PHYLOGEOGRAPHY OF THE LARGE MOUSE-EARED BATS IN TURKISH THRACE AND ANATOLIA

by

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For my doorbell

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ABSTRACT

PHYLOGEOGRAPHY OF THE LARGE MOUSE-EARED BATS IN TURKISH THRACE AND ANATOLIA

The main objective of this study is to determine the exact number of species/subspecies within the Large Mouse-eared Bat Complex that inhabit Anatolia and the Turkish Thrace. In this study we also explore the historical relationships among these taxa, and how their geographical distribution changed over time. Defining precise methods for identification of these species/subspecies in the field and in laboratory settings is among the aims of the study. We also focus on the discrepancy in the information acquired from the nuclear and mitochondrial DNA in terms of species identification and try to explain the processes that cause this discrepancy. The results of our study reveal that in the study area, there are two distinct species one of which is further divided into two evolutionarily significant units. Besides the previously accepted two taxa, there is a separate unit residing in Anatolia that is morphologically similar to the *Myotis myotis* in the west but genetically isolated from this ancestral population. We conclude that some of the alleged subspecies in previous studies do not exist, but Anatolia is occupied by a genetically isolated branch of the greater form that might be defined as a different species/subspecies with the previously suggested name, *M. (m.) macrocephalicus*. This study also highlights the importance of these findings that redefine the species distribution, with regard to conservation studies. A mathematical method including morphological measurements is also suggested for differentiation of these species in the field.

ÖZET

TRAKYA VE ANADOLU'DAKİ BÜYÜK FARE KULAKLI YARASALARIN FİLOCOĞRAFYASI

Bu araştırmanın asıl amacı, Anadolu ve Trakya'da Büyük Fare Kulaklı Yarasa grubuna mensup kaç tür/alt tür olduğunun belirlenmesidir. Ayrıca, bu alt grupların birbirleriyle tarihsel ilişkileri, zaman içindeki coğrafi hareketleri, sahada ve laboratuvar ortamında birbirlerinden ayırt edilme yöntemleri araştırılmıştır. Bu türler/alt türler arasında nükleer ve mitokondrial DNA'ların tür ayrımı açısından sergilediği farklılığa da odaklanılmış ve bunun sebepleri açıklanmaya çalışılmıştır. Sonuçlar, çalışmanın yürütüldüğü coğrafyada iki farklı tür olduğunu ancak bunlardan birinin evrimsel açıdan anlamlı iki farklı gruba ayrıldığını göstermektedir. Kabul gören iki tür haricinde, morfolojik olarak *Myotis myotis* ile yakın benzerlik göstermesine karşın sadece Anadolu coğrafyasında bulunan ve batıdaki benzerlerinden genetik olarak ayrışan izole bir grup olduğu görülmektedir. Önceki çalışmalarda var olduğu öne sürülen bazı alt türlerin aslında bilinen türlerden farklı olmadığı gösterilmiştir. Bu yarasa grubunun sadece Anadolu'da bulunan büyük formunun ise farklı bir tür /alt tür statüsüne sahip olabileceği ve daha önce önerilmiş olan *M. (m.) macrocephalicus* isminin bu grup için uygun olduğu sonucuna varılmıştır. Çalışmada ayrıca, tür dağılımını yeniden tanımlayan bu bulguların koruma çalışmaları açısından önemi vurgulanmakta ve bu türlerin sahada ayırt edilebilmesi için morfolojik ölçümlere dayanan matematiksel bir yöntem önerilmektedir.

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LIST OF SYMBOLS/ABBREVIATIONS

Symbol	Explanation	Unit
CM^3	Distance from Canine to 3rd Molar	(mm)
Q	Posterior Probability	
F_{st}	Fixation Index (Nuclear DNA)	
Φ_{st}	Fixation Index (Mitochondrial DNA)	
K	Number of Clusters	

Abbreviation	Explanation
FA	Forearm
HVII	Hypervariable Control Region II
M.m.	*Myotis myotis*
M.b.	*Myotis blythii*
s.l.	*sensu lato*
MMW	*Myotis myotis* (western branch)
MME	*Myotis myotis* (eastern branch)
PCR	Polymerase Chain Reaction
DNA	Deoxyribonucleic Acid
mtDNA	Mitochondrial DNA
Hd	Haplotype Diversity
n	Number of Samples
SD	Standard Deviation
Nh	Number of Haplotypes
Min	Minimum
Max	Maximum
Nd	Nucleotide Diversity
Km	Kilometers
Mm	Millimeters

1. INTRODUCTION

The main focus of this study is the large mouse eared bat complex, which is part of the order Chiroptera that includes all flying mammal species. This topic is selected because the taxonomic statuses and the geographical distribution of the representatives of this bat complex that reside in Europe, Anatolia and the Middle East have been under debate for a long time.

This bat complex comprises at least two known subgroups in Anatolia and continental Europe, and the proper method for differentiating these two common forms in the field, whether they hybridize with each other or not, and how they are distributed in certain regions are currently unclear. Other than two widely agreed-upon species, namely the greater (*Myotis myotis*) and the lesser forms (*Myotis blythii / Myotis b. oxygnathus*), there are also some alleged species/sub-species within this complex. On the other hand, the research held until now show that the mitochondrial and nuclear markers used for genetic identification of these species tell different stories about the taxonomy of these bats.

The bats are a very important part of ecological balance and being agents of pest control, they also play an economical role in agriculture. Today, many bat species are under threat of extinction or at least to some extent, vulnerable to some environmental changes mostly induced by human actions. Since a comprehensive knowledge of their taxonomic statuses and geographical distributions is also critical for the conservation efforts the studies on the phylogeography of bats are not only important for purely scientific reasons, but an essential part of conservation of these species.

This study aims to provide a better understanding of the species distribution of Large Mouse-eared bats in Anatolia and the Turkish Thrace, determine the number of species that inhabit these regions, and shed light on the historical and current relationships of these species with each other. For this purpose, many samples from the Large Mouse-eared bat complex were collected from Anatolia and the Turkish Thrace in field studies performed over three years, and some samples were received from other researchers in neighboring countries. Genetic and morphologic data acquired from samples used in previous studies by various researchers were also included in the data analyses to achieve a clearer picture of this bat complex in a wider scope within the western Palearctic region.

Genetic data acquired from specific control regions of nuclear and mitochondrial DNA was analyzed in conjunction with the morphological measurements obtained in the field. With the help of

a more comprehensive data set compared to previous studies, a precise species delimitation and correct geographical distribution of these species was determined for the area of interest. Besides this, a relatively precise morphometric method for species identification in the field is offered. The results of this study are meant not only to be helpful in resolving scientific debates on this bat complex, but to contribute to the conservation efforts regarding these bat species.

2. STATEMENT OF PROBLEM AND LITERATURE REVIEW

2.1. History of the Taxonomic Studies on Large Mouse-eared Bats

The Large Mouse-eared bats belong to the genus Myotis that embrace all mouse-eared bats. Large Mouse-eared bats (genus *Myotis)* are part of the family Vespertilionidae, which is the largest bat family under the order Chiroptera, which comprise all bat species. The genus *Myotis* is divided into three subgenera: *Myotis*, *Selysius* and *Leuconoe* (Findley, 1972). Members of the subgenus *Myotis* are distinguishable from other *Myotis* bats by their larger size, longer ears, broader wings and more derived dentition (Menu, 1987). The members of this genus are generally gleaners, picking up their prey from solid surfaces or even from the ground (Arlettaz et.al., 1997a). The genus *Myotis* represents a total of about 90 bat-species spread all over the world (Ruedi & Mayer, 2001). The Large Mouse-eared bats include several species and subspecies, occupying large areas in Europe and Asia (Mitchell-Jones *et al*., 1999; Dietz *et. al*., 2007). But the taxonomic statuses of these bats in Europe and Anatolia is only partly resolved.

Before presenting the literature on the current taxonomy of this bat group, it is worth mentioning the difference between species and subspecies concepts. According to the biological species concept, "species are groups of actually or potentially interbreeding populations that are reproductively isolated from other such groups" (Mayr, 1942). The subspecies concept, on the other hand, has a more arbitrary definition and is used for defining morphological variations in distinct geographical populations of a species; but it may as well be considered as a stage in the speciation process (Mayr, 1982).

The greater form of Large Mouse-eared bats inhabiting Europe was the first one to be identified as a distinct species, and was named *Myotis myotis* (Borkhausen, 1797; terr. typ.: Germany). The second, or the "lesser" form of Large Mouse-eared bats in Europe was first identified by Monticelli (1885) and named as *Myotis oxygnathus* (terr. typ.: Italy). Later on, because of their morphological similarity to *Myotis blythii* (Tomes, 1857; terr. typ.: India) from Central Asia, , *Myotis oxygnathus* began to be considered a synonym of *Myotis blythii* (Ellerman & Morrison-Scott, 1951; Strelkov, 1972) and from that time on, named by many authors as *Myotis blythii oxygnathus*.

However, further studies showed that Asian *Myotis blythii* is genetically (according to mitochondrial genome) different from the lesser form of mouse-eared bats in Europe and Anatolia

(Bogdanowicz *et al*., 2009; Furman *et al*., 2011). Simmons et.al (2005) proposed that the lesser mouse-eared bats in Europe should be attributed species status (*Myotis oxygnathus,* as a different species than *Myotis blythii* in central Asia) but this approach is not accepted by all authors. In current scientific literature, both names are still used when referring to the lesser form of Large Mouse-eared bats. While the greater mouse-eared bats are believed to be endemic to Europe and Middle East (Ruedi, 2011), the eastern boundaries for the geographical distribution of the lesser form are unclear due to lack of more precise taxonomic studies. Because of this uncertainty in nomenclature, in this study, all members of the lesser mouse-eared bats are referred to as *Myotis blythii sensu lato* (or *M.blythii s.l.*) to avoid confusion.

A third species within the Large Mouse-eared bat complex, which resides in Northern Africa and Mediterranean islands, is *Myotis punicus* (Felten *et al*., 1977). Because this species has been proven to reside outside of Europe and Anatolia (with the exception of some Mediterranean islands) and do not mix up with European Large Mouse-eared bats (Castella et.al., 2000), it is not included to the scope of this study.

Besides the three species mentioned above, several local populations of mouse-eared bats were attributed species or subspecies statuses by some researchers, due to their deviances in their morphological characteristics with respect to the known averages for the already identified species. Two different populations of Large Mouse-eared bats from Iran were suggested subspecies statuses in 1905 and 1921, with the names *Myotis myotis omari* and *Myotis myotis risorius*, respectively (Thomas, 1905; Cheesman, 1921). After a while, these alleged subspecies were assigned to *Myotis blythii* (Ellerman & Morrison-Scott, 1951; Harrison and Lewis, 1961; Strelkov, 1972). Strelkov, as a result of their study focusing on the geographic variation of the lesser form of mouse-eared bats, suggested that *Myotis (b.) oxygnathus* occupied a geographical range limited to the area extending from Spain to Crimea (including the Thrace), and the rest of Anatolia, Middle East and some Mediterranean islands were inhabited by the subspecies *Myotis bylthii omari.* The distinguishing characteristic of *M. b. omari*, according to Strelkov, was their larger size, which was comparable to *M. myotis.* Some authors also argued that the taxon *omari* was the representative of the greater form (i.e. *M. myotis*) and resided in Turkey, Iran and the Middle East (Arlettaz et. al, 1997b).

Another suggested subspecies belonging to the Large Mouse-eared bat complex was *Myotis myotis macrocephalicus* (Harrison and Lewis, 1961; terr. typ.: Lebanon), a morphologically distinct subspecies residing in the Levantine countries (and Hatay province in Turkey). The differentiating morphological characteristics of this alleged subspecies were described as their bigger cranial

measurements and the paleness of the fur at the back. Nevertheless, later on it was claimed by some authors that *Myotis myotis macrocephalicus* -at least for the studied populations occupying a significant part of Anatolia- is a synonym of *M. myotis* (Koopman, 1993; Albayrak, 1998).

In a more recent study conducted in Lesvos Island in the Aegean Sea, Iliopoulou and Georgudaki (1984) claimed that the island was inhabited by a possible intermediary subspecies between *M.b. oxygnathus* residing in Greece and *M.b. omari* residing in Anatolia. They suggested that this intermediary form could be named as *M. blythii lesviacus*. The distinguishing features of this subspecies, according to these researchers, were their paler color and cranial measurements, which were different from both mainland species.

2.2. Ecology of Large Mouse-eared Bats

Most of the studies focusing on the ecology of Large Mouse-eared bats were conducted in Europe and Mediterranean islands and in limited areas. While many of these studies lack a molecular component in species differentiation and lean mostly on morphological measurements, they point out to a clear separation between the diets and preferred habitats of the two already confirmed species.

The greater form of Large Mouse-eared bats, according to several authors, prey mostly on forest-dwelling beetles such as carabid beetles (Bauerova 1978, Audet 1990, Arlettaz 1996). Nevertheless, studies also reveal that *M. myotis* can change its diet opportunistically, depending on the availability of the food sources and habitats they exploit (Arlettaz, 1996: Spitzenberger, 1996). According to these studies, *M. myotis* can prey on smaller beetles, which are also preyed on by *M. blythii s.l.* The habitats occupied by *M. myotis* are expected to be more humid areas with “freshly cut meadows, mown grass in intensively cultivated orchards, and forests without undergrowth” (Arlettaz, 1999).

M. blythii s.l., on the other hand, is a grass-dwelling species, feeding mostly on orthopterans such as bush crickets (Arlettaz et.al., 1993). Because of this prey choice they inhabit warm steppe belt and colonize secondary grassland habitats such as meadow and pastureland (Arlettaz et.al., 1995; Arlettaz, 1999). It was also shown that while the two sibling species exists sympatrically, *M.blythii s.l.* feeds mostly on primary consumers whereas *M. myotis* has a more enriched diet comprising both primary and secondary consumers, indicating that they have a clear separation between their trophic ecology involving different trophic levels of prey (Björn et.al., 2011).

A study conducted in southwestern Switzerland indicates that the greater and lesser forms of Large Mouse-eared bats also differ in the time they give birth to their offspring. According to this study, the parturition time of the two species differ according to the availability of the different preys they consume and the parturition time of *M. blythii s.l.* changes from year to year associated with the existence of cockchafers (Arlettaz et al., 2001).

It is worth mentioning that almost all the research on the foraging habits and food preferences of the Large Mouse-eared bats were conducted in specific regions in continental Europe, while the studies suggesting the existence of other species/subspecies within the Large Mouse-eared Bat complex do not rely on such ecological data. Therefore, rather than the ecological difference between the potential species, in this study, we gave priority to the molecular and morphological data in order to achieve a better resolution regarding the taxonomic delimitation within this bat complex. The distribution of the species and climatic conditions of the areas they inhibit, on the other hand, are also noted.

2.3. Molecular Studies on Large Mouse-eared Bats

2.3.1. Mitochondrial DNA

The earlier studies on the taxonomy of Large Mouse-eared bats, as it was in many other species, depended solely on morphological and ecological characteristics. Because of their very similar morphology and the difficulty of differentiating the species by their ecology, these characteristics were not functional in many cases. Another method used for species differentiation was the comparison of karyotypes -a method involving the inspection of chromosome shapes- also proved uninformative for these species because their karyotypes were identical (Zima & Horacek 1985).

The introduction of molecular methods focusing on the nuclear and mitochondrial DNA, on the other hand, promised a more reliable source of information regarding the ancestral and phylogeographical relationships of cryptic species or subspecies. The use of allozymes (enzymes coded by different alleles from the same locus) was the first molecular method widely used for phylogeographic studies on bats, as well as many other species. For many years, bat researchers used allozyme data to facilitate species identification, alone or along with other molecular methods (Ruedi et.al. 1990, Arlettaz et.al. 1997b, Castella et.al., 2000). After the use of Polymerase Chain Reaction became more feasible, “surveying variation at the DNA level” replaced allozyme studies that had limited capabilities (Zink & Barrowclough, 2008).

Mitochondrial DNA, which is transmitted maternally and includes certain regions that evolves rapidly in higher animals, has been the main object of focus in most phylogenetic studies for a long time (Avise, 1998). The efforts to identify animal species through DNA barcoding focused on a certain part of mitochondrial DNA (Hebert et.al., 2003) for the sake of using a standard, easily identifiable marker for all species. Many researchers used the cytochrome b and ND 1 regions of the mitochondrial DNA to map the diversification of bat species (e.g. Ruedi & Mayer, 2001; Mayer & Halversen 2001; Ibáñez et. al. 2006). The studies focusing on differences between the sibling species *M. myotis* and *M. blythii s.l.* or intraspecific diversity within these taxa, first utilized these regions of the mitochondrial DNA. More recently, hypervariable domain (HVII) of the control region, which accumulates mutations with a greater pace compared to other regions of the mitochondrial DNA, was also included in such studies.

The earlier molecular studies utilizing cytochrome b and ND1 regions of mitochondrial DNA (Castella et.al., 2000; Mayer & Helversen, 2001) showed that *M. blythii s.l.* and *M. myotis* shared similar mitochondrial haplotypes in most of the regions that they cohabit. Moreover, the attempts to make taxonomic classifications according to these markers were not backed by the findings acquired from cranial measurements (Evin et. al., 2008) or allozyme studies (Castella et. al., 2000).

Studies also revealed that there is a distinction in the mitochondrial DNA (cytochrome b) separating western and central European *M. myotis* and *M. blythii s.l.* from the representatives of the same species in the eastern regions such as eastern Europe, Asia Minor, Transcaucasia, Crimea, etc. This finding indicated that two separate (western and eastern) lineages existed within this bat complex according to this mitochondrial marker (Furman et.al., 2013). The same study also showed that the mtDNA of *M. blythii s.l.* in Europe and Anatolia was substantially different from *M. blythii* from Central Asia. This result indicated that the taxonomic nomenclature, which referred to the European and middle Asian forms of lesser Mouse-eared Bats as the same species, was problematic.

In addition to these findings, Berthier and colleagues (2006), in their study covering regions where *M. myotis* and *M. blythii s.l.* were sympatric, reached the conclusion that the mitochondrial DNA of *M. blythii s.l.* was recently replaced by the mitochondrial DNA of *M. myotis* as they expanded westward into continental Europe. Few studies suggest that the two species can still hybridize, at least in certain regions in Europe (Berthier et.al., 2006) though some of these rely only on morphological data (Bachanek and Postawa, 2010). These findings support the hypothesis that gene flow between the two taxa, which is a requirement for the replacement of the mitochondrial DNA, is possible. The

existence of this molecular replacement process known as introgression, which was confirmed by studies conducted in the following years, showed that it was not possible to achieve a clear species delimitation merely by mitochondrial markers.

While it proves uninformative in species delimitation, the HVII domain of the mitochondrial DNA was utilized most of the time in phylogeographical studies and several haplotypical clades were assigned to certain groups according to their similarities of the DNA sequence within this domain. These clades were useful in identifying the post-glacial movements of the bat populations and direction of the introgression events. First three of these clades; A, B and C were introduced to explain the differences in HVII domain of *M. myotis* bats in central Europe (Castella et.al., 2001). To these, D, E and F clades corresponding to eastern European samples were added in a following study (Ruedi & Castella, 2003). The overall picture reveals that the same haplotypes were shared by both taxa everywhere, but certain haplotypes were limited to certain regions: Clade A: All Europe from Iberia to the Turkish Thrace; Clade B: Italy and Switzerland; Clades C and E: Northern Italy, Switzerland and Greece; Clade D: Syria, Iran, Turkey, and Balkans; Clade F: Italy and the Balkans. In addition to these six clades, Clade G (in Italy; Bücs et.al., 2015) and Azokh Clade (in Armenia; Bogdanowicz et.al., 2009) were also suggested as additional haplogroups with very limited geographical distributions.

2.3.2. Nuclear DNA

Microsatellites, or "1 to 6 tandem repeats found at high frequency in the nuclear genomes of most taxa," is another molecular tool widely utilized by researchers to analyze the phylogeography and migration patterns of several species (Selkoe and Toonen, 2006). Since they are rapidly evolving and non-coding regions of nuclear DNA, the microsatellites offer a reliable way to study the recent phylogeography of many species. Therefore, in accordance with the fact that the female bats are generally more philopatric than the males, while mtDNA markers which are maternally inherited gives more information about the past interaction, the microsatellite markers better reflect recent interactions and related gene flow (Ruedi & Castella, 2003).

The first study utilizing microsatellite regions for the genetic study of Large Mouse-eared Bats was conducted to check the patterns of south-to-north expansion pattern of *M. myotis* (Castella and Ruedi, 2000). The 13 microsatellite markers developed for this study (A13, B11, B22, C113, D9, D15, E24, F19, G9, G25, G30, H19, and H29) were also used by many researchers afterwards, shedding more light on the phylogeography of this bat complex than any other measures.

One of the further studies utilizing different subsets of these microsatellite markers revealed that there was no gene flow between European and North African representatives of Large Mouse-eared bats (Castella et.al., 2000). Berthier et.al. (2006), with a relatively narrow set of markers, found that C113 was fixed for *Myotis myotis* while it is polymorphic for *M. blythii*, and E24 showed only 2-base-pair repeat alleles for *M. myotis*, while *M. blythii* had both 1- and 2-base-pair repeats. Thus, it was possible to differentiate the species (at least the ones with non-fixed traits in these markers) with rather high accuracy using these markers (Berthier et.al., 2006).

