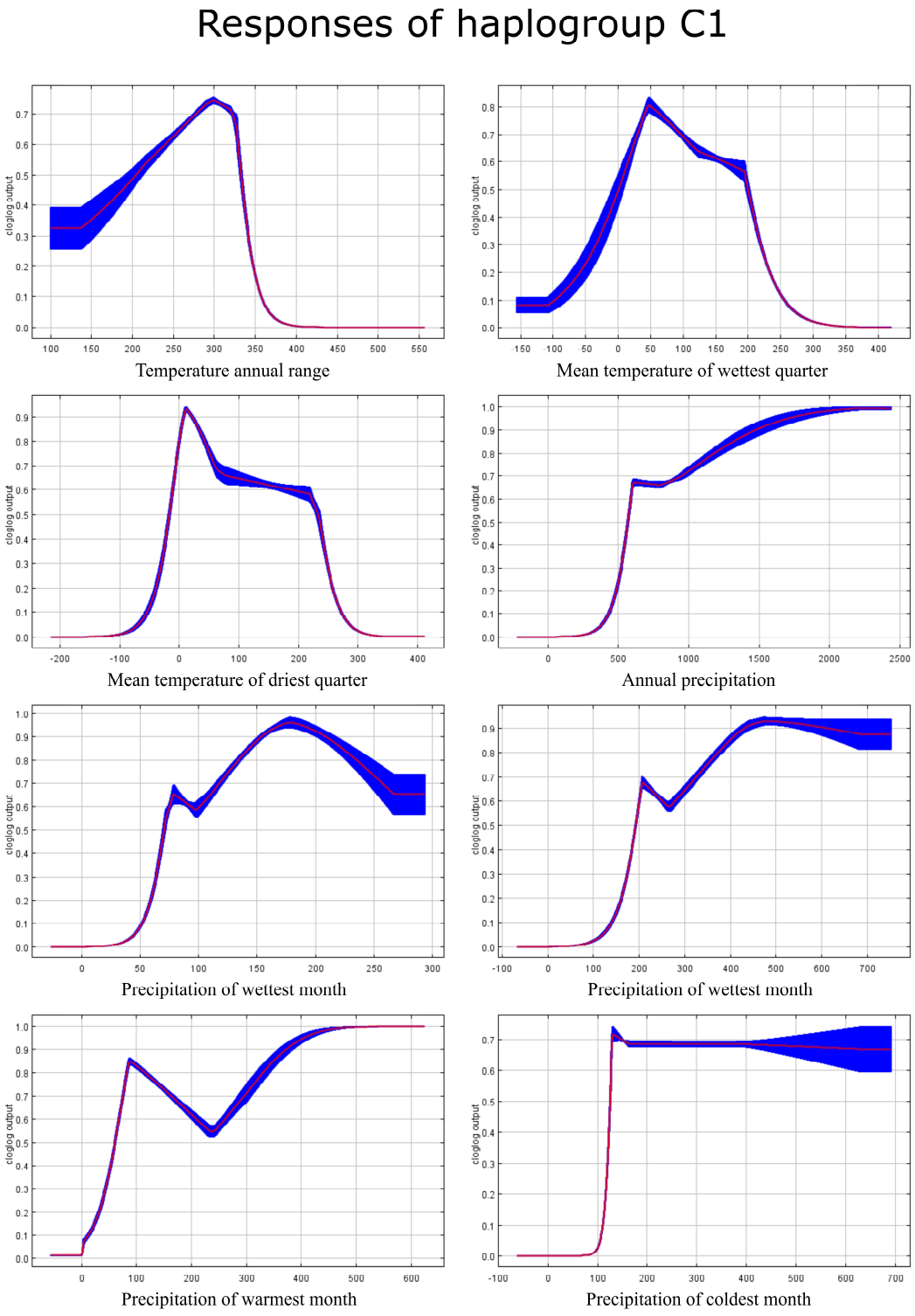


Figure A3. Response curves obtained in Maxent for the four environmental factors related to *Myotis capaccinii* haplogroup C1 presence. Blue bands indicate the standard deviation of the curves (see Methods).

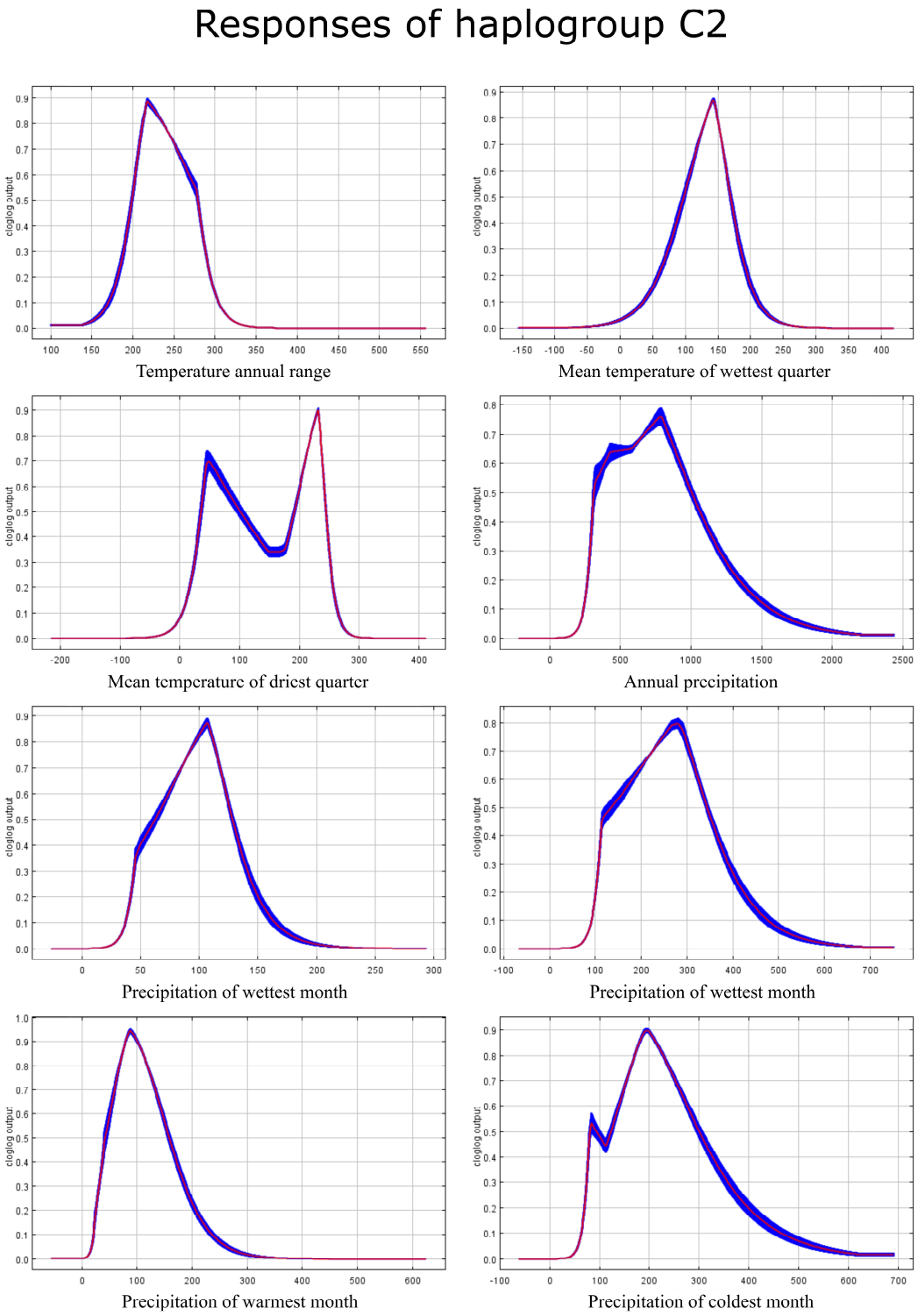


Figure A4. Response curves obtained in Maxent for the four environmental factors related to *Myotis capaccinii* haplogroup C2 presence. Blue bands indicate the standard deviation of the curves (see Methods).