2.3.3. Cyto-nuclear discordance

Cyto-nuclear (or mito-nuclear) discordance refers to the situation when markers from nuclear DNA and mitochondrial DNA (located in the cytoplasm) imply different historical relationships between taxonomic units. One important factor introducing a controversial element to the taxonomic differentiation within the Large Mouse-eared bat complex was the discovery of the discordance between what the information gathered from mtDNA and nuclear DNA of the samples indicated about their phylogeography.

The cyto-nuclear discordance may occur due to few reasons, including introgression and incomplete lineage sorting. Introgression requires introduction of alleles from one taxonomic unit into the gene pool of the second (Anderson 1949) while some portion of the gene pool of both units remains untouched, so the differentiation between the two as distinct taxonomic units is still possible (Harrison & Larson, 2014). As a part of this process, the mtDNA of one species (or sub-specific evolutionary unit) may partly or totally be replaced by the mtDNA of the other (Petit & Excoffier, 2009). Meanwhile, in incomplete lineage sorting, multiple population of the same species go through evolutionary steps in separation from one another, leading to the appearance of similar and dissimilar traits in their genome at the same time. Therefore, while introgression implies historical hybridization between taxonomic units, incomplete lineage sorting requires parallel evolution of parapatric units.

Several studies conducted on Large Mouse-eared Bat complex in different regions showed that the nuclear markers could be employed to differentiate between greater and lesser forms. However, their mitochondrial markers were not in accordance with this differentiation indicated by the nuclear markers, and were even identical in several locations (Castella et al., 2000; Berthier et al., 2006; Bogdanowicz et al., 2009; Furman et al., 2011). The reason for the cyto-nuclear discordance in Large Mouse-eared bats were attributed to both processes by different authors.

2.4. Current Theories on the Phylogeography of Large Mouse-eared Bats

Phylogeography is defined as the "historical aspects of the contemporary spatial distributions of gene lineages" (Avise 1996). In other terms, this line of study aims to reveal the historical occurrences that lead to the current distribution of the species and the current picture regarding the genetic differences and similarities between taxonomic units. Earlier paleontological, morphological and genetic studies conducted on Large Mouse-eared bats suggest that the *M. blythii s.l.* and *M. myotis* were separated during the Pleistocene, meaning that they split from a single ancestor during this era (Arlettaz et.al., 1997b).

The species differentiation between two sibling (or recently split) species may be attributed to either sympatric or allopatric speciation events. Sympatric speciation, which occurs when populations are not geographically separated from each other, requires disruptive selection via habitat specialization and assortative mating (Rice, 1987). Allopatric speciation event, on the other hand, requires a long-time geographic separation between populations of a single taxonomic unit due to any reason (ice age, migration, tectonic movements, etc.)

According to Arlettaz and colleagues (1997b), due to their colonial habits (i.e. mixed maternity colonies), sympatric speciation is not possible for Large Mouse-eared bats. These authors argue that the ice age separated this ancestral species into two, causing them to turn into two distinct species through mutations and ecological adaptations during the Pleistocene. According to this view, the part of Large Mouse-eared bats that took refuge in western Europe speciated into *M. myotis*, and the part that took refuge in eastern regions speciated into *M. blythii s.l.* and began expending eastward and westward, respectively, after the Last Glacial Maximum.

Berthier and colleagues, in one of the first studies involving both mitochondrial and nuclear genetic studies on these species (2006), suggest that the central Asian *M. blythii* invaded the areas formerly occupied only by *Myotis myotis*, and during this process, the mitochondrial genome of *M. blythii* was totally replaced by the mitochondrial genome of the latter, through repeated asymmetric hybridization events.

In another hypothesis aimed at resolving the cytonuclear discordance problem, Bogdanowicz and colleagues (2009) suggest that the lesser and greater forms of Large Mouse-eared bats are separated only at subspecies level and the discordance is due to incomplete lineage sorting. According to this scenario, the variability of mitochondrial haplotypes had its roots in the genome of the ancestral

species and survived more than one (ecological) speciation event occurring in separate locations. This scenario was further scrutinized by Furman et.al (2013), raising the possibility that full speciation events might have occurred in different regions, keeping the standing genetic variation.

In more recent studies, including the papers published during the course of this study, the idea that the two forms evolved into totally different species by geographical segregation gained dominance. These studies suggest that the cyto-nuclear discordance is caused by introgression as a result of asymmetric hybridization events in two separate directions. According to this view, the lesser form (*M. blythii s.l.*) that took refuge in east expanded into west, gaining the mitochondrial genome of the greater form (*M. myotis*) during this process, and vice versa (Furman et.al., 2014). A survey on introgression events (Currat et. al. 2008) supports this idea, showing that even small hybridization rates can result in substantial replacement of the mitochondrial DNA of the invading species, by the mitochondrial DNA of the resident species.

2.5. Aim of the Study

Following from the current literature on the taxonomy and phylogeography of Large Mouse-eared bats, the objective of this study is to determine the number of species and/or evolutionarily significant units within this group inhabiting the study area, which remained controversial up to date. While the morphological and molecular data we acquired are not intended to describe a species/subspecies, we try to find clues for the existence of previously suggested ones by checking gene-flow between populations and morphological leaps or trends in few traits, which might support or negate the existence of such species/subspecies. Besides this, the most appropriate way to identify one species from another by both molecular and morphological traits is tried to be determined. Revealing the historical relationship between these species/subspecies, the processes through which they differentiated from one another, and the current level of gene flow between them is among the aims of the study.

The species delimitation within the Large Mouse-eared bats is examined with a deeper focus on the nuclear genome, as this was the suggested method in former studies. The recent history of gene flow and recent movements of these taxa, on the other hand, are explored using mitochondrial markers. The level of observed differentiation (or fixation) between populations are utilized to acquire information on the phylogeography of this group of animals. Also, within the scope of this study, we try to determine the actual cause of the discordance between the data acquired from mitochondrial and nuclear markers.

We analyzed the patterns of morphological characteristics with reference to molecular findings and tried to achieve a more consistent and convenient method for identification of these species in the field, based on their morphology. Also, need for further studies and conservation implication of these findings are presented.

3. MATERIALS AND METHODS

3.1. Sample Collection

The sample set used in this study comprises three major components. Besides tissue samples and morphological measurements acquired directly in the field, we included samples and measurements gathered by other researchers. Data published in previous studies were also combined with the acquired data in order to achieve more precise results about the taxonomical statuses of the Large Mouse-eared bats in the region. The biopsy samples from within Turkey were collected with a permit issued by the Turkish General Directorate of Nature Conservation and National Parks (B.23.0.DMP.0.15.01-12 510.02-14138) and approved by the Boğazici University Ethics Committee on Animal Research (BUHADYEK).

Since the original contact zone of possible different taxonomic units within this species complex was expected to be in the Balkans or the Turkish Thrace, tissue samples and measurements were requested from researchers in the Balkan countries such as Bulgaria and Romania. Samples acquired from Poland were also included in the study. In addition to these, measurements and genetic data (acquired from the tissue samples processed in the same laboratory) that were used in a previous study (Furman et al., 2014) were also used in the analysis.

Overall, the number of individual bats whose data were included in this study is 475. The geographical locations of samples are given in Figure 3.1 and 3.2. Among these, 264 were used exclusively for this study (152 were collected in Turkey, and 112 were sent by other researchers), while the remaining were also used in a formerly published research (Furman, et. al., 2014). The sample numbers, locations and morphological measurements of all the specimens used in this study are listed in APPENDIX A.

Figure 3.1. The distribution of roosts that the samples were collected. Figure includes samples from Anatolia and the Turkish Thrace (+), and the roosts from which samples were sent by other researchers from Balkan Countries and Poland (□).

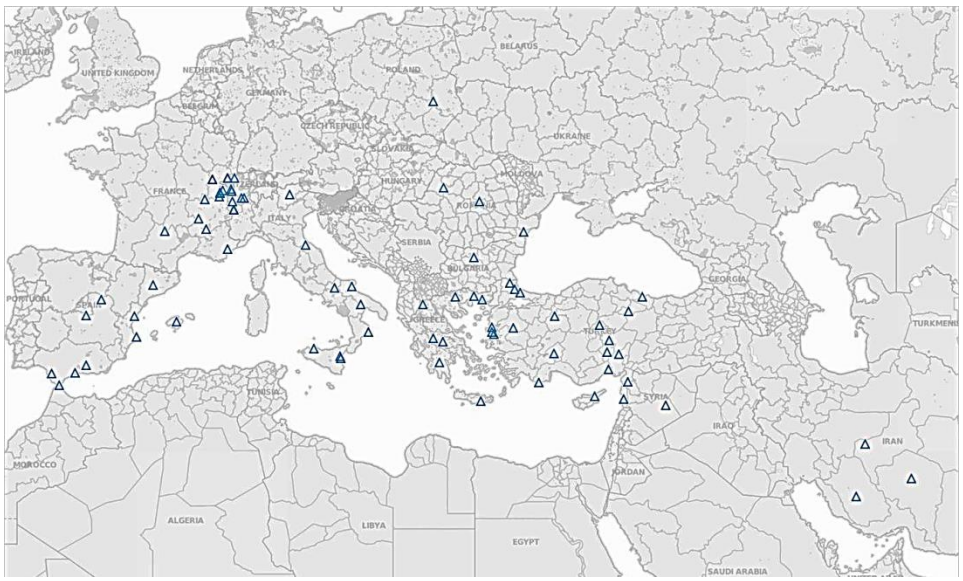


Figure 3.2. The distribution of all samples used in this study (including ones from previously published studies).

3.1.1. Field Studies

The samples from Anatolia and the Turkish Thrace were collected in field studies conducted between 2012 and 2014. The field studies were done during summer, avoiding the hibernation periods of the bats. Several caves throughout Anatolia and the Turkish Thrace were determined as the locations for field studies.

The bats were captured using hand nets within the caves. When this was not possible, mist nets were used to capture them in the vicinity of the caves during the night. The bats captured were placed in bags made of soft fabric and moved to a suitable location within or just out of the cave for measurement and tissue sampling. The females with pups attached were released immediately after capture and were not included in the study to prevent any harm to the pups. All bats were released within maximum 3 hours of capturing.

3.1.2. Morphological Measurements

The captured bats were measured for their forearm length (FA) and the distance from the Canine tooth to the last molar tooth (CM^3). The teeth measurements were taken from the upper left jaw. Although some additional measurements such as tail length, ear length, tragus length, etc. were also taken during the first field studies, these measurements were dropped later on, since they proved non-informative regarding species delimitation. All the measurements were taken using a high-accuracy digital caliper.

The morphological measurements were noted alongside with other nominal data such as the sex, estimated life stage (juvenile vs. adult), and lactation status (for females). The sex of the specimens were noted to be able to check for sexual dimorphism and the juveniles were not included in the analysis of morphological traits. The captured bats were also photographed by a high-resolution digital camera in order to be examined for noticeable differences that could be of help for species assignment in the field. The echolocation sounds of the bats were also recorded upon release, but these recordings were not included within the scope of this study and were kept for future reference.

3.1.3. Tissue Sampling

Following the capturing of bats, a 3 mm biopsy punch was taken from their wing membranes. These biopsies are conducted in a manner that would not have a negative effect on the flight and foraging abilities of the bats and heals in a few weeks (Pierce & Keith, 2011). The biopsy punches were sterilized by swapping with alcohol and then exposing to high heat by a lighter prior to use, in order to eliminate remaining tissues or other contaminants from the previous punches.

The tissues were then put into 2 ml-tubes and kept in 90% ethyl alcohol during the field study. The tubes were labeled in the field as to show the specifications of individual tissue samples, and were kept in a refrigerator in the laboratory until DNA extraction.

3.2. Acquisition of the Molecular Data

3.2.1. DNA Extraction

The DNA extraction from all 475 tissues used in this study was made in the Molecular Ecology Laboratory in the Institute of Environmental Sciences, Boğaziçi University. The tissues kept in ethyl alcohol were first washed with distilled water and then the extraction process was conducted using Roche High Pure PCR Template Preparation Kits, following the manufacturer's protocols (Roche Applied Science).

Following the extraction process, the eluted DNA was placed into 1.5 ml microcentrifuge tubes and stored in -18 degrees Centigrade. When necessary, the amount of DNA was measured using a Picodrop Pico 100 (Picodrop Ltd.) spectrophotometry device.

3.2.2. Amplification of the Mitochondrial Control Region

The 2nd hypervariable region (HVII) from the Control Region of the mitochondrial DNA was amplified using Polymerase Chain Reaction (PCR). In the first set of samples that was also used for a published study (Furman et al., 2014), the hypervariable region was amplified with the primers L16517: 5′-CATCTGGTTCTTACT- TCAGG-3′ (Fumagalli et al., 1996) and sH651: 5′-AAGGCT-AGGACCAAACCT-3′ (Castella et al., 2001). Amplifications were made in 20 µL reaction volumes, each containing 1,3 µL of template DNA, 2 µL of PCR buffer with NH_4, 1,6 µL $MgCl_2$ (25mM), 0,24 µL Taq Polimerase enzyme, 0,4 µL from each primer, and 0,4 µL dNTP (10 mM), using two thermal

cycler units (Bio-Rad). The PCR amplification process comprised of following steps: initial denaturation at 95°C for 5 minutes, 35 cycles of 94°C for 30 seconds, 57°C for 30 seconds and 72°C for 60 seconds; and a final extension phase at 72°C for 10 minutes.

For the rest of the samples, the primers Myohvr2L: 5′AAAATCAAGATCGCCCACTC3′ and Myohvr2H: 5′GTTGTGTTGTATGTCCTGTAACCA3′ (Bogdanowicz et al. 2009) were used for the amplification of the same region. In this second batch, the PCR reactions were carried out with the same amounts of DNA, primers and enzymes, with the following reaction steps: initial denaturation at 95°C for 3 minutes, 35 cycles at 94°C for 45 seconds, 50°C for 60 seconds and 72°C for 60 seconds; and a final extension phase at 72°C for 7 minutes.

The amplification of PCR products was tested visually by electrophoresis using 3 µL of each product on a 1.5% agarose gel, stained by Ethidium Bromide. After the visual verification, the PCR products were sent to Macrogen, South Korea for DNA sequencing. The accession numbers of the mitochondrial sequences used in the study are listed in APPENDIX B.

3.2.3. Amplification of the Nuclear Loci

For the exploration of nuclear data, a total of 17 microsatellite loci were tested at the first step; D15, C113, D9, A13, E24, G25, H19, H29 designed specifically for *M. myotis* (Castella & Ruedi 2000); and A2-Mluc, G31-Mluc, EF15-Mluc, G30-Mluc, G2-Mluc, G6-Mluc, H23-Mluc, and A24-Mluc designed for multiple bat species belonging to the family Vespertilionidae (Jan et al., 2012). The primers were dyed with 6-FAM and HEX fluorescent dyes.

The loci were amplified using a Roche AptaTaq Fast PCR Master according to the manufacturer's protocols (Roche Applied Science). The reactions were carried out in 10 µL volumes using 1 µL of template DNA, 2 µL AptaTaq Polymerase (5U/ µL), 0,5 µL of reverse and forward primers. The PCR amplification process contained the following steps: initial denaturation at 95°C for 30 seconds, 35 cycles at 94°C for 1 second and at 60°C for 25 seconds. Two thermocyclers (Bio-Rad) were used simultaneously for the amplification process. The PCR amplifications were first visually verified by electrophoresis with 1.5% agarose gel stained by Ethidium Bromide. Later, the products were sent to Microgen, South Korea for chromatography.

The markers H29 and H19 were discarded at the first step since they did not amplify for a significant number of samples. The markers D9, A13, E24, G31, G30, and B11, on the other hand,

were excluded from the analysis since they were either unreadable or had null alleles. The marker EF15 was also discarded because it proved to be fixed throughout all populations and thus uninformative. At the end, 8 markers were chosen to be used in further analysis as the markers giving the best results. The markers, primer sequences and relevant fluorescent dyes used for amplifying and dying the microsatellite regions used in this study are given in Table 3.1.

Table 3.1. Sequences and dyes used in amplification of microsatellite control regions

Marker	Primer Sequences	Dye
C113	F: ACCTCCCTGCCCTGCAC R: GCAATGCTTCCTCCAAGTCC	[6FAM]
A2-Mluc	F: TTTGTTGAGTAAATGAGTGGATGAATG R: GTCTCCCTCTCCCCTGGAAC	[6FAM]
G2-Mluc	F: TGAAAAGAACTGGAGAGGCTTT R: AGATTGATGAATGTGAAAGGTCAG	[6FAM]
G6-Mluc	F: GGCTTTTTGAAAAGACTGAGG R: ACATCAGCCAGTTCCTGTTC	[HEX]
G25	F: TCCTTCCCATTTCTGTGAGG R: CCATTTCATCCATCCAGTCC	[HEX]
H23-Mluc	F: TTGTCTACTAGCATTTGTCCAGTG R: ATAGCTATGTTGCCTAAC	[HEX]
A24-Mluc	F: GTGGTATGAAATAACCAGTTCACTTTG R: CAGACTGCATTACTGAAGAAATTATGG	[HEX]
D15 Mluc	F: AAATTCTTTCCCTCCAAAGTGG R: GCACGCTCAGACTCCTTCC	[HEX]

3.3. Analysis of the Data

3.3.1. Analysis of the Mitochondrial Control Region

After obtaining the sequences for the second hypervariable region of the mtDNA (HVII), these sequences were cleaned, aligned and trimmed manually, using Sequencher v. 4.1 (Gene Codes Corp.). To be able to compare the sequences with even the shortest fragments published in previous studies, a 291-base-pair fragment was used in the analyses. The mtDNA sequences were analyzed in conjunction with sequences acquired in several previous studies, adding up to a total of 470 individuals.

Arlequin 3.5 (Excoffier & Lischer 2010) software was used to evaluate the DNA polymorphism of the mitochondrial control region, utilizing indicators such as the nucleotide diversity. The haplotype network with reference to the mitochondrial control region is generated using the software TCS v. 1.21 (Clement, Posada and Crandall, 2000). Populations of *M. myotis* and *M. blythii* inhabiting the roosts in the Balkan countries and Turkey were analyzed separately to check for the relation between geographical and genetic distances. DnaSP v.5 (Librado & Rozas, 2009) software was used to calculate genetic distances between these populations.

3.3.2. Analysis of the Tandem Repeats in the Nuclear DNA

The species assignment according to the nuclear data was handled first, in order to be able to interpret the morphological and mitochondrial data based on an appropriate taxonomic classification of the specimens. The lengths of the tandem repeats in the nuclear DNA, also called the microsatellites, were scored manually using the software GeneMarker (Softgenetics). Afterwards, those scores were collapsed to repeat numbers using Flexibin (Amos et. al., 2007). The results were checked for the existence of null alleles and allelic drop-outs by Microchecker software (Oosterhout et al., 2004).

Most probable number of subgroups that are substantially different from each other according to the molecular data were determined using STRUCTURE v. 2.3.4; a software utilizing a Bayesian clustering approach for identifying K number of subpopulations from the molecular data set and assigning individuals to these subpopulations via posterior probabilities (Pritchard et al., 2000). Data from eight microsatellite loci were used in this analysis. Only the samples that multiplied in seven or all of the eight loci were included in the analyses (N=475).

When running Structure software for inferring the number of clusters, K was set from 1 to 6, and four simulations were run for each value of K. During the run, 30,000 burn-in length period and 60,000 run length with the admixture and correlated allele frequency model was used, in accordance with the recommendations of the developers (Pritchard, 2010). Five simulations were run for each K value. The most probable number of subpopulations/clusters were determined according to the method suggested by Evanno et al. (2005). For the calculations using this method and visual presentation of the results, an online tool, Structure Harvester, developed by Earl & vonHoldt (2012) was utilized.

After STRUCTURE analysis, NEW HYBRIDS (v. 1.1) software (Anderson & Thompson 2002) was used to identify potential hybrids and pure-bred individuals with regard to subgroups acquired by STRUCTURE. In this analysis, 50,000 generations following 10,000 burn-ins were run, using uniform priors. DISTRUCT v1.1 (Rosenberg, 2004) was utilized for the graphical display of summary data acquired by STRUCTURE and NEW HYBRIDS software.

The possible taxonomic sub-groups inferred by Bayesian statistics were later analyzed using Arlequin 3.5 (Excoffier & Licher 2010) with regard to their nuclear and mitochondrial genetic diversity. In the analysis of eight nuclear microsatellite loci, Analysis of Molecular Variance (AMOVA) with F statistics was used.

3.3.3. Analysis of the Morphological Data

The forearm lengths were analyzed separately for females and males, due to sexual dimorphism between the two sexes, which is evident in many species. After a statistical inquiry revealed that the sexual dimorphism was not effective in dental measurements, the CM^3 distances were analyzed for both sexes combined. The morphological measurements of samples from previous studies were included in the analysis only when their sex and geographical coordinates were available. The morphological data were divided into further subgroups in accordance with the group memberships acquired from the analyses of the nuclear data. The effects of taxonomic classification, sex and geographical coordinates on the Forearm Lengths and CM^3 distances were checked for, using general linear models (effect size estimates are given as partial eta-squared values).

For deriving a mathematical formulation for determining probability of group memberships based on morphological and geographical data, binary logistic regression models were utilized.

Principal component analysis was used to check for the contribution of different factors to the variance in morphological features. The statistical analysis of the morphometric data was handled with the SPSS statistics software (IBM Corp., 2013).

4. RESULTS

4.1. Analysis of the Overall Nuclear Genetic Data

4.1.1. Structure

The STRUCTURE analysis of the eight microsatellite regions from the nuclear DNA reveals that the Large Mouse-eared bats in the study area forms two distinct clusters. Meanwhile, the interpretation of the results according to Evanno's criteria shows that separating the overall population to three distinct clusters is almost equally helpful in explaining the variance in nuclear data (Table 4.1 & Figure 4.1).

Table 4.1. Evanno table output for STRUCTURE results including all samples that multiplied for at least seven microsatellite loci (N=475).

K	Reps	Mean LnP(K)	Stdev LnP(K)	Ln'(K)	|Ln''(K)|	Delta K
1	6	-14238.466667	0.307679	—	—	—
2	6	-12462.116667	1.022578	1776.350000	904.950000	884.968785
3	6	-11590.716667	0.966264	871.400000	829.583333	858.547032
4	5	-11548.900000	6.725325	41.816667	6.763333	1.005651
5	5	-11500.320000	7.191801	48.580000	52.380000	7.283294
6	5	-11504.120000	7.206386	-3.800000	40.080000	5.561734
7	5	-11548.000000	31.678699	-43.880000	—	—

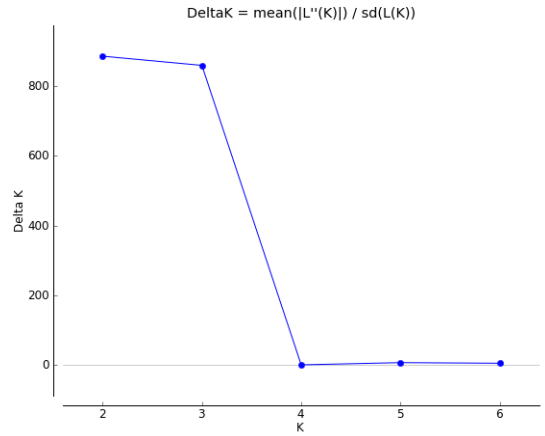


Figure 4.1. Graphic representation of Delta K values vs number of clusters.

The individual posterior probabilities (Q) of cluster membership acquired from STRUCTURE for two clusters (K=2) indicate that 139 of the samples belong to one group, and 327 to the other.

Nine individuals, on the other hand, are not assigned to any group with a high probability (Q < 0.75). While two of these nine individuals are from Central Europe, seven of them are from Eastern Europe and Turkish Thrace. The comparison of these group memberships with species associations made in the field shows that first group overlaps to a great extent with *Myotis blythii s.l.* and the second one with *Myotis myotis.* Both clusters contained members from all western Palearctic region, with the exception that no representatives of the greater form are found to the east of Central Anatolia (see Figure 4.2).

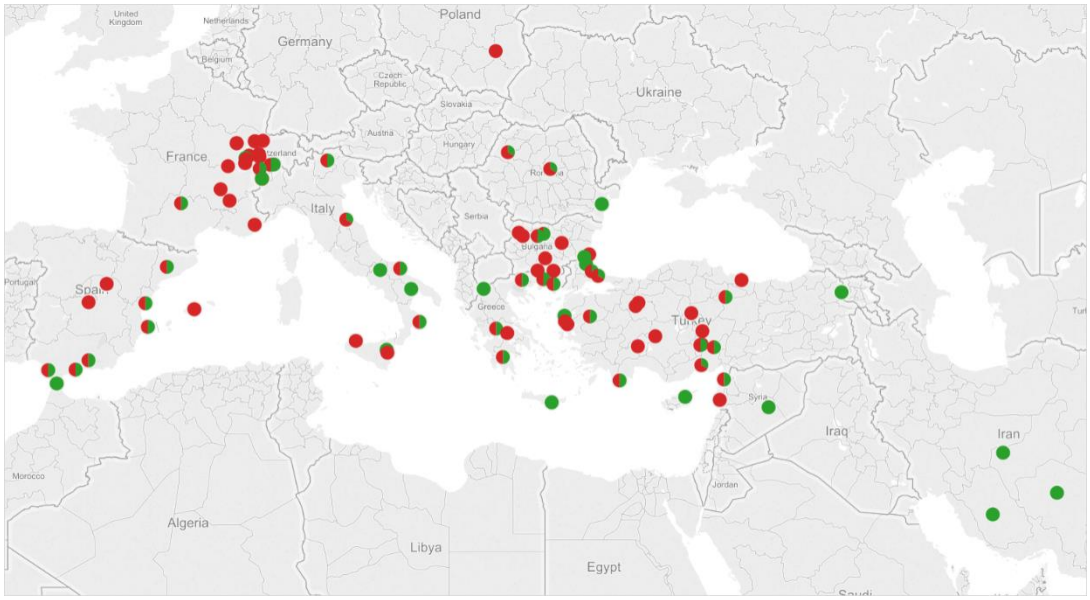


Figure 4.2. Distribution of *Myotis myotis* (red) and *Myotis blythii s.l.* (green) samples used in the study (with posterior probability of cluster membership (Q) > 0.75). The dots represent existence data and the sizes are not correlated to sample counts.

Since there is a minute difference between the Delta K values for K=2 and K=3, the posterior probabilities for group membership of three clusters were also analyzed, revealing a third distinct group within *Myotis myotis.* According to the individual posterior probabilities for K=3, the exact same 139 individuals that were assigned to Group 1 for K=2, were again included in a distinct cluster. While 160 and 163 of the remaining samples were assigned to second and third groups respectively, 2 individuals were assigned to neither of the groups, and 11 individuals' group memberships were ambiguous within the greater form (Q < 0.75). One of the unassigned individuals in this run is from central Europe (Italy), and the rest are from the contact zone of these groups -Bulgaria, Romania, and Turkey. First of the two groups within the greater form occupies only three countries: Turkey, Syria and Greece (limited to Lesvos Island which is in close vicinity of Anatolia). Meanwhile, the second group consists of individuals residing in all European countries except for longitudes to the east of Turkish Thrace (Figure 4.3).

Since the samples belonging to *M. blythii s.l.* were clearly isolated from the rest of the samples, a second STRUCTURE run was performed to make a clearer sense of the separation within the greater form. In this second run, the samples which belonged to the greater form with a high probability (Q > 0.75) according to K=2 or K=3 were included (N=334). This run indicated that existence of two clusters (K=2) was most likely as expected, again pointing out to a very similar separation within the greater mouse-eared bats. The clustering within the greater form revealed that this group was divided into Eastern and Western clusters, comprising 165 and 160 members, respectively. In this run nine individuals were not assigned to any group. The unassigned individuals in both STRUCTURE runs (with K=2 and K=3) were overlapping.

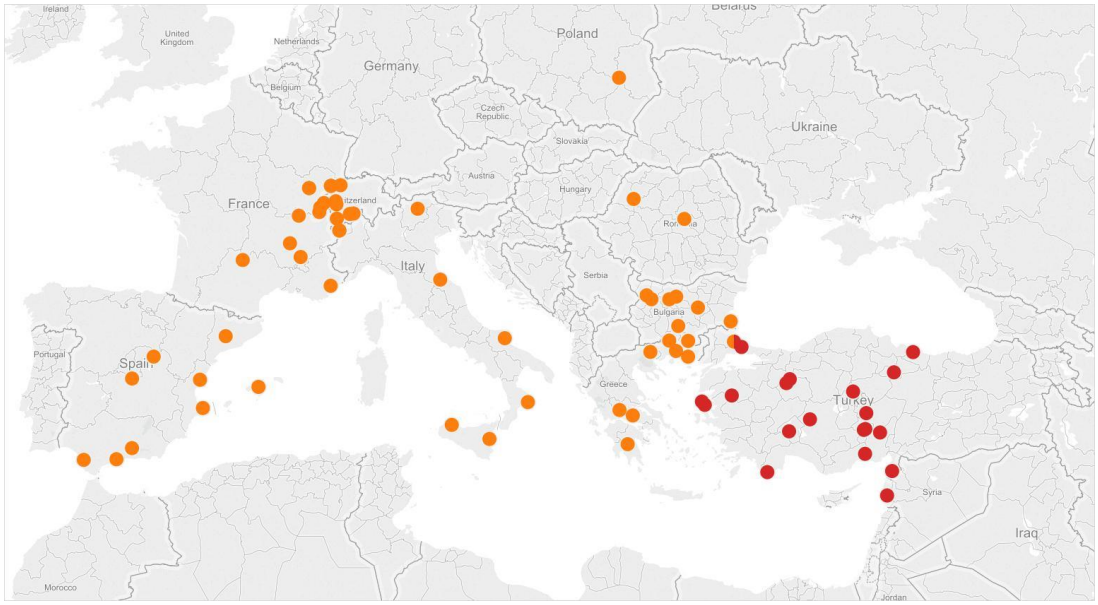


Figure 4.3. Distribution of eastern (red) and western (orange) lineages of *Myotis myotis* (with posterior probability of cluster membership (Q) > 0.75). The dots represent existence data and the sizes are not correlated to sample counts.

When STRUCTURE was run only for samples from Turkey, Balkan countries and Poland, the three clusters comprised exactly the same individuals as previous runs. Ambiguous individuals were again the same, with the exception that one individual previously unassigned, was included in one of the *M. myotis* clusters in this new run.

4.1.2. NewHybrids

In order to confirm cluster memberships and to identify possible hybrids of greater and lesser forms and the hybrids of two subgroups within the greater form, an analysis with NewHybrids was

performed. In this analysis, the samples assigned as pure-bred individuals belonging to the groups *M. blythii s.l.*, eastern cluster of *M. myotis* and western cluster of *M. myotis* west almost exactly the same as STRUCTURE results. Similarly, the individuals not assigned to any of the groups in STRUCTURE were overlapping with the ones not assigned to any categories (pure-bred, F1 or back-cross) by the NewHybrids.

According to NewHybrids analysis, none of the individuals identified as possible hybrids were F1 hybrids; and most were identified as back-cross hybrids (F1s hybridizing with pure-bred individuals). Only one out of 4 individuals with relatively high probabilities (> 0.6) of being a back-cross hybrid between the lesser and greater forms in NewHybrids is also not attributed to any group in any of the STRUCTURE runs. This individual from Turkish Thrace, according to our results, is the only probable evidence of hybridization between these species. When the two lineages of *M. myotis* were compared, NewHybrids results show that there is no individual with a relatively high probability of being a hybrid of western and eastern branches of *M. myotis*. Out of four ambiguous specimens not clearly assigned to any category by NewHybrids, three were assigned to one of the groups with a high probability (> 0.8) in STRUCTURE analysis, and only one individual's group membership was ambiguous according to both software. Since this single individual from Romania was not in the contact zone of the *M. myotis* lineages, we consider this individual to be a member of the western lineage. The bar representations of posterior probabilities are shown in Figure 4.4 and 4.5.

Figure 4.4. The graphical representation of posterior probabilities* assigned to individuals (*Myotis blythii s.l.* [green] vs *Myotis myotis* [red]).

**The length of colored bars are proportional to posterior probabilities assigned to cluster membership, for K=2 in STRUCTURE and pure bred and hybrid categories for Myotis blythii s.l. vs Myotis myotis in NewHybrids. Green and red colors represents Myotis blythii s.l. and Myotis myotis respectively. The blue color represents probability of being an F1 hybrid while other colors represents belonging to F2 and back-cross hybrid categories in NewHybrids. The bars are ordered according to the magnitude of probabilities and do not represent geographical distribution. Individuals with low posterior probabilities are scattered in different regions.*

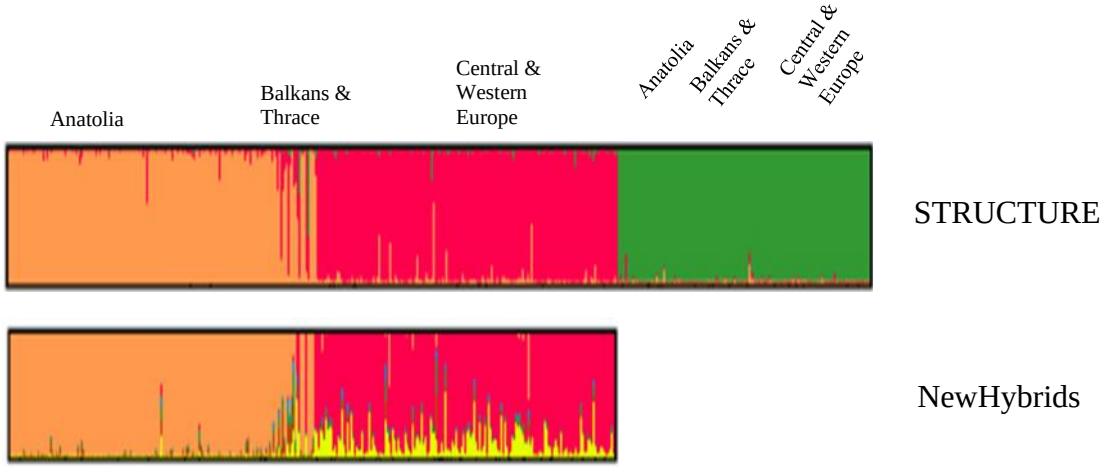


Figure 4.5. The graphical representation of posterior probabilities* assigned to individuals (*Myotis blythii s.l.*, *Myotis myotis* West and *Myotis myotis* East).

**The length of colored bars are proportional to posterior probabilities assigned to cluster membership, for K=3 in STRUCTURE and pure bred and hybrid categories for Eastern and Western clades of Myotis myotis in NewHybrids. Green, red and orange colors represents M. blythii s.l., western M. myotis and eastern M. myotis, respectively. The blue color represents probability of being an F1 hybrid while other colors represents belonging to F2 and back-cross hybrid categories in NewHybrids. The bars are ordered according to longitude (from East to West) for Myotis myotis*

4.1.3. Nuclear Genetic Diversity

Seven loci for both lineages of the greater form (*Myotis myotis*) are highly polymorphic, except for C113 which is fixed for all samples but one (this polymorphic individual was assigned to the western clade of *M. myotis* with a high posterior probability). Similarly, seven loci (except for G25) are highly polymorphic for *Myotis blythii s.l.*

While the fixation index (F_{st}) between the greater and lesser forms varies from 0.021 to 0.793 for individual loci, the overall F_{st} is 0.224. On the other hand, the individual fixation indexes for the lineages of greater form vary between 0 (for the fixed allele) and 0.447. The overall F_{st} for eastern and western lineages of the greater form is 0.197, which is almost as high as the fixation index between *Myotis myotis* and *Myotis blythii s.l.* This fact also supports the existence of two distinct clades within the greater form. The haplotype diversity and F_{st} values for the three taxa are shown in 4.2 and 4.3)

Table 4.2. Locus-based genetic diversity and F statistics for *Myotis blythii s.l.* vs *Myotis myotis*. N_a represents number of unique haplotypes; H represents Haplotype diversity and F_{st} represents Fixation index.

	Myotis blythii s.l.		***Myotis myotis***		
	n=139		**n=325**		
Locus	**N_a**	**H**	**N_a**	**H**	**F_{st}**
C113	3	0.482	2	0.003	0.793
A2-Mluc	14	0.842	11	0.452	0.288
G2-Mluc	17	0.694	17	0.695	0.046
G6-Mluc	11	0.783	7	0.512	0.104
G25	7	0.182	9	0.664	0.398
H23-Mluc	18	0.849	17	0.854	0.021
A24-Mluc	19	0.854	13	0.497	0.172
D15-Mluc	17	0.790	19	0.789	0.079
Overall					**0.224**

Table 4.3. Locus-based genetic diversity and F statistics for eastern and western clades of *Myotis myotis*. N_a represents number of unique haplotypes; H represents Haplotype diversity and Fst represents Fixation index.

	Myotis myotis (East)		***Myotis myotis (West)***		
	n=160		**n=165**		
Locus	**N_a**	**H**	**N_a**	**H**	**F_{st}**
C113	1	0.000	2	0.006	0.000
A2-Mluc	3	0.125	11	0.770	0.447
G2-Mluc	7	0.579	17	0.811	0.066
G6-Mluc	4	0.425	7	0.598	0.292
G25	8	0.606	8	0.720	0.135
H23-Mluc	13	0.829	16	0.879	0.025
A24-Mluc	11	0.606	11	0.390	0.378
D15-Mluc	11	0.739	18	0.836	0.065
Overall					**0.197**

4.2. Morphology

4.2.1. Forearm Lengths

The forearm lengths of *Myotis blythii s.l.* samples varied between 53 mm and 63.80 mm, and the average FA length was 58.73 mm (± 2.18 mm). On the other hand, the FA lengths of eastern *Myotis myotis* varied between 58.80 mm and 71.30 mm, while the FA lengths of western *Myotis myotis* varied between 57.16 mm and 65.86 mm. The average FA lengths for eastern and western

lineages of *Myotis myotis* were 64.70 mm (± 2.16 mm) and 61.80 mm (± 2.09 mm), respectively Table 4.4).

Table 4.4. FA lengths* of *Myotis blythii s.l. and Myotis myotis.*

	Sex	n	Mean	SD	Min	Max
Myotis blythii	**F**	64	59.50469	2.203794	53	63.8
	M	57	57.85667	1.801939	53.8	60.4
	Total	121	58.72835	2.178851		
Myotis myotis West	**F**	94	62.77223	1.610225	58.87	65.86
	M	49	59.94449	1.5808	57.16	63.2
	Total	143	61.80329	2.087244		
Myotis myotis East	**F**	77	65.98429	1.873341	60.55	71.3
	M	83	63.50096	1.681579	58.8	68.75
	Total	160	64.69606	2.164487		

**All measurements are in millimeters. n is sample size and SD is the standard deviation*

The forearm length measurements show that the average FA length of *Myotis blythii s.l.* varies significantly from both eastern and western subpopulations of *M. myotis* ($P < 0.01$). Meanwhile, the average forearm length of eastern subpopulation of *M. myotis* is significantly higher than the western population as well (see figures 4.6 and 4.7).

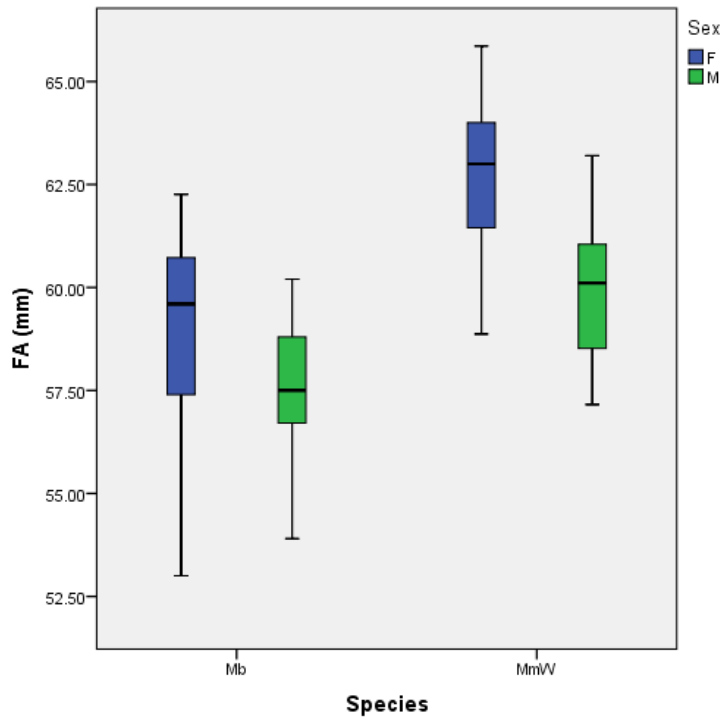


Figure 4.6. Boxplot representation of the forearm length comparison between *M. blythii s.l.* and western branch of *M. myotis* (below 26 degrees longitude).

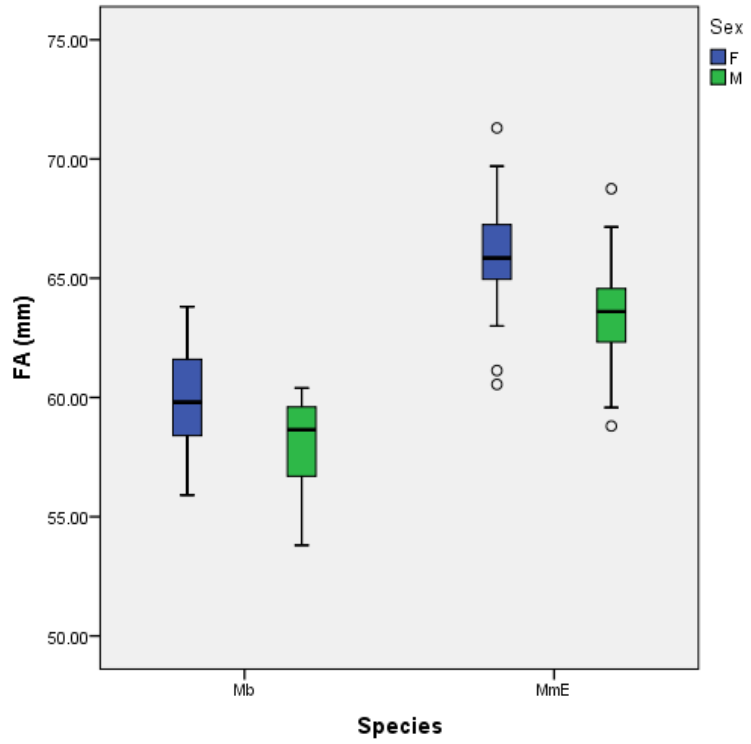
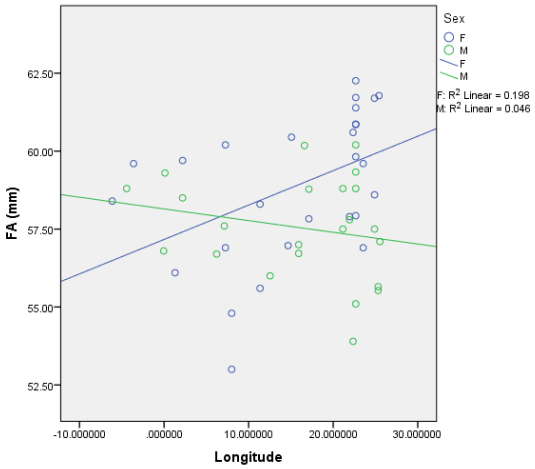


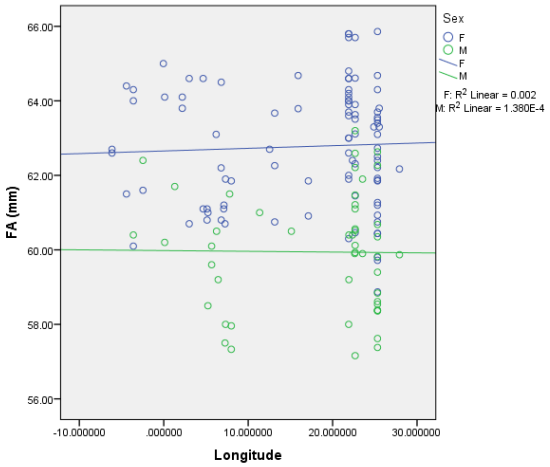
Figure 4.7. Boxplot representation of the forearm length comparison between *M. blythii s.l.* and eastern branch of *M. myotis* (above 26 degrees longitude).

The FA length also shows a gradual increase (see Figures 4.8) eastward for *M. blythii s.l.* in Anatolia and the eastern *M. myotis*, in accordance with the findings of former studies (Benda & Horacek, 1995; Furman et al, 2014). Notwithstanding, the FA length for the western *M. myotis* remains almost constant with respect to longitude. The trend of FA length of *M. blythii s.l.* to the west of 26th meridian (to the west of Turkish Thrace) is not clear, as females tend to have higher forearm length measurements while the FA length for the males tend to get shorter from west to east. We believe that this controversial situation might have arisen from inadequate/poor sampling and inclusion of some juvenile male specimens.

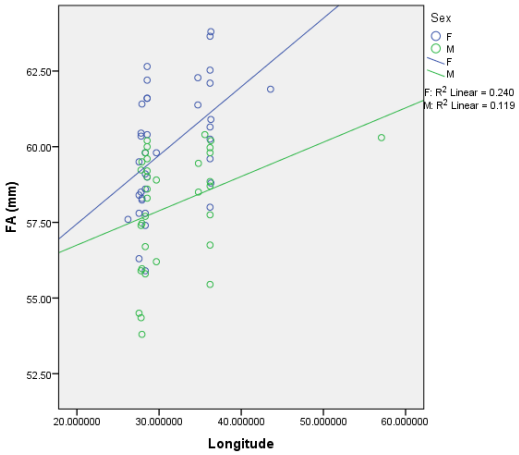
Myotis blythii s.l. (Below 26 degrees longitude)*



*Myotis myotis west**



Myotis blythii s.l. (Above 26 degrees longitude)



Myotis myotis east

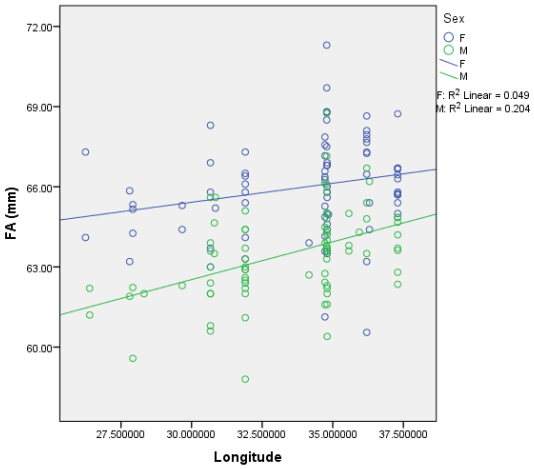


Figure 4.8. Change in forearm length with longitude.

**The first two graphs are included in order to represent all relevant data in continuum, but it is important to note that the these graphs might not be representing the actual situation due to insufficient number of samples and poor sampling methods used in western Europe, as confirmed by the researchers providing the data from the region. P values for all samples in Anatolia and female M. blythii s.l. are less than 0.05).*

The statistical analysis of the morphological data gathered from the area we focused on (above 26 degrees longitude), reveals that the change in longitude accounts for 45.9% of the change and sex accounts for 27.8% of the change in FA length for the lesser form (*Myotis blythii s.l.*) For the greater form, these percentages are 34.9% and 16.5%, respectively.

The linear regression model shows that, for the lesser form, the average increase in FA length per one degree of longitude change is approximately 0.23 mm for females and 0.11 mm for males. For the greater form, the FA length increase per one degree of longitude is 0.14 mm for females and 0.28 mm for males. The regression line equations for average FA length for the lesser form are:

FA = 52.923 + (0.226 x Degrees Longitude) for males, and

FA = 53.483 + (0.113 x Degrees Longitude) for females.

For the greater form, the equations for the average FA lengths are as follows:

FA = 61.158 + (0.142 x Degrees Longitude) for males, and

FA = 54.041 + (0.283 x Degrees Longitude) for females.

4.2.2. CM^3 distances

The average length between the canine and the 3rd molar teeth are smallest for *M. blythii s.l.* Similar to the FA measurements, there is also a significant difference between eastern and western subgroups of *M. myotis*, eastern specimens having significantly longer CM^3 distances $P < 0.01$).

Table 4.5. CM^3 distances* of *Myotis blythii s.l.* and *Myotis myotis*

	Sex	n	Mean	SD	Min	Max
Myotis blythii	**F**	49	8.735714	0.437283	7.6	10
	M	47	8.827872	0.466807	7.36	9.53
	Total	96	8.780833	0.451966		
Myotis myotis West	**F**	88	9.814659	0.426881	8	10.52
	M	45	9.939111	0.450201	8.77	10.9
	Total	133	9.856767	0.437217		
Myotis myotis East	**F**	70	10.33186	0.338955	9.19	10.95
	M	72	10.31222	0.296782	9.38	10.95
	Total	142	10.3219	0.317289		

*All measurements are in millimeters. n is sample size and SD is the standard deviation

The mean CM^3 distance for *M. blythii s.l.* is 8.78 mm (± 0.45 mm) while the mean CM^3 distances are 9.86 mm (± 0.44 mm) and 10.32 mm (±0.32 mm) for western and eastern subgroups of *M. myotis*, respectively (see Table 4.5.). The average CM^3 distances again show that *M. blythii s.l.* varies significantly from *M. myotis*, both in eastern and western regions ($P < 0.01$) in this morphological trait (see figures 4.9 and 4.10).

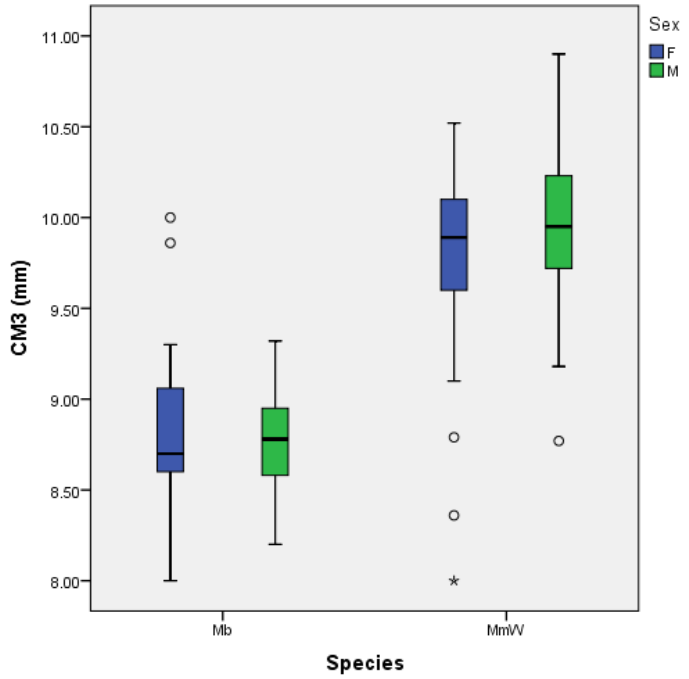


Figure 4.9. Boxplot representation of the CM^3 length comparison between *M. blythii s.l.* and western branch of *M. myotis* (below 26 degrees longitude).

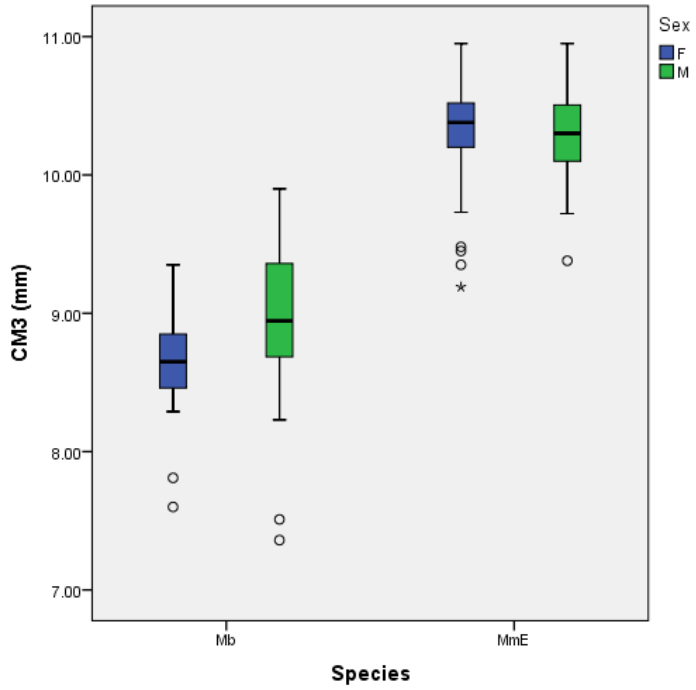
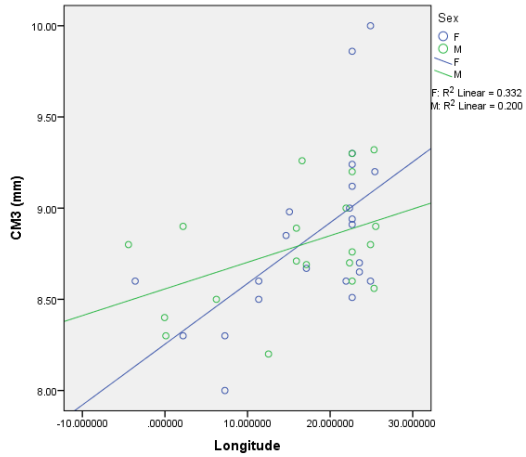


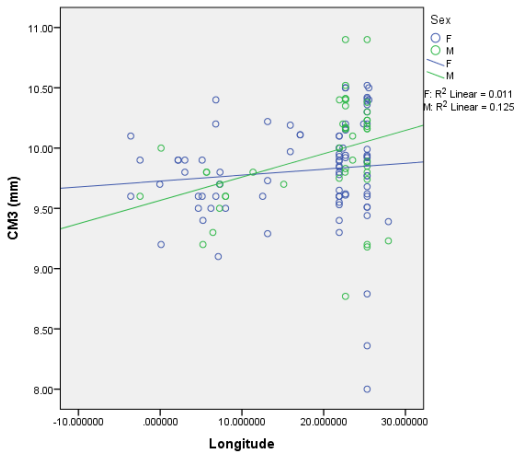
Figure 4.10. Boxplot representation of the CM^3 distance comparison between *M. blythii s.l.* and eastern branch of *M. myotis* (above 26 degrees longitude).

The difference between The CM^3 distances, just like forearm lengths, tend to have a gradual increase from west to east (see Figure 4.11), with the exception that the western lineage of *M. myotis* does not show a prominent increasing trend below 26 degrees longitude.

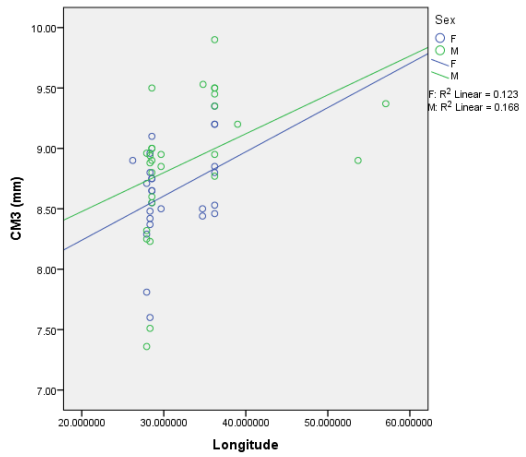
Myotis blythii s.l. (Below 26 degrees longitude)



Myotis myotis west



Myotis blythii s.l. (Above 26 degrees longitude)



Myotis myotis east

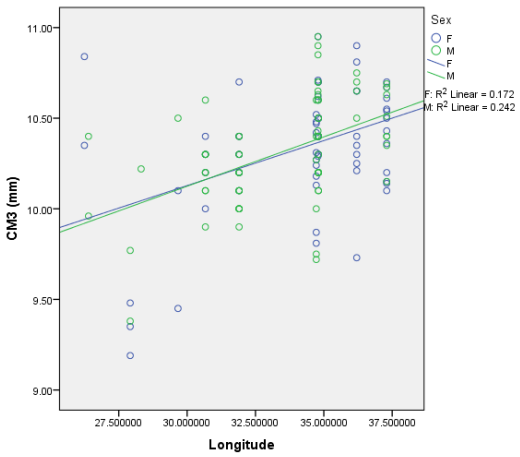


Figure 4.11. Change in CM^3 distance with longitude.

**The first two graphs are included in order to represent all relevant data in continuum, but it is important to note that these two graphs might not be representing the actual situation due to insufficient number of samples and poor sampling methods used in western Europe, as confirmed by the researchers providing the data from the region. P values are less than 0.05 for all but western M. myotis.*

For the specimens collected from the east of 26 degrees longitude, our statistical analyses show that the 54.9% of the variance in CM^3 distance can be explained by change in longitude for the greater form. This ratio is 47.3% for the lesser form. The distance between the canine and the third molar teeth increases by 0.052 mm per one degree of longitude for the lesser form, and 0.036 mm per one degree of longitude for the greater form. The linear regression model reveals that the average CM^3 distance follows the lines with the following equations:

8.578 + (0.052 x Degrees Longitude) for the lesser form, and

7.628 + (0.036 x Degrees Longitude) for the greater form.

4.2.3. Other Morphological Features

Cryptic species, by definition, are very hard -if not impossible- to differentiate from one another merely by visual indicators, and *M. myotis* and *M. blythii s.l.* are no exception to this. One of the visual clues offered by some researchers for the identification of the lesser form (i.e. *M. blythii s.l.*) in the field was a white spot between the ears (Arlettaz et al., 1991). During our field studies, most of the bats belonging to this group, as confirmed by nuclear studies later, showed no sign of such a spot. This trait is probably a local feature of the lesser mouse-eared bats inhabiting a limited region in Europe and hence is not applicable for species identification in the whole span of this species' distribution.

Another visual indicator suggested for identifying greater form of mouse-eared bats was a dark spot at the tip of the tragus (Dietz & von Helversen, 2004). This spot was also missing in most of the specimens collected from Anatolia and later assigned to the greater form by nuclear markers. This spot again might be a local trait, or a trait that might be lost by the eastern subgroup or gained by the western subgroup after they diverged from each other. Whatever the reason is, this trait also proves inapplicable for on-the-field species delimitation.

During the course of this study, high resolution photographs of the captured bats were used in order to look for similar visual clues that would help differentiate the lesser and greater forms, and no such morphological feature was discovered. These facts led us to the conclusion that there is probably no way of discriminating lesser and greater forms of Large Mouse-eared bats from one another just by looking at them.

4.2.4. Species delimitation by morphometric measurements

The only method for the precise identification of greater and lesser forms of Large Mouse-eared bats is utilizing nuclear markers (Arlettaz et al., 1997b; Berthier et al., 2006). But this method depends on tissue sampling, laboratory work and analysis, making it very difficult to make quick identifications in the field for surveys related to population dynamics, biodiversity, conservation, etc. So, it is important to have an easier method to discriminate these species on the field.

There is but one obvious physical difference between the lesser and greater forms within the Large Mouse-eared bats: as the nomenclature implies, the average size of the lesser form is smaller than the other. Nevertheless, factors such as the age of the individual bats, sexual dimorphism, and

clinal change in size with longitude complicates this identification, rendering mere visual identification on the field unreliable. So, a proper species delimitation by morphometric data requires making precise measurements and combining multiple variables.

The first method to differentiate between the two species by morphometric measurements was suggested by Arlettaz and colleagues. (1997b). The discriminant function offered by these authors included forearm and ear measurements, and accurately identified 98% of the individuals. But this study covered specimens collected from a limited geographical area. Because of the considerable change in the dimensions of the bats with longitude, this method is not applicable for the bats in a wider scope, and particularly proved unreliable for identification of these species in Anatolia. Also, in these early studies, the intermediate sized bats were excluded in the field in order to discriminate clearly between the two forms and the high accuracy of the method may be reflecting this bias in sampling.

As part of an effort to find a reliable method for species delimitation based on physical traits, during our early field studies, morphometric measurements such as ear length, tragus length and C^1-C^1 distance (distance between the two canine teeth of the upper tooth raw) were also taken along with the forearm and CM^3 (distance between the canine and the third molar). The comparison of these measurements across taxonomic classifications based on nuclear markers, revealed that only forearm and CM^3 distance showed significant difference among different species/subgroups. Hence, measurements other than these two were discontinued and dropped from the analysis.

We performed Principal Component Analysis for CM^3 distance and FA length to reveal the variance structure with regard to morphological data. Since longitude is an effective factor on the morphological measurements, we eliminated the effect of clinal change from the data by using residual distances from the regression line both for FA length and CM^3 distance. According to this analysis that included the morphometric data from a total of 194 individuals , only one factor was enough to explain 86.5% of the total variance. The scatter-plot graphic of the regression scores colored according to species memberships reveals that the morphological data can separate the two species almost perfectly (Figure 4.12).

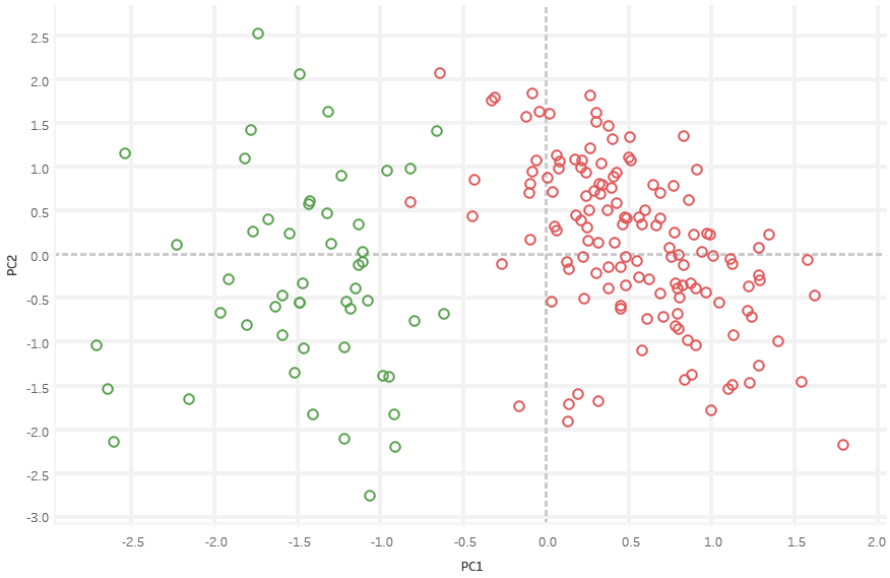


Figure 4.12. The scatter-plot distribution of the regression scores from the Principal Component Analysis for FA length and CM^3 distance. Green dots represent the lesser form and the red dots represent the greater form in Anatolia.

In order to derive a reliable discriminant function for identification of these species who cohabit the same roosts and whose sizes vary according to longitude and sex, we included all relevant features in a binary logistic regression model. The analyses were conducted only for specimens that were collected from the Turkish Thrace and Anatolia (above 26 degrees longitude) and were assigned to either lesser or greater form with high posterior probabilities by STRUCTURE software.

When the forearm lengths of both species are analyzed with a general linear model, it is shown that the difference between species accounted for 43.8% of the variance in forearm length ($p < 0.001$). The contribution of longitudinal change to this variance is 32%, and the contribution of sex is 20.7% ($p < 0.001$ for both). For CM^3 distances, these percentages were 61.9% for differences between species and 47.4 for change in longitude ($p<0.001$ for both). Sex had no significant effect on CM^3 distance ($p > 0.5$).

According to the binary logistic regression model for FA length and longitude, logarithmic probability of belonging to either of the groups can be derived from the following formulae:

Males: **log (p/1-p) = -151.514 - (0.149 x Degrees Longitude) + (2.591 x FA)**
Females: **log (p/1-p) = -96.972 - (0.33 x Degrees Longitude) + (1.565 x FA)**

Table 4.6. The resulting classification table and included variables* of the logistic regression model for forearm lengths of male individuals.

			Predicted		
			Species		Percentage
	Observed		Mb	MmE	Correct
Step 1	Species	Mb	33	1	97.1
		MmE	3	80	96.4
	Overall Percentage				96.6

		B	S.E.	Wald	df	Sig.	Exp(B)
Step 1	EW	-.149	.144	1.060	1	.303	.862
	FA	2.591	.754	11.796	1	.001	13.347
	Constant	-151.514	44.358	11.667	1	.001	.000

** Mb and MmE represents the smaller and greater (eastern) forms. EW stands for Degrees Longitude and FA stands for the forearm length. The model is constructed in the SPSS software.*

Table 4.7. The resulting classification table and included variables* of the logistic regression model for forearm lengths of female individuals.

			Predicted		
			Species		Percentage
	Observed		Mb	MmE	Correct
Step 1	Species	Mb	34	3	91.9
		MmE	2	75	97.4
	Overall Percentage				95.6

		B	S.E.	Wald	df	Sig.	Exp(B)
Step 1	EW	-.033	.120	.073	1	.787	.968
	FA	1.565	.357	19.164	1	.000	4.783
	Constant	-96.972	22.377	18.781	1	.000	.000

** Mb and MmE represents the smaller and greater (eastern) forms. EW stands for Degrees Longitude and FA stands for the forearm length. The model is constructed in the SPSS software.*

The cutoff value was set to 0.5, meaning that when the probability ($p = e^{\ln(p/1-p)} / 1 + e^{\ln(p/1-p)}$) for an individual is above 0.5 the individual is assigned to the greater form, and if it is below this value, the individual is assigned to the lesser form. This model assigned 95.6% of female individuals and 96.6% of male individuals correctly (Table 4.6 and 4.7).

Three of 83 males belonging to the greater form were erroneously assigned to the lesser form, and 1 of 34 males belonging to the lesser form was erroneously assigned to the greater form. It is worth mentioning that p values of two out of four individuals erroneously assigned to the wrong group were the closest values to the cutoff value (0.45 and 0.55). For females, 3 out of 37 individuals belonging to the lesser form and 2 out of 77 individuals belonging to the greater form were assigned

to the wrong group. One out of these 5 erroneous assignments, one was again in the 0.45 - 0.55 range. Omitting the individuals with probability scores closest to the cutoff value may result in a more reliable species delimitation.

Since there is no significant difference in CM^3 distances among sexes, sex was not included as a variable in the binary logistic regression model including CM^3 distances and longitudes. This second model was able to assign individuals more precisely than the FA length. Out of 196 individuals (54 belonging to the lesser form and 142 belonging to the greater form), 192 were correctly assigned. This yields a correct assignment rate of 98% (Table 4.8). Two individuals from each group were assigned erroneously. The individual probabilities of group membership were derived from the below formula:

$\log (p/1-p) = -90.66 - (0.737 \times \text{Degrees Longitude}) + 12.051 \times CM^3 \text{ distance}$

Table 4.8. The resulting classification table and included variables* of the logistic regression model for CM^3 distances.

			Predicted		
			Species		Percentage
	Observed		Mb	MmE	Correct
Step 1	Species	Mb	52	2	96.3
		MmE	2	140	98.6
	Overall Percentage				98.0

		B	S.E.	Wald	df	Sig.	Exp(B)
Step 1	EW	-.737	.293	6.311	1	.012	.479
	CM3	12.051	3.321	13.167	1	.000	171217.228
	Constant	-90.660	24.594	13.588	1	.000	.000

** Mb and MmE represents the smaller and greater (eastern) forms. EW stands for Degrees Longitude and CM3 stands for the distance between the molar and third canine teeth. The model is constructed in the SPSS software.*

4.3. The Analysis of the Mitochondrial Genetic Data

4.3.1. Overview

The 470 specimens, for which the second hypervariable region (HVII) of the mitochondrial DNA were successfully amplified, had 128 unique haplotypes for the 291-base-pair-long sequence examined. The haplotype network of all mitochondrial haplotypes is given in Figure 4.13.

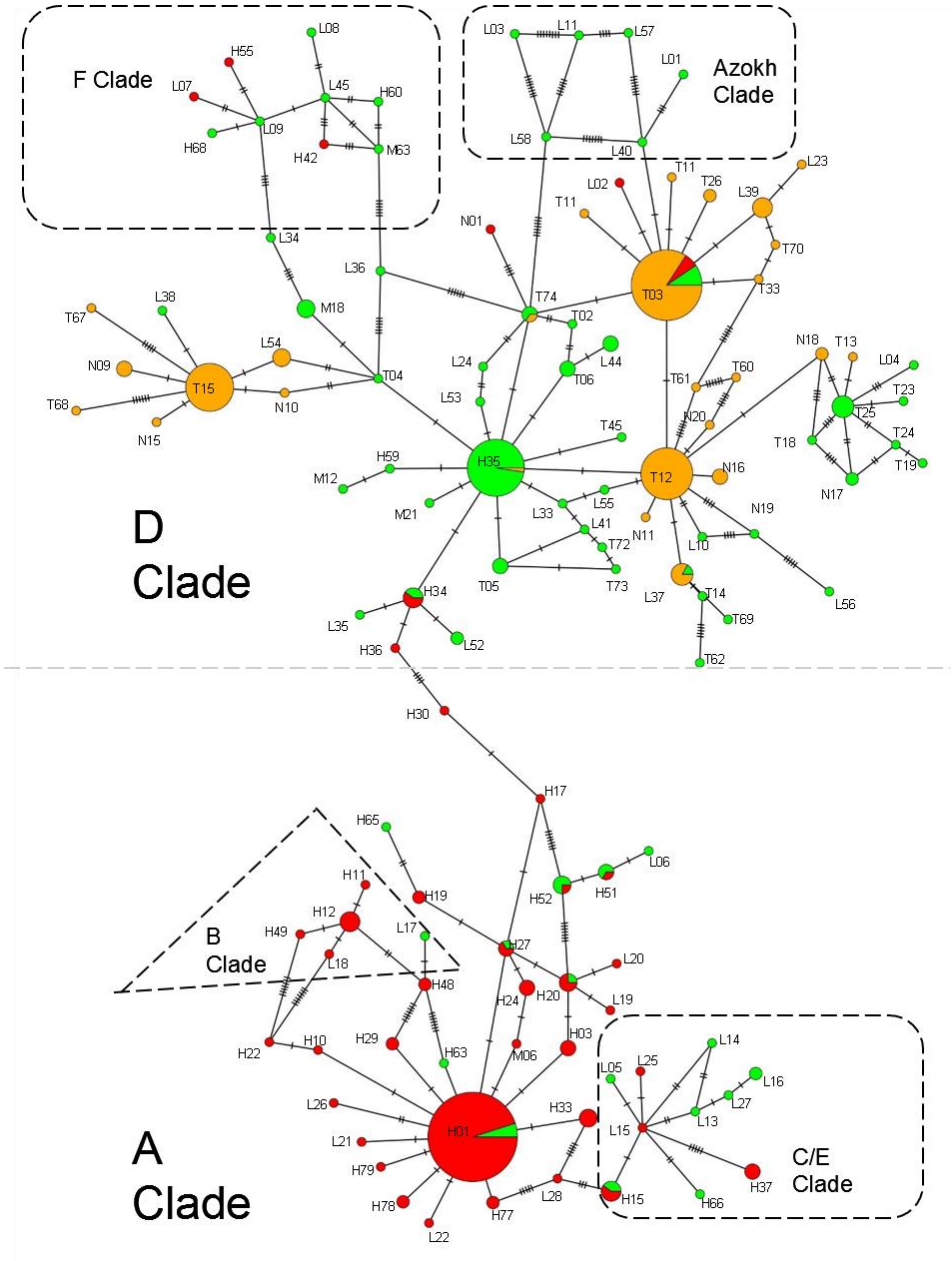
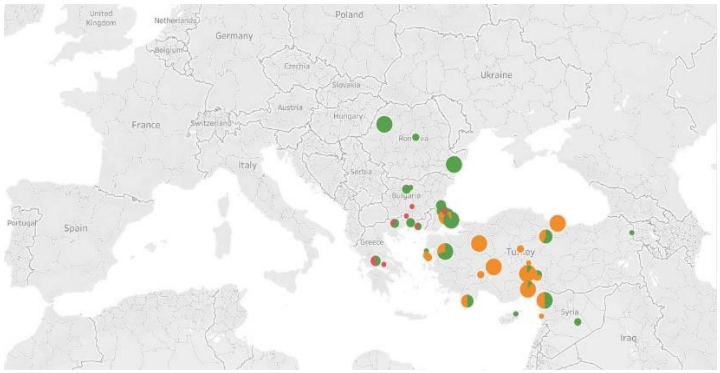


Figure 4.13. The haplotype network of all mitochondrial haplotypes according to maximum parsimony. Network is acquired by TCS software (Clement et. al., 2000). *Myotis blythii s.l.*, western clade of *Myotis myotis* and eastern clade of *Myotis myotis* are represented with green, red and orange color, respectively.

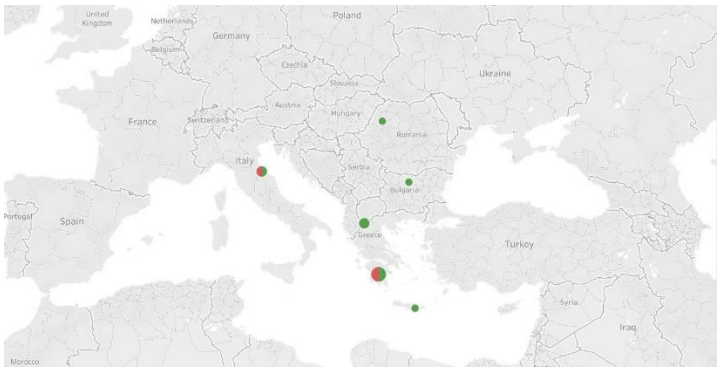
The breakdown of these haplotypes according to species, clade memberships and locations as suggested in current literature, are given in Table 4.9. Eleven of the haplotypes were new haplotypes that were not reported in previous studies, and all of these new haplotypes were part of the D clade. Two of these new haplotypes belonged to western *M. myotis* from Bulgaria, two belonged to *M. blythii s.l.* from Hatay Province and the rest belonged to eastern *M. myotis* from Central Anatolia. The sequences of these haplotypes are listed in APPENDIX C.

Clade A

Clades B/C/E



Clade D



Clade F

Figure 4.14. Geographical and species distribution of HVII Clades. *M. blythii s.l* is shown with green, eastern (MmE) and western (MmW) branches of *M. myotis* are shown with orange and red, respectively. The Azokh clade is not included in these maps as it is restricted to Iran. The maps are created by the Tableau Software v 2018.1.1 (www.tableau.com).

The geographical distribution of clades (Figure 4.14) reveals that it does not reflect the distribution of species, but certain clades are peculiar to certain regions. A, B, C/E are native to western and central Europe, while D clade is native to Balkans and Anatolia. A clade can be encountered all over the European continent and the clades B, C/E seem to be peculiar to Apennine Peninsula and the Alps. While the F clade is seen around the Adriatic and in some Balkan countries, the Azokh Clade is represented only by *M. blythii s.l.* residing in Iran. The distribution of mitochondrial haplotypes are given in Table 4.6.

Table 4.9. Distribution of unique haplotypes according to taxonomic units, clades and locations.

Taxonomic Unit (nH)	**Clades (nH)**	**Location**
Myotis myotis west (44)	Clade A (24)	Spain, France, Switzerland, Poland, Romania, Bulgaria, Greece
	Clade B (7)	Italy, Switzerland
	Clade C/E (5)	Italy, Switzerland, Bulgaria, Greece
	Clade D (5)	Bulgaria, Greece, Turkey
	Clade F (3)	Italy, Greece
Myotis blythii s.l. (70)	Clade A (5)	Spain, France
	AZOKH (4)	Iran
	Clade B (4)	Italy
	Clade C/E (7)	Italy, Switzerland
	Clade D (42)	Romania, Bulgaria, Cyprus, Greece, Turkey, Syria, Iran
	Clade F (8)	Italy, Romania, Bulgaria, Greece
Myotis myotis east (27)	Clade D (27)	Greece, Turkey, Syria

Myotis blythii s.l. and western *M. myotis* has the highest number (8) of shared haplotypes (H34 and T03 belonging to Clade D; H01, H20, H27, H51 and H52 belonging to Clade A; and H15 blonging to Clade C/E). T3 is the only haplotype shared by all three taxonomic units, which is also the sole haplotype shared between western and eastern lineages of *M. myotis*. The number of haplotypes shared by *Myotis blythii s.l.* and the eastern form of *M. myotis* is 3 (T03, T74, and L37). As can be seen from the clade distribution given in Figure 4.15, eastern lineage of *M. myotis* consists only of one Clade (D).

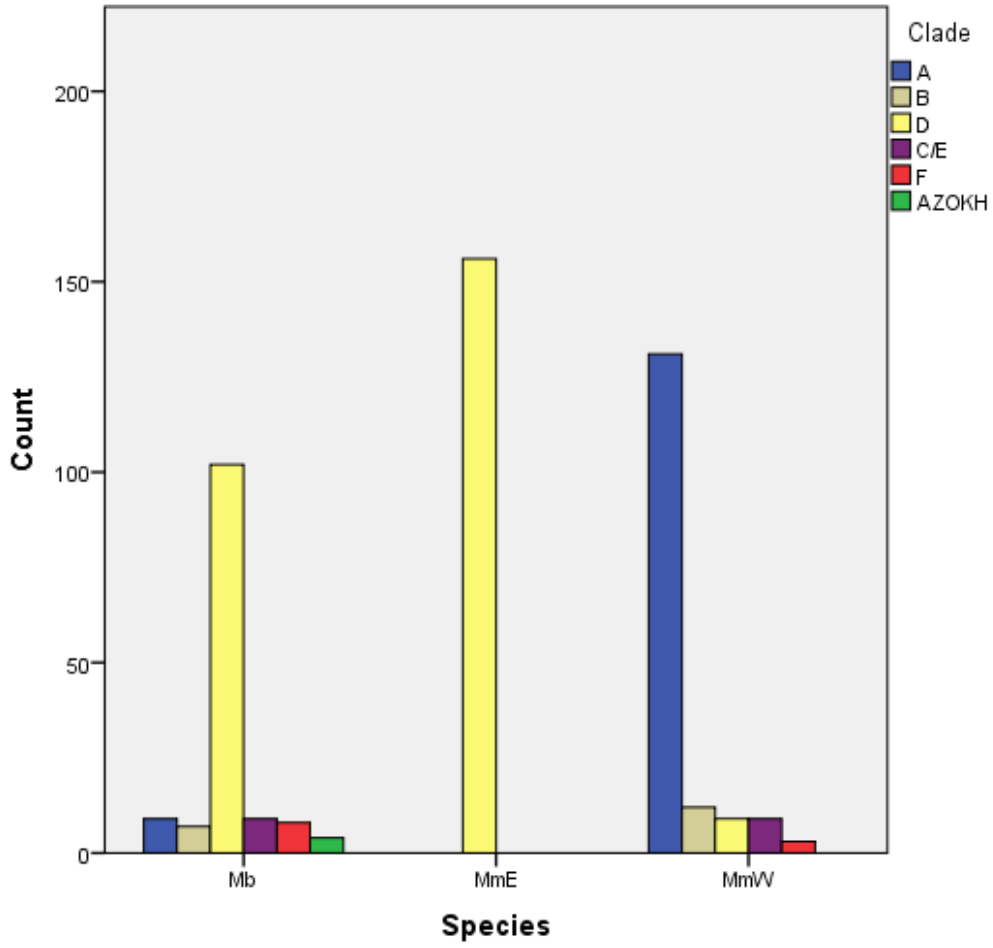


Figure 4.15. Bar chart representation depicting the distribution of mitochondrial clades among species/taxonomic units comprising Large Mouse-eared bat complex.

The Φ Statistics calculated by Arlequin Software reveals that the fixation index between *Myotis blythii s.l.* and the eastern lineage of *M. myotis* is relatively low, (Φ_{st} = 0.128) while the same score is much higher between *M. blythii s.l.* and western *Myotis myotis*, and between the western and eastern lineages of *M. myotis* (with Φ_{st} scores 0.41 and 0.60, respectively). The overall fixation index between

M. blythii s.l. and all *M. myotis*, on the other hand, is 0.119. This low score indicates that the lesser and greater forms share several haplotypes from different clades where they cohabit, both in the east and in the west.

M. blythii s.l., occupying a large area expending from Iberian Peninsula to Iran, has the highest number of haplotypes (70 haplotypes) and highest haplotype diversity (Hd = 0.917) with 60 polymorphic sites and a total of 63 mutations in these sites. The eastern lineage of *M. myotis* that is mostly confined to Anatolia and Levant, has the least number of haplotypes (H = 27) with a relatively high haplotype diversity score of 0.808, 34 polymorphic sites and 34 mutations. The western *M. myotis* inhabiting almost all European continent has 41 different haplotypes but the lowest haplotype diversity (Hd = 0.655) with 43 polymorphic sites and 43 mutations (see Table 4.10).

Table 4.10. Number of haplotypes, Haplotype diversities, Nucleotide diversities, and pairwise Φ_{st} values***** for the three taxonomic units

***M. blythii s.l.* (n=139)**			***M. myotis* (East) (n=156)**			
H	**Hd**	**Nd**	**H**	**Hd**	**Nd**	**Φ_{st}**
70	0.917	0.024	27	0.808	0.012	0.12838
***M. blythii s.l.* (n=139)**			***M. myotis* (West) (n=164)**			
H	**Hd**	**Nd**	**H**	**Hd**	**Nd**	**Φ_{st}**
70	0.917	0.024	41	0.655	0.014	0.41108
***M. myotis* (East) (n=156)**			***M. myotis* (West) (n=164)**			
H	**Hd**	**Nd**	**H**	**Hd**	**Nd**	**Φ_{st}**
27	0.808	0.012	41	0.655	0.014	0.60209

* H: Number of unique haplotypes, Hd: Haplotype diversity, Nd: Nucleotide diversity, and Φst: Fixation index

The low haplotype diversity of western *M. myotis* arises from the fact that more than half of the western *M. myotis* specimens share H01 haplotype. H01 is also the most common haplotype along all samples, distributed from the Iberian Peninsula to the Balkans, with 102 representatives (97 of which are western *M. myotis* and 5 are M. blythii s.l.) The second most common haplotype is T03, which is encountered only in Turkey and Greece, and shared by 52 eastern *M. myotis*, four *M. blythii s.l.*, four western *M. myotis*, and four of the unidentified specimens. Other most common haplotypes are H35 (one eastern *M. myotis*, two unidentified species and rest are *M. blythii s.l.*), and T12 (34 eastern *M. myotis*).

The haplotype network (Figure 4.13) and distribution maps based on the mitochondrial HVII region show that certain clades are restricted to certain geographical regions, and clade memberships do not overlap with species memberships assigned by nuclear markers. The A clade includes most ancestral haplotypes in the western part of the distribution, and mostly represented by the specimens belonging to the western lineage of *M. myotis*. Only eight *M. blythii s.l.* that belong to this clade, are close to the western end of this species' distribution range (Spain and France). Meanwhile, the nine individuals of western *M. myotis* that have haplotypes belonging to Clade D -which includes the ancestral haplotypes for the eastern lineage- are at the eastern end of this species' distribution range (Greece, Bulgaria and the Turkish Thrace).

The haplotypes in B and C/E clades are genetically closest to A clade haplotypes, and the F and Azokh clades are genetically close to the central D clade haplotypes. There is a clear longitudinal separation between these clade groups, with A clade haplotypes (alongside with B, C, and E) dominating western and central Europe and D clade dominating Anatolia and the Balkans. The A Clade has its farthermost eastern representative around 28 degrees longitude.

The D clade, on the other hand, has its westernmost representative around 22 degrees longitude. Within the large geographic extend of the samples used in this study (from -6 degrees to 57 degrees longitude), the overlap zone occupies a relatively narrow width (about 6 degrees). The only exception to this almost clear-cut separation between the eastern and western lineages of mtDNA is the F clade, which belongs to the eastern lineage but is only seen in central and eastern Europe (between 12 to 25 degrees longitude).

4.3.2. Large Mouse-eared Bat Complex in Turkey and the Contact Zone

Apart from the two western *M. myotis* representatives from a particular cave in the Turkish Thrace, the Large Mouse-eared bat complex in Turkey consists only of the eastern *M. myotis* and *M. blythii s.l.* All mitochondrial haplotypes in Turkey, (other than one unassigned individual from the same cave), belong to D Clade.

The samples used in this study include 56 *M. blythii s.l.* samples and 155 western *M. myotis* samples from Anatolia and the Turkish Thrace. Out of the 56 *M. blythii s.l.* haplotypes, 25 were unique, as compared to 27 unique haplotypes in 151 *M. myotis*. The significant difference in the ratio of unique haplotypes show that the haplotype diversity of the lesser form is much higher even in this

limited area, supporting the idea that this form is older in this geography and introgressed the mitochondrial genome of the greater form.

In another study conducted with an overlapping but wider data set from Anatolia and the Turkish Thrace, it was found that 165 *M. myotis* samples yielded 29 unique haplotypes whereas 162 *Myotis blythii* s.l. samples yielded a total of 60 different haplotypes (Çelik et.al., 2014). The haplotype network acquired from the data used in this study is given in Figure 4.16. This study also reveals that, despite the larger number of samples, there are only two haplotypes shared by the greater and the lesser forms.

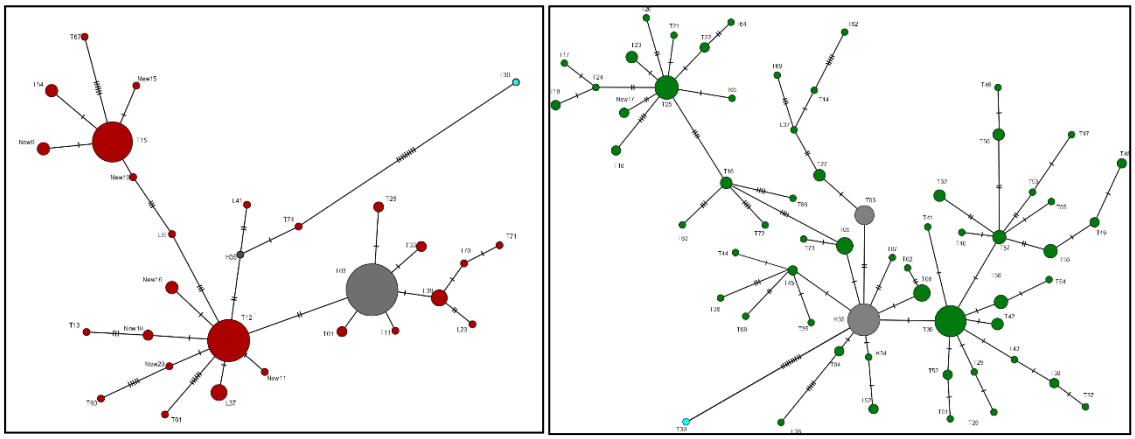


Figure 4.16. Haplotype networks for *Myotis myotis* and *Myotis blythii s.l.* residing in Anatolia and the Turkish Thrace (Çelik et.al., 2014). *Myotis myotis* haplotypes are represented by red, *Myotis blythii s.l.* haplotypes are represented by green, and shared haplotypes are represented by grey. Blue color represents one unidentified individual belonging to Clade A.

A separate analysis comparing the populations inhabiting the roosts in the Balkans and Anatolia reveals that observed genetic (mitochondrial) distance between *M. myotis* and *M. blythii s.l.* populations can be as high as 0.7 at a cave that they inhabit in a mixed colony, while this distance can be as low as 0.33 between the populations occupying two caves 1400 kilometers apart. The analysis of variance (ANOVA) conducted on these populations show that the observed mitochondrial genetic distance between populations of the same species is correlated with the geographical distance for eastern *M. myotis* and *Myotis blythii s.l.*

Since the populations of western *M. myotis* in the region consist almost only of A-Clade individuals, the same result does not apply to this group. On the other hand, the genetic and physical distances between populations of eastern *M. myotis* and *M. blythii s.l.* are not correlated ($P > 0.05$).

The physical and genetic distance matrix for the populations residing in the contact zone and Anatolia are given in APPENDIX D.

5. DISCUSSIONS AND CONCLUSION

5.1. Morphology and Ecology

The morphological data examined in this study shows that the species comprising the Large Mouse-eared bat complex are hard to distinguish by their looks. In Anatolia, it is not possible to attribute species memberships according to visual identifiers on the field, as suggested in previous studies. Although some overlaps can occur, making an proper identification of the lesser and greater forms on the field is possible through fine measurements of the CM^3 distance and/or the FA length, which exhibits a significant difference between species.

The morphological measurements also reveal that there is a clinal change in forearm length and cranial measurements in Anatolia (and for only lesser form in Europe), indicating an increase in size, as reported formerly by some researchers (Spitzenberger, 1996; Benda et al., 2011). While all key morphological features sufficient to define a species/subspecies are not checked in this study, this finding indicates that the reason for greater cranial or other morphological measurements encountered in the East might be this clinal increase. Hence, most of the subspecies suggested formerly are not supported by the findings of this study. When the clinal change is taken into account, *M. blythii s.l.* samples gathered from Lesvos, Anatolia or Iran exhibit no leap in their morphological features from those in Europe and are exhibiting a gradual increase throughout their distribution range. Meanwhile, the western lineage of the greater form differentiates from the eastern form with lack of such prominent clinal increase from west to east.

Although this study does not particularly focus on the ecology of the Large Mouse-eared bats, our observations show that the region preferences attributed to the greater and lesser forms (i.e. *M. myotis* is expected to occupy humid regions, *M. blythii* is expected to occupy arid regions) in previous researches conducted in Europe (Arlettaz et.al., 1995; Arlettaz, 1999), are not valid for the species residing in Anatolia.

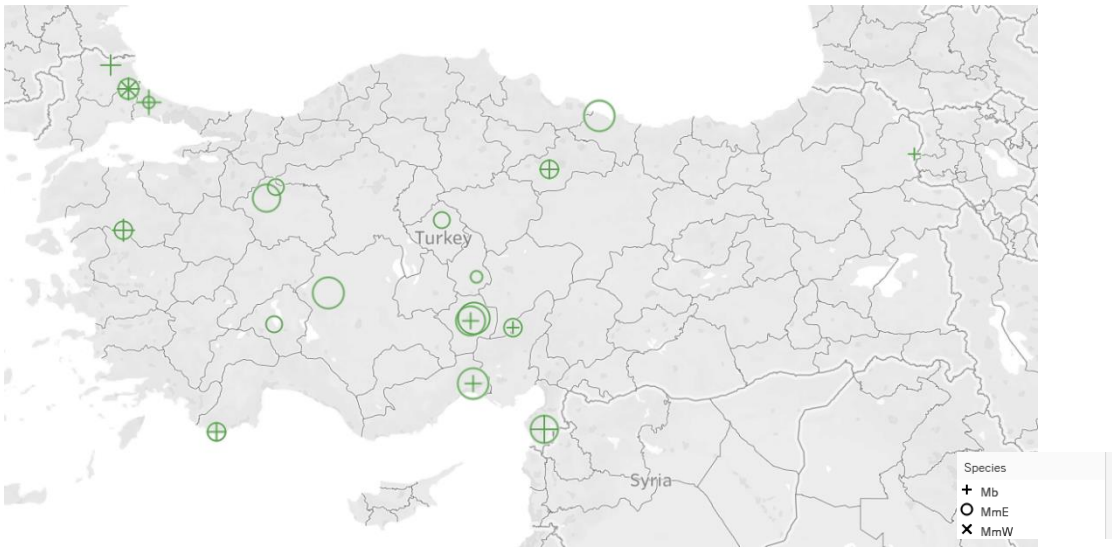


Figure 5.1. The distribution of the three taxa residing in Anatolia and the Turkish Thrace. The shapes "+", "O" and "X" represents *M. blythii s.l.* (Mb), eastern *M. myotis* (MmE), and western *M. myotis* (MmW), respectively.

In Anatolia, *M. myotis* appears to occupy arid environments, as we were not able to find any *M. blthii s.l.* individuals in many of these arid regions, especially in the Central Anatolia (five roosts found in more arid regions were occupied only by the greater form). This may be due to the difference in foraging habits of the western and eastern lineage of *M. myotis*, and/or different ecological competition and exclusion dynamics between the lesser and greater forms throughout this region. Also, *M. blythii s.l.* roosts we were able to find in Anatolia were almost always shared by the *M. myotis* in mixed colonies (see Figure 5.1). *M. myotis*, on the other hand, was the only resident species in some of the roosts.

The eastern edge for the distribution range of *M. myotis* seems to be around the 37th meridian. *M. blythii s.l.*, on the other hand, is existent in Eastern Anatolia and even in Iran. To reveal a more precise picture of food preferences and their ecological traits of the two species in Anatolia, which would probably supply more information for the conservation of the species, further research focusing on the ecology of these species is required.

5.2. Genetic Data and Phylogeography of Large Mouse-eared Bats

5.2.1. Taxonomy and Distribution of Taxonomic Units

The analysis of the nuclear data gathered from all collected or acquired samples (including all specimens from the Iberian Peninsula to Iran) indicate that there are three distinct clusters within the

Large Mouse-eared bat complex residing in this geographical range. One obvious distinction is between the previously recognized species: *M. myotis* and *M. blythii s.l.* One other prominent distinction was found between the two lineages of *M. myotis*: the western and eastern lineages, pointing out to the lack of gene flow between these two groups. The genetic distance between the eastern and western lineages of the greater form was almost as high as the total difference between the lesser and greater forms. This finding, along with the difference in clinal change of morphological characteristics, supports the idea that Anatolia may be occupied by a different species/subspecies than the European *M. myotis*.

The C113 marker for *M. myotis* (both lineages), and G25 marker for *M. blythii s.l.* are almost fixed for these species, which may be helpful in genetic identification. This is also indicative of the current reproductive isolation between the greater and the lesser forms. The fact that there are no individuals with definitive high probability of being hybrids, even though these species cohabit in the same caves most of the time, also supports this conclusion. The most plausible theory is that these sibling species which originated from the same ancestor have been geographically separated for some time and were subject to a speciation event resulting in almost total reproductive isolation, as suggested by Furman and colleagues (2013) .

Bearing in mind that *M. myotis* cannot be found to the east of central Anatolia whereas *M. blythii s.l.* specimens can be encountered even in Iran (leaving aside the suggested *M. blythii* from central Asia), it is reasonable to conclude that the lesser form took refuge in the east, and greater form was restricted to west during the last glacial maximum (as also suggested by Furman et.al., 2013). Most probably, both species geographically remerged after the glacial period, as a result of an expansion to their former habitats.

The nuclear genetic data also show that there is a clear separation and very few to no hybridization between the eastern and western lineages of *M. myotis,* but this time with the exception that they inhabit almost totally separated areas. The only roost shared by both species included two western *M. myotis* individuals at the eastern extreme of this species' distribution (see Figure 5.1.) This differentiation within the greater form is probably due to a secondary geographical isolation event that occurred during the post-glacial west-to-east expansion of the greater form. The current distribution of the two lineages of the greater form indicates that after a group invaded Anatolia, their connection with the western ancestral population was cut off.

The contact zone of the eastern and western lineages of *M. myotis* is in the Turkish Thrace. The Marmara Sea and the two straits (Bosphorus and Dardanelles) appear to be forming the cutting line between them. Since Large Mouse-eared bats can travel great distances, the two straits separating Anatolia from the European continent are not impassible barriers for Large Mouse-eared bats (Castella et.al., 2001). Lack of suitable roosts, and more recently, dense human settlements around the straights might be a reason for this separation.

5.2.2. Distribution of the HVII Haplotypes

Our results are in concordance with previous studies stating that it is not possible to barcode the species within the Long Mouse-eared Bat Complex according to the mitochondrial markers. But the distribution of clades and the mitochondrial genetic distance between individuals can still shed light on relatively recent history and interactions of the species and subpopulations within this bat complex.

The haplotypes belonging to Clades B, C, and E are probably evolved from the central haplotypes within Clade A, which are ancestral to *M. myotis*. Similarly, the F and Azokh clades are genetically close to the central D clade haplotypes and probably have evolved from ancestral *M. blythii s.l.* haplotypes. The clear geographical separation between these clade groups implies that most probably, two separate mtDNA lineages evolved in strict geographical separation as *M. myotis* was restricted to roosts in the west with suitable climatic conditions for some time, and *M. blythii s.l.* was restricted to similar roosts in western Asia and/or Anatolia for the same time period. The same separation might account for the allopatric speciation event for these two forms during the Pleistocene -which is, according to Bogdanowicz and colleagues (2009), took place some 560 thousand years ago - and the lack of lesser Mouse-eared bat fossils in Europe before the Holocene (Topal and Ruedi, 2001).

The F clade haplotypes distributed around the Adriatic, which is ancestral to *M. blythii* and shared by the greater form, may indicate the existence of a separate and possibly more recent refugium in this region. This refugium could have been occupied by *Myotis blythii s.l.* before the greater form, and the mitochondrial DNA of *M. myotis* could have been replaced during its re-expansion into the Apennine and Balkan peninsulas. Because there are no representatives of the F Clade in Anatolia, these haplotypes may have evolved in this region or may be originating from a different expansion route of the lesser form, probably located to the north of Black Sea. Notwithstanding, the existence of B and C/E clades in a restricted region may be an indicator of refugia around the Alps and/or

Apennine Peninsula that are occupied by *M. myotis* prior to the emergence of *M. blythii s.l.* in this region. Existence of such refugia in Apennine Peninsula is also in accordance with the previous findings of Ruedi and colleagues (2008). These smaller haplotype groups and possible secondary invasion routes/refugia related with these groups require further research.

The low haplotype diversity of eastern *M. myotis* as compared to the lesser form in Anatolia, supports the hypothesis that *M. blythii s.l.* was present in this region prior to the greater form. Meanwhile, the very low number of shared haplotypes indicates that the greater form spent enough time in the region to develop their own haplotypes through mutations. This low number of shared haplotypes is also an evidence of current reproductive isolation between these species.

5.2.3. Cytonuclear Discordance

Because the mtDNA is uniparentally inherited, it has a smaller effective population size and can complete the process of lineage sorting much faster than the nuclear DNA, incomplete lineage sorting may, to some extent, explain some of the discrepancy between the information acquired from nuclear and mitochondrial markers (Toews & Brelsford, 2012). On the other hand, discordance occurring as a result of incomplete lineage sorting "is not expected to leave any predictable biogeographic pattern" (Funk & Omland 2003).

Since there is a clear geographic separation between the two lineages of mtDNA haplotypes, incomplete lineage sorting is not likely to be the cause of cytonuclear discordance among Large Mouse-eared bats. The incomplete lineage sorting scenario regarding Large Mouse-eared bat complex also includes a non-completed speciation (Bogdanowicz et.al., 2009) hypothesis, which is not the case according to our findings. In many roosts that the lesser and greater forms cohabit, there is no concrete evidence of current hybridization, indicating that the speciation process between these species is completed. Very low number of shared HVII haplotypes between these individuals and the mitochondrial genetic distance between the representatives of the two forms that cohabit the same roosts also support this conclusion.

Toews and Brelsford (2012) suggest that in most cases, clear biogeographic patterns (such as the separation we encountered in the eastern and western lineages of mitochondrial markers of the Large Mouse-eared bats) indicate adaptive introgression of mitochondrial DNA, along with "demographic disparities and sex-biased asymmetries." The female philopatry and male-biased

dispersal observed in the large mouse-eared bats (Castella et. al., 2001) is in agreement with this introgression scenario.

The hypothesis that the lesser mouse-eared bats originating from central Asia invaded Anatolia when *M. myotis* was already in the region (Ruedi et al. 1990; Arlettaz et al. 1997b; Berthier et al. 2006) is not supported by our findings. The D Clade haplotypes that are ancestral to the *M. blythii s.l.* are present in the eastern parts of Anatolia and Iran where *M. myotis* is not existent, and the replacement of the mitochondrial DNA of a resident species by that of the invading species is highly unlikely. The scenario, which is more concordant with our findings is that the western lineage of *M. myotis* emerged in Anatolia when *Myotis blythii s.l.* was already there and lost its mtDNA to those of lesser form during this process.

So, according to the most plausible scenario, after the physical separation of the two forms (or the ancestral species these forms originate from), the lesser form that took refuge in the east and the greater form that took refuge in the west during the last glacial maximum, and began invading regions already occupied by the other form by the end of glacial maximum. Graphic representation of this two-way expansion is given in Figure 5.2. Since the allopatric speciation event was not fully completed, a small amount of hybridization as a result of male-biased dispersal caused the mitochondrial DNA of the invading species to be replaced by the mitochondrial DNA of the resident species.

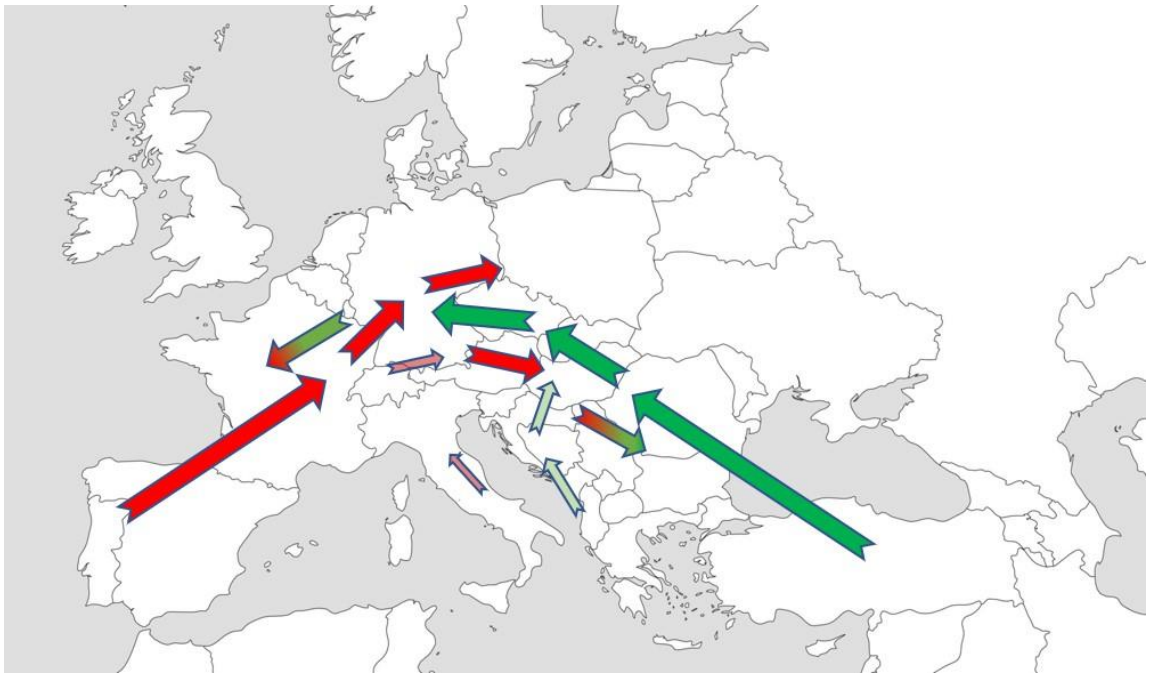


Figure 5.2. Possible post-glacial expansion routes for *Myotis blythii s.l.* (indicated by green) and *Myotis myotis* (indicated by red). The lighter colors represent expansion from possible smaller refugia

and related haplotypes (F Clade for *M. blythii* and B, C/E Clades for *M. myotis*) originating from these refugia. The transition in colors represents introgression events.

As *M. blythii s.l.* went further west in Europe they acquired A, B and C/E haplotypes from the greater form, and as *M. myotis* reached the Balkan Peninsula and the Turkish Thrace, they acquired the D and F haplotypes that are ancestral to the lesser form. This limited hybridization between the two species was not sufficient for these species to remerge into one species, and they were able to cohabit in the same roosts as different species without competition, due to their adaptive abilities and mutually exclusive ecological niches.

A second dispersal was within the *M. myotis*. As they expanded into Anatolia, the *M. myotis* separated into two branches. Probably, the branch that invaded Anatolia was separated from the original population and with time, might have evolved into a separate species, or at least a subspecies that is biologically isolated from the western representatives of the greater form. As the microsatellite data indicates, the nuclear genetic distance between these two lineages is almost as high as the distance between the lesser and greater forms in total, so the Anatolian branch of *M. myotis* might need to be considered a different taxonomical unit.

Since these two branches are geographically isolated (except for only one cave in Thrace that might be inspected for existence of hybrids in the future), possible hybridization between the representatives of these groups could not be observed in their natural environment, and potential reproductive compatibility cannot be confirmed or denied. Hence, whether the eastern lineage of the great form that is distributed throughout Anatolia and Levant is a subspecies or a separate species needs further research by taxonomists.

5.3. Conservation of Mouse-eared Bats

Although this study does not particularly focus on the conservation of the relevant bat species, it has some implications regarding conservation efforts. Obviously, no conservation strategy can be developed without having information about the number of species within an animal complex, the geographical space they occupy, and specific threats these species are facing. Discovery of cryptic or new lineages within a bat complex is directly related to the endangerment status of and conservation strategies for these animals (Ibáñez et.al. 2006), and the distribution of these lineages are of critical importance regarding their risk of extinction (Jones et.al., 2003).

5.3.1. Distribution of Species

According to the IUCN Red List of Threatened Species (IUCN, 2019), *M. myotis* occupies an area ranging from the Iberian Peninsula to eastern Anatolia (Figure 5.3) and is listed as "least concern" (with stable population trend) regarding its level of endangerment. In this list, there is no information about the two lineages of *M. myotis* and they are considered as part of the same population, which is not in accordance with our findings. Since there is a clear separation between the western and eastern clades of *M. myotis* and there is little to no gene flow between these clades, the current distribution information and assessment criteria should be updated accordingly.

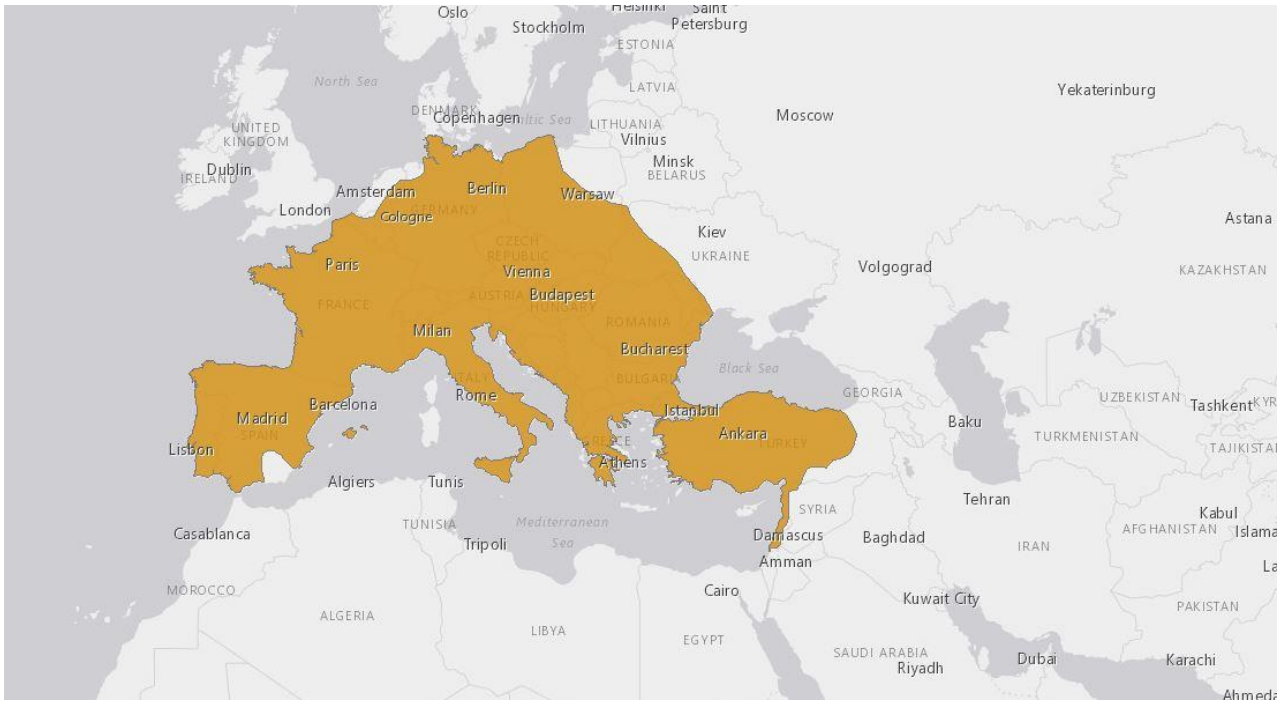


Figure 5.3. The distribution map of *Myotis myotis* according to the IUCN Red List of threatened species (IUCN, 2019).

The Lesser Mouse-eared Bat (*M. blythii s.l.*), on the other hand is considered as "least concern" with decreasing population trend in the same resource. However, the distribution pattern of lesser form is depicted in a patchy manner, ranging from the Iberian Peninsula to central Asia (Figure 5.4), despite the literature revealing the genetic separation between the European and Asian representatives of this species. It is obvious that more research is necessary to determine the taxonomic statuses and genetic relationship of the eastern and western representatives of the Lesser Mouse-eared bats. This being the case, the current distribution information and endangerment assessment about this species should also be subject to revision.

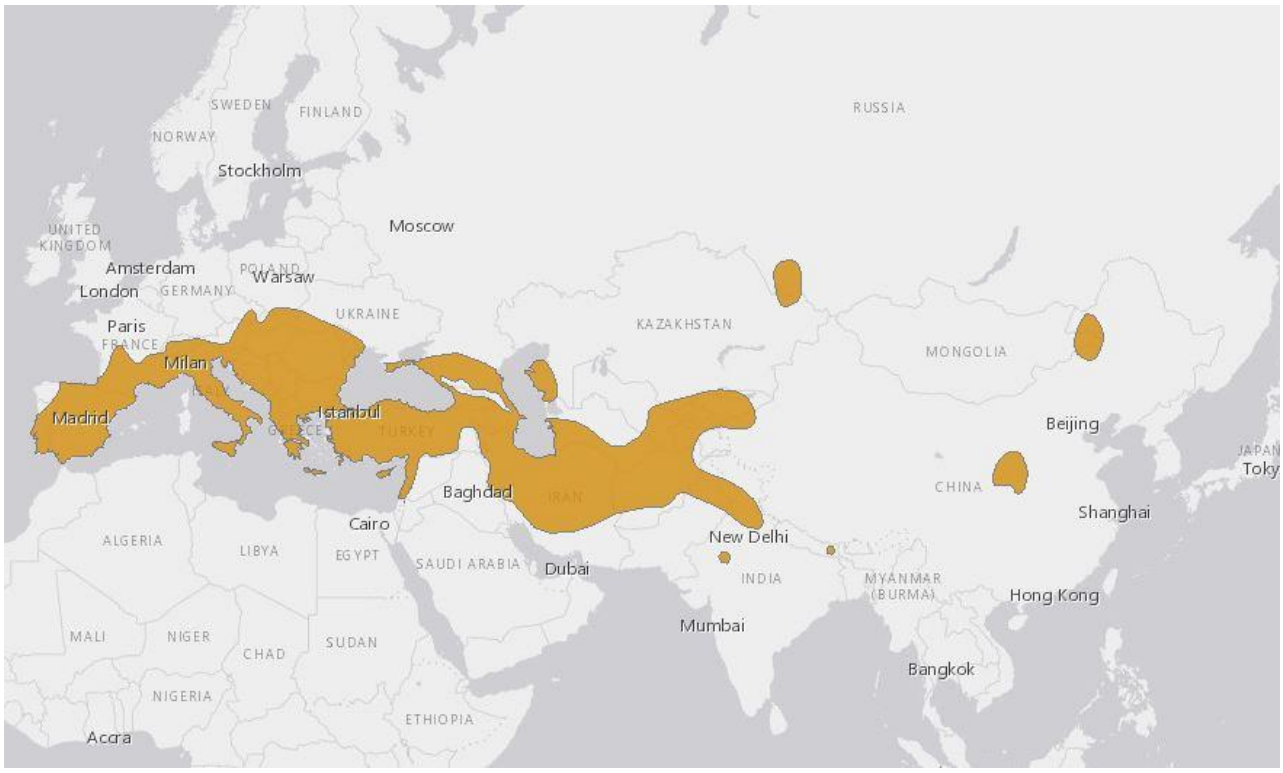


Figure 5.4. The distribution map of *Myotis blythii (s.l.)* according to the IUCN Red List of threatened species (IUCN, 2019).

5.3.2. Threats Against Large Mouse-eared Bats

During our field studies, we found that the bat populations in Anatolia (including, but not limited to Large Mouse-eared bat complex) are facing some major threats:

Loss of roosts and habitat: Because of extensive construction of new houses or other buildings by people, and with the effect of accompanying deforestation, bats, as well as other species, continuously lose their roosts or foraging grounds.

Overuse of pesticides: While bats serve as a pest diminishing factor themselves, extensive use of chemical pesticides causes build-up of toxins in their metabolism, leading to death, infertility or other conditions.

Cave tourism: Caves are one of the most important roosts for many bat species. Opening caves to tourism without proper identification of the bat populations that reside inside the caves, use of objects that are not part of the cave habitat (such as heat-emitting lighting), and human actions during hibernation period disturb the well-being of bat populations.

Hunting of bats due to several reasons: Hearsay about the “dangers” that bats pose to humans, or about the “healing power” of bat blood, bat organs, etc., some people hunt down bats, which is a phenomenon we encountered more than once in several parts of Anatolia.

Illegal guano mining: Bat feces can be a beneficial ingredient in certain types of fertilizers, but unconscious mining of bat guano may destruct cave habitats and disturb bat populations, causing them to leave their roosts or wake them up during hibernation.

Cave tourism, hunting of bats, and guano mining are factors that are more pronounced in (if not peculiar to) the Turkish Thrace and Anatolia, as compared other European countries. Therefore, existence of a different species or subspecies isolated in Anatolia (such as the *Myotis myotis macrocephalicus*, existence of which is supported by this study) points out to the necessity of a new assessment perspective and protective measures in terms of conservation. A more appropriate assessment of this evolutionary unit calls for further studies on the population size, genetic variety of and location-specific threats against this group of bats.

5.4. Conclusion

The results of our study do not support the existence of formerly suggested species/subspecies (such as *M. b. lesviacus* or *M. b. risorius*) within the lesser form. The limited molecular and morphological data we acquired show that there are no geographically isolated units, and no morphologically distinct specimens to support these suggestions. But the east-to-west clinal increase might be the reason for the morphological differences identified in eastern representatives of this form.

Molecular data we gathered during this study reveal that there are three distinct subunits of the Large Mouse-eared bat complex in the Western Palearctic region. Aside from the lesser form (*Myotis blythii s.l.*), there are two possible evolutionarily significant units (i.e. eastern and western lineages) within the greater form that seems to be genetically isolated from each other. The geographical barrier separating these appears to be the İstanbul and Çanakkale Straits (or the urban development around these straits).

While we did not explore all criteria for identifying different taxonomic units, the lack of gene flow and different morphological trends between these two lineages support the idea that the eastern lineage of *M. myotis* may be a subspecies (if not species) that might be named *Myotis myotis*

macrocephalicus or *Myotis macrocephalicus,* as suggested in former studies (Harrison and Lewis, 1961; Spitzenberger, 1996), yet with a different range of distribution than previously suggested.

The lesser form has a continuous distribution in the whole range of our study with no distinct subgroups, but since the representatives of this form in the western Palearctic region is shown to have no genetic resemblance with the *Myotis blythii* from central Asia -where its name is acquired from- probably this taxonomic unit might also be named differently. In case further studies clearly show that these are different taxa, the species name *Myotis (blythii) omari* with the type locality in Iran (Thomas, 1905) appears to be a better alternative than *M. (blythii) oxygnathus* for the lesser form, since our data supports the hypothesis that it took refuge in the eastern part of its current known distribution range during the event of speciation.

The cytonuclear discordance evident in the Large Mouse-eared bat complex arises from multiple introgression events. During their post-glacial expansion to their former habitats, the sex-biased dispersal caused the mitochondrial DNA of the pioneer specimens to be replaced by the already resident group. That's why the use of mitochondrial markers for species delimitation, such as mtDNA barcoding, proves to be an inappropriate method for this bat complex. The most accurate way to identify these species is using nuclear genetic markers such as microsatellites.

When it comes to the field identification of the lesser and greater forms of Large Mouse-eared bats (particularly in Anatolia), the CM^3 (canine to 3^{rd} molar teeth) distance, combined with longitude seems to be the most precise indicator. The forearm length (FA) on the other hand, when combined with sex and longitude data, is also a good indicator for species delimitation, with a little less precision. With the use of binary logistic regression models, species delimitation can be made with at least 95% confidence without using molecular methods, given that the specimens are mature, and longitude is known.

We believe that the findings of this study, as well as recent literature on the geographical distribution of this bat complex should be taken take into consideration for any conservation study, as the current distribution maps for the Large Mouse-eared bats do not represent the actual situation. The separation within the greater form and the difference between Asian and western Palearctic representatives of the lesser form, most probably indicate different conservation needs (related to their habitats, active population sizes, ecological needs, etc.) for these taxonomic units.

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APPENDIX A: GEOGRAPHICAL COORDINATES, GENDER, FOREARM AND CM^3 LENGTHS OF SAMPLES USED IN THE STUDY

Code	Lattitude	Longitude	Sex	FA	CM^3	Haplotype	Species*
LM1	36.69	-6.13	F	58.4	N/A	H01	*Mb*
LM2	36.02	-5.60	F	N/A	N/A	H27	*Mb*
LM3	36.72	-4.42	M	58.8	8.8	H01	*Mb*
LM4	37.19	-3.61	F	59.6	8.6	H63	*Mb*
LM5	39.98	-0.05	M	56.8	8.4	H65	*Mb*
LM6	38.84	0.11	M	59.3	8.3	H20	*Mb*
LM7	41.71	1.30	F	56.1	N/A	H01	*Mb*
LM8	44.62	2.19	F	59.7	8.3	H01	*Mb*
LM9	44.62	2.19	M	58.5	8.9	H01	*Mb*
LM10	46.60	6.22	M	56.7	8.5	L05	*Mb*
LM11	46.14	7.12	M	57.6	N/A	L16	*Mb*
LM12	45.71	7.26	F	56.9	8	H66	*Mb*
LM13	45.71	7.26	F	60.2	8.3	L16	*Mb*
LM14	46.31	7.80	N/A	54.9	N/A	H15	*Mb*
LM15	46.33	8.00	F	54.8	N/A	L27	*Mb*
LM16	46.33	8.00	F	53	N/A	H15	*Mb*
LM17	46.50	11.35	F	58.3	8.6	L13	*Mb*
LM18	46.50	11.35	F	55.6	8.5	L14	*Mb*
LM19	41.56	14.66	F	57	8.9	H51	*Mb*
LM20	37.71	15.07	F	60.5	9	L17	*Mb*
LM21	41.63	15.92	M	57	8.7	H51	*Mb*
LM22	41.63	15.92	M	56.7	8.9	L06	*Mb*
LM23	40.67	16.60	M	60.2	9.3	H52	*Mb*
LM24	39.08	17.13	M	58.8	8.7	H52	*Mb*
LM25	39.08	17.13	F	57.8	8.7	H52	*Mb*
LM26	40.67	21.15	M	57.5	N/A	L34	*Mb*
LM27	40.67	21.15	M	58.8	N/A	M63	*Mb*
LM28	38.76	21.93	F	57.9	8.6	H34	*Mb*
LM29	38.76	21.93	M	57.8	9	L01	*Mb*

LM30	37.35	22.35	M	53.9	8.7	L08	*Mb*
LM31	37.35	22.35	F	60.6	9	H68	*Mb*
LM32	46.85	22.67	F	60.9	8.9	L44	*Mb*
LM33	46.85	22.67	M	58.8	9.3	T74	*Mb*
LM34	46.85	22.67	F	57.9	8.5	L45	*Mb*
LM35	41.09	23.54	F	59.6	8.7	L33	*Mb*
LM36	41.09	23.54	F	56.9	8.7	M21	*Mb*
LM37	41.13	24.89	F	58.6	10	L24	*Mb*
LM38	41.13	24.89	M	57.5	8.8	L40	*Mb*
LM39	41.13	24.89	F	61.7	8.6	H34	*Mb*
LM40	43.24	24.90	N/A	N/A	N/A	H59	*Mb*
LM41	43.24	24.90	N/A	N/A	N/A	H60	*Mb*
LM42	46.13	25.32	M	55.7	9.3	H35	*Mb*
LM43	46.13	25.32	M	55.5	8.6	T74	*Mb*
LM44	35.05	25.41	F	61.8	9.2	L36	*Mb*
LM45	40.90	25.52	M	57.1	8.9	H35	*Mb*
LM46	39.38	26.21	F	57.6	8.9	L53	*Mb*
LM47	41.84	27.56	F	58.4	N/A	H35	*Mb*
LM48	41.84	27.56	F	56.3	N/A	T73	*Mb*
LM49	39.35	27.81	M	54.4	N/A	T27	*Mb*
LM50	39.35	27.81	M	55.9	N/A	H35	*Mb*
LM51	39.35	27.81	F	58.5	N/A	H35	*Mb*
LM52	41.50	27.92	F	58.3	8.3	H35	*Mb*
LM53	41.50	27.92	M	59.5	9	T05	*Mb*
LM54	41.50	27.92	M	53.8	8.3	T04	*Mb*
LM55	41.29	28.32	F	59.1	8.4	H35	*Mb*
LM56	41.29	28.32	F	57.4	8.4	H35	*Mb*
LM57	41.29	28.32	F	57.8	9	H35	*Mb*
LM58	41.29	28.32	M	55.8	8.9	H35	*Mb*
LM59	44.58	28.55	F	59	9.1	H35	*Mb*
LM60	44.58	28.55	M	59.2	9.5	H35	*Mb*
LM61	44.58	28.55	M	59	8.6	H35	*Mb*

LM62	44.58	28.55	M	59.6	9	H35	*Mb*
LM63	44.58	28.55	F	62.7	8.6	H35	*Mb*
LM64	44.58	28.55	F	62.2	8.7	H35	*Mb*
LM65	36.18	29.67	F	59.8	8.5	L52	*Mb*
LM66	36.18	29.67	M	56.2	8.9	L52	*Mb*
LM67	35.33	33.77	N/A	N/A	N/A	L55	*Mb*
LM68	37.94	34.72	F	62.3	8.5	T03	*Mb*
LM69	37.94	34.72	F	61.4	8.4	T14	*Mb*
LM70	36.95	34.79	M	58.5	N/A	T72	*Mb*
LM71	37.83	35.57	M	60.4	N/A	L37	*Mb*
LM72	36.22	36.20	M	60	9.9	T18	*Mb*
LM73	36.22	36.20	F	62.5	9.4	T24	*Mb*
LM74	40.28	36.30	F	60.9	N/A	T62	*Mb*
LM75	40.28	36.30	F	58.8	N/A	T69	*Mb*
LM76	40.28	36.30	M	60.2	9.8	T03	*Mb*
LM77	34.80	39.00	M	N/A	9.2	L04	*Mb*
LM78	34.80	39.00	N/A	N/A	N/A	L56	*Mb*
LM79	40.51	43.57	F	61.9	N/A	L38	*Mb*
LM80	29.10	53.05	N/A	N/A	N/A	L58	*Mb*
LM81	32.43	53.69	M	N/A	8.9	L03	*Mb*
LM82	32.43	53.69	M	N/A	N/A	L10	*Mb*
LM83	30.28	57.07	N/A	N/A	N/A	L57	*Mb*
LM84	30.28	57.07	M	60.3	9.4	L11	*Mb*
LM85	36.69	-6.13	F	62.7	N/A	H01	*MmW*
LM86	36.69	-6.13	F	62.6	N/A	L21	*MmW*
LM87	36.72	-4.42	F	64.4	N/A	H19	*MmW*
LM88	36.72	-4.42	F	61.5	N/A	H30	*MmW*
LM89	37.19	-3.61	F	60.1	9.6	H20	*MmW*
LM90	37.19	-3.61	F	64.3	10.1	L19	*MmW*
LM91	40.03	-3.60	M	60.4	N/A	H19	*MmW*
LM92	40.03	-3.60	F	64	N/A	H17	*MmW*
LM93	40.91	-2.47	F	61.6	9.9	L20	*MmW*

LM94	40.91	-2.47	M	62.4	9.6	H20	*MmW*
LM95	39.98	-0.05	F	65	9.7	H24	*MmW*
LM96	38.84	0.11	M	60.2	10	H22	*MmW*
LM97	38.84	0.11	F	64.1	9.2	H20	*MmW*
LM98	41.71	1.30	M	61.7	N/A	H24	*MmW*
LM99	41.71	1.30	N/A	N/A	N/A	N/A	*MmW*
LM100	39.70	3.02	F	64.6	9.8	H27	*MmW*
LM101	39.70	3.02	F	60.7	9.9	H24	*MmW*
LM102	45.24	4.67	F	61.1	9.5	H01	*MmW*
LM103	45.24	4.67	F	64.6	9.6	H01	*MmW*
LM104	46.25	5.13	F	60.8	9.9	H03	*MmW*
LM105	46.25	5.13	F	61.1	9.6	H03	*MmW*
LM106	44.73	5.23	F	61	9.4	H01	*MmW*
LM107	44.73	5.23	M	58.5	9.2	H29	*MmW*
LM108	47.24	5.67	M	59.6	9.8	H01	*MmW*
LM109	47.24	5.67	M	60.1	9.8	H01	*MmW*
LM110	46.38	6.21	F	63.1	9.5	H11	*MmW*
LM111	46.55	6.25	M	60.5	N/A	H01	*MmW*
LM112	43.65	6.80	F	62.2	10.2	H29	*MmW*
LM113	47.32	6.81	F	64.5	10.4	H01	*MmW*
LM114	47.32	6.81	F	60.8	9.6	L22	*MmW*
LM115	46.77	7.07	M	N/A	N/A	H01	*MmW*
LM116	46.66	7.10	F	61.1	9.1	H01	*MmW*
LM117	46.66	7.10	F	N/A	N/A	H10	*MmW*
LM118	46.14	7.12	F	61.2	N/A	H12	*MmW*
LM119	45.71	7.26	M	57.5	9.5	L25	*MmW*
LM120	45.71	7.26	F	60.7	9.7	L15	*MmW*
LM121	47.34	7.32	F	61.9	9.8	H01	*MmW*
LM122	47.34	7.32	M	58	9.7	H01	*MmW*
LM123	46.31	7.80	M	61.5	N/A	L28	*MmW*
LM124	46.33	8.00	M	58	9.6	H15	*MmW*
LM125	46.33	8.00	F	61.9	9.5	H15	*MmW*

LM126	46.33	8.00	M	57.3	9.6	H15	*MmW*
LM127	46.50	11.35	M	61	9.8	L18	*MmW*
LM128	43.88	12.54	F	62.7	9.6	H55	*MmW*
LM129	38.15	13.14	F	63.7	9.7	H48	*MmW*
LM130	38.15	13.14	F	60.8	9.3	H48	*MmW*
LM131	38.15	13.14	F	62.3	10.2	H12	*MmW*
LM132	37.57	15.11	M	60.5	9.7	H12	*MmW*
LM133	37.57	15.11	N/A	64.2	10.8	H12	*MmW*
LM134	41.63	15.92	F	64.7	10	H52	*MmW*
LM135	41.63	15.92	F	63.8	10.2	H51	*MmW*
LM136	39.08	17.13	F	60.9	10.1	H12	*MmW*
LM137	39.08	17.13	F	61.9	10.1	H49	*MmW*
LM138	51.02	21.90	F	64.2	10.1	H01	*MmW*
LM139	51.02	21.90	F	64	9.6	H27	*MmW*
LM140	51.02	21.90	F	64.3	10	H01	*MmW*
LM141	38.76	21.93	M	60.4	10.4	T03	*MmW*
LM142	37.35	22.35	F	62.4	10	L07	*MmW*
LM143	37.35	22.35	M	60.4	10.2	H42	*MmW*
LM144	38.54	22.62	M	59.9	9.8	L02	*MmW*
LM145	46.85	22.67	M	60.1	10.2	H01	*MmW*
LM146	41.09	23.54	M	61.9	10.1	H37	*MmW*
LM147	41.09	23.54	M	59.9	9.9	H36	*MmW*
LM148	41.13	24.89	F	63.3	10.2	H01	*MmW*
LM149	43.24	24.90	N/A	N/A	N/A	H01	*MmW*
LM150	43.24	24.90	N/A	N/A	N/A	H01	*MmW*
LM151	46.13	25.32	F	58.9	8.4	H01	*MmW*
LM152	46.13	25.32	F	59.7	8	H01	*MmW*
LM153	46.13	25.32	M	62.3	10.2	H01	*MmW*
LM154	46.13	25.32	F	63.3	9.9	H35	*Mm?*
LM155	40.90	25.52	F	63.8	10.5	H33	*MmW*
LM156	41.50	27.92	M	58.8	N/A	T30	*M?*
LM157	43.88	12.54	M	62	10.2	H12	*Mm?*

LM158	39.10	26.24	F	64.1	10.4	T03	*MmE*
LM159	39.10	26.24	F	67.3	10.8	T03	*MmE*
LM160	38.97	26.39	M	61.2	10	T03	*MmE*
LM161	38.97	26.39	M	62.2	10.4	T03	*MmE*
LM162	39.35	27.81	F	65.9	N/A	T26	*MmE*
LM163	39.35	27.81	F	63.2	N/A	T03	*MmE*
LM164	39.35	27.81	M	61.9	N/A	T26	*MmE*
LM165	41.50	27.92	M	63.9	10.2	T03	*Mm?*
LM166	41.50	27.92	F	64.3	9.2	T03	*MmE*
LM167	41.50	27.92	F	67.5	9.4	T03	*Mm?*
LM168	41.29	28.32	M	62	10.2	T03	*MmE*
LM169	36.18	29.67	F	65.3	9.5	T27	*MmE*
LM170	36.18	29.67	M	62.3	10.5	T03	*MmE*
LM171	36.18	29.67	F	64.4	10.1	T03	*MmE*
LM172	37.88	30.81	M	64.7	N/A	T12	*MmE*
LM173	37.88	30.81	M	63.5	N/A	T12	*MmE*
LM174	40.01	30.85	F	65.2	N/A	T60	*MmE*
LM175	40.01	30.85	M	65.6	N/A	T61	*MmE*
LM176	39.51	34.16	F	63.9	N/A	T74	*MmE*
LM177	39.51	34.16	M	62.7	N/A	T68	*MmE*
LM178	37.94	34.72	F	65.3	10.2	T03	*MmE*
LM179	37.94	34.72	M	62.8	9.7	T15	*MmE*
LM180	37.94	34.72	M	61.6	9.8	T11	*MmE*
LM181	36.95	34.79	F	68.8	11	L23	*MmE*
LM182	36.95	34.79	M	64.3	10.9	T03	*MmE*
LM183	36.95	34.79	M	63.8	10.2	T03	*MmE*
LM184	36.95	34.79	M	67.2	11	T03	*MmE*
LM185	36.95	34.79	M	63.3	N/A	T70	*MmE*
LM186	38.63	34.85	F	65	N/A	T15	*MmE*
LM187	37.83	35.57	M	65	N/A	T03	*MmE*
LM188	37.83	35.57	M	63.8	N/A	L54	*MmE*
LM189	36.22	36.20	F	67.7	10.8	T03	*MmE*

LM190	36.22	36.20	F	67.8	10.2	T03	*MmE*
LM191	36.22	36.20	F	66.5	9.7	T03	*MmE*
LM192	40.28	36.30	F	64.4	N/A	T33	*MmE*
LM193	40.28	36.30	M	66.2	N/A	T67	*MmE*
LM194	40.28	36.30	F	65.4	N/A	T15	*MmE*
LM195	41.08	37.30	F	66.5	10.7	T15	*MmE*
LM196	41.08	37.30	F	65.4	10.4	T15	*MmE*
LM197	41.08	37.30	F	66.7	10.1	T15	*MmE*
LM198	43.88	12.54	M	56	8.2	L09	*Mb*
LM202	46.85	22.67	F	61.4	9.1	L44	*Mb*
LM204	41.84	27.56	M	54.5	N/A	T06	*Mb*
LM205	36.18	29.67	M	58.9	9	T27	*Mb*
LM206	36.95	34.79	M	59.5	9.5	L41	*Mb*
LM208	40.28	36.30	F	63.8	10.1	L35	*Mb*
LM210	44.62	2.19	F	64.1	9.9	H01	*MmW*
LM211	44.62	2.19	F	63.8	9.9	L26	*MmW*
LM213	46.71	6.44	M	59.2	9.3	L51	*MmW*
LM216	38.76	21.93	M	59.2	10	T03	*MmW*
LM217	40.90	25.52	F	63.3	10.4	H34	*MmW*
LM218	41.29	28.32	M	64.3	10.7	T01	*Mm?*
LM220	36.95	34.79	F	71.3	10.6	L39	*MmE*
LM221	35.18	35.94	M	64.3	N/A	T03	*MmE*
LM222	37.83	35.57	M	63.6	N/A	L54	*MmE*
LM224	36.95	34.79	M	66.1	10.7	L39	*MmE*
LM225	36.95	34.79	M	63.7	10.4	T03	*MmE*
LM226	36.95	34.79	M	64.2	10.3	T03	*Mm?*
LM227	36.95	34.79	F	69.7	10.6	T03	*MmE*
LM228	36.95	34.79	F	68.5	10.3	T03	*MmE*
LM229	36.95	34.79	F	68.8	10.4	L39	*MmE*
LM230	36.95	34.79	M	65.8	10.6	T12	*MmE*
LM231	36.95	34.79	M	63.6	10.6	L39	*MmE*
LM232	36.95	34.79	F	67.5	10.7	T03	*MmE*

LM233	36.95	34.79	F	68.8	10.4	T03	*MmE*
LM234	36.95	34.79	M	64.8	10.7	T03	*MmE*
LM235	36.95	34.79	F	66.8	10.7	T03	*MmE*
LM236	36.95	34.79	M	65	10.9	T03	*MmE*
LM237	36.95	34.79	M	68.8	10.7	L39	*MmE*
LM238	36.22	36.20	M	58.7	9	T25	*Mb*
LM239	36.22	36.20	M	59.8	8.8	T23	*Mb*
LM240	36.22	36.20	M	65.4	10.8	T03	*MmE*
LM241	36.22	36.20	F	60.6	10.3	T03	*MmE*
LM242	36.22	36.20	M	64.8	10.7	T03	*MmE*
LM243	36.22	36.20	F	68	10.7	T03	*MmE*
LM244	36.22	36.20	M	58.9	9.4	N17	*Mb*
LM245	36.22	36.20	F	68.1	10.7	T03	*MmE*
LM246	36.22	36.20	F	63.2	10.4	T03	*MmE*
LM247	36.22	36.20	F	60.3	9.2	T19	*Mb*
LM248	36.22	36.20	M	57.8	9.5	T25	*Mb*
LM249	36.22	36.20	M	55.5	9.5	N19	*Mb*
LM250	36.22	36.20	F	67.3	10.9	T03	*MmE*
LM251	36.22	36.20	M	66.7	10.5	T03	*MmE*
LM252	36.22	36.20	F	67.3	10.3	T03	*MmE*
LM253	36.22	36.20	M	63.5	10.7	T03	*MmE*
LM254	36.22	36.20	M	56.8	9.5	T25	*Mb*
LM255	36.22	36.20	F	58	8.8	N17	*Mb*
LM256	36.22	36.20	F	60.7	8.5	T03	*Mb*
LM257	36.22	36.20	F	68.7	10.4	T03	*MmE*
LM258	36.22	36.20	F	62.1	9.2	T25	*Mb*
LM259	36.22	36.20	F	59.6	8.9	T25	*Mb*
LM260	36.22	36.20	F	63.7	8.5	T25	*Mb*
LM261	37.98	34.80	M	64.2	10.2	T03	*MmE*
LM262	37.98	34.80	F	63.5	10.4	T15	*MmE*
LM263	37.98	34.80	M	65	10.5	T15	*MmE*
LM264	37.98	34.80	F	65	10.5	T03	*MmE*

LM265	37.98	34.80	M	64	10.4	T03	*MmE*
LM266	37.98	34.80	M	63.7	10.6	T12	*MmE*
LM267	37.98	34.80	M	63.6	10.2	N09	*MmE*
LM268	37.98	34.80	M	62.3	10.3	T15	*MmE*
LM269	37.98	34.80	F	64.6	10.5	N09	*MmE*
LM270	37.98	34.80	F	64.4	10.4	T15	*MmE*
LM271	37.98	34.80	F	65.8	10.5	T15	*MmE*
LM272	37.98	34.80	M	62	10.2	T03	*MmE*
LM273	37.98	34.80	F	65.6	10.3	T15	*MmE*
LM274	37.98	34.80	M	61.6	10.4	N10	*MmE*
LM275	37.98	34.80	M	63.6	10.2	T12	*MmE*
LM276	37.98	34.80	M	62.2	10.1	T03	*MmE*
LM277	37.98	34.80	F	66.9	10.7	T15	*MmE*
LM278	37.98	34.80	M	64.4	10.4	T15	*MmE*
LM279	37.98	34.80	F	66.8	10.5	T15	*MmE*
LM280	37.98	34.80	M	60.4	10.2	T15	*MmE*
LM281	37.98	34.80	M	65	10.1	N09	*MmE*
LM282	37.98	34.80	M	63.6	10.5	N/A	*MmE*
LM283	37.98	34.80	F	66	10.3	T12	*MmE*
LM284	38.38	31.90	M	61.1	10.3	T12	*MmE*
LM285	38.38	31.90	F	67.3	10.7	T12	*MmE*
LM286	38.38	31.90	F	66.5	10.2	T12	*MmE*
LM287	38.38	31.90	M	62.5	10.4	T12	*MmE*
LM288	38.38	31.90	M	63.7	10.1	T12	*MmE*
LM289	38.38	31.90	M	63	10	T12	*MmE*
LM290	38.38	31.90	M	58.8	10	T12	*MmE*
LM291	38.38	31.90	M	62.4	10.1	N16	*MmE*
LM292	38.38	31.90	F	66.1	10.4	N11	*MmE*
LM293	38.38	31.90	M	65.1	10.2	N16	*MmE*
LM294	38.38	31.90	M	62.9	10	T12	*MmE*
LM295	38.38	31.90	F	64.1	10.3	N18	*MmE*
LM296	38.38	31.90	F	65.4	10.4	T12	*MmE*

LM297	38.38	31.90	M	62.2	10.2	T12	*MmE*
LM298	38.38	31.90	M	64.4	10.1	N18	*MmE*
LM299	38.38	31.90	M	64.4	9.9	T12	*MmE*
LM300	38.38	31.90	F	66.4	10.2	T12	*MmE*
LM301	38.38	31.90	M	62.9	10.4	T12	*MmE*
LM302	38.38	31.90	M	62	10	T12	*MmE*
LM303	38.38	31.90	M	63.3	10.2	N16	*MmE*
LM304	38.38	31.90	F	65.8	10.1	N/A	*MmE*
LM305	38.38	31.90	M	62.6	10.3	N/A	*MmE*
LM306	38.38	31.90	F	63.3	10.3	T12	*MmE*
LM307	39.85	30.67	M	63.9	10.3	T12	*MmE*
LM308	39.85	30.67	M	60.8	10.2	T12	*MmE*
LM309	39.85	30.67	F	63	10.3	T12	*MmE*
LM310	39.85	30.67	F	65.8	10.3	T12	*MmE*
LM311	39.85	30.67	M	62	10.2	N/A	*MmE*
LM312	39.85	30.67	F	66.9	10.2	T12	*MmE*
LM313	39.85	30.67	M	62.4	10.3	T12	*MmE*
LM314	39.85	30.67	M	65.6	10.6	T12	*MmE*
LM315	39.85	30.67	M	62	10.1	T12	*MmE*
LM316	39.85	30.67	M	63.6	9.9	N20	*MmE*
LM317	39.85	30.67	M	63	10.1	T12	*MmE*
LM318	39.85	30.67	F	63.7	10	T12	*MmE*
LM319	39.85	30.67	F	68.3	10.4	T12	*MmE*
LM320	39.85	30.67	M	60.6	10.3	T12	*MmE*
LM321	41.54	24.53	F	N/A	N/A	N01	*MmW*
LM322	41.54	24.53	M	N/A	N/A	N02	*MmW*
LM323	42.29	27.75	F	N/A	N/A	H01	*MmW*
LM324	43.14	23.60	N/A	N/A	N/A	H01	*MmW*
LM325	43.29	23.35	F	N/A	N/A	H79	*MmW*
LM326	42.11	25.00	M	N/A	N/A	H01	*MmW*
LM327	42.11	25.00	F	N/A	N/A	H34	*MmW*
LM328	41.53	25.52	M	N/A	N/A	H01	*MmW*

LM329	42.82	26.03	M	N/A	N/A	H01	*MmW*
LM331	43.14	24.53	N/A	N/A	N/A	H01	*MmW*
LM332	43.14	24.53	N/A	N/A	N/A	H37	*MmW*
LM333	43.14	24.53	N/A	N/A	N/A	H35	*Mb*
LM334	43.14	24.53	N/A	N/A	N/A	H35	*Mb*
LM335	43.14	24.53	N/A	N/A	N/A	H01	*MmW*
LM336	43.14	24.53	N/A	N/A	N/A	H37	*MmW*
LM337	43.14	24.53	N/A	N/A	N/A	H35	*Mb*
LM339	43.14	24.53	N/A	N/A	N/A	H33	*MmW*
LM340	43.14	24.53	N/A	N/A	N/A	H01	*MmW*
LM341	43.14	24.53	N/A	N/A	N/A	H01	*MmW*
LM342	42.17	27.45	N/A	N/A	N/A	H35	*Mb*
LM344	42.17	27.45	N/A	N/A	N/A	H35	*Mb*
LM345	42.17	27.45	N/A	N/A	N/A	H35	*Mb*
LM346	42.17	27.45	N/A	N/A	N/A	H35	*M?*
LM347	42.17	27.45	N/A	N/A	N/A	M11	*Mb*
LM348	44.58	28.55	F	61.6	8.8	H35	*Mb*
LM349	44.58	28.55	M	58.3	9	H35	*Mb*
LM350	44.58	28.55	F	61.6	8.8	H35	*Mb*
LM351	44.58	28.55	M	58.6	8.6	H35	*Mb*
LM352	44.58	28.55	M	60.2	8.9	H35	*Mb*
LM353	44.58	28.55	F	60.4	8.7	H35	*Mb*
LM354	44.58	28.55	M	60	8.8	H35	*Mb*
LM355	51.02	21.90	F	63	9.7	H01	*MmW*
LM356	51.02	21.90	F	63.9	9.5	H01	*MmW*
LM357	51.02	21.90	F	64.6	10	H01	*MmW*
LM358	51.02	21.90	F	63	9.4	H01	*MmW*
LM359	51.02	21.90	F	63.7	9.9	H01	*MmW*
LM360	51.02	21.90	F	65.8	10.1	H01	*MmW*
LM361	51.02	21.90	F	64.1	10	H01	*MmW*
LM362	51.02	21.90	F	65.8	9.9	H01	*MmW*
LM363	51.02	21.90	F	64.8	9.9	H01	*MmW*

LM364	51.02	21.90	F	65.7	9.8	H01	*MmW*
LM365	51.02	21.90	F	62	9.3	H01	*MmW*
LM366	51.02	21.90	M	58	9.8	H01	*MmW*
LM367	51.02	21.90	F	60.3	9.6	H01	*MmW*
LM368	51.02	21.90	F	62.6	9.6	H01	*MmW*
LM369	51.02	21.90	F	64	9.8	H01	*MmW*
LM370	51.02	21.90	F	64.6	9.8	H01	*MmW*
LM371	51.02	21.90	F	61.9	9.9	H01	*MmW*
LM372	51.02	21.90	F	63.6	9.6	H01	*MmW*
LM373	46.85	22.67	F	61.7	9.9	H35	*Mb*
LM374	46.85	22.67	M	59.3	8.6	M18	*Mb*
LM375	46.85	22.67	F	62.3	8.9	M18	*Mb*
LM376	46.85	22.67	F	59.8	9.2	L44	*Mb*
LM377	46.85	22.67	M	62.6	9.8	H33	*MmW*
LM378	46.85	22.67	F	65.7	10.2	H01	*MmW*
LM379	46.85	22.67	M	60.5	10.4	H01	*MmW*
LM380	46.85	22.67	F	64.6	10.2	H01	*MmW*
LM381	46.85	22.67	M	59.9	10.9	H01	*MmW*
LM382	46.85	22.67	M	61.2	10.4	H01	*MmW*
LM383	46.85	22.67	M	61.1	10.5	H01	*MmW*
LM384	46.85	22.67	M	55.1	9.2	M18	*Mb*
LM385	46.85	22.67	F	62.3	9.6	H01	*MmW*
LM386	46.85	22.67	M	62.2	10.4	H01	*MmW*
LM387	46.85	22.67	M	60.6	10.2	H01	*MmW*
LM388	46.85	22.67	F	63.6	9.6	M06	*MmW*
LM389	46.85	22.67	M	61.5	10.2	H01	*MmW*
LM390	46.85	22.67	F	61.5	9.9	H01	*MmW*
LM391	46.85	22.67	F	60.9	9.3	M18	*Mb*
LM392	46.85	22.67	F	60.5	10.5	H77	*MmW*
LM393	46.85	22.67	F	63.9	9.9	H77	*MmW*
LM394	46.85	22.67	F	63.1	10.5	H01	*MmW*
LM395	46.85	22.67	M	63.2	10.4	H33	*MmW*

LM396	46.85	22.67	M	60.2	8.8	M12	*Mb*
LM397	46.85	22.67	F	65.7	9.4	H01	*Mm?*
LM398	46.85	22.67	M	57.2	8.8	H78	*MmW*
LM399	46.85	22.67	F	63.5	10.2	H78	*MmW*
LM400	37.94	34.72	M	64.5	10.3	T12	*MmE*
LM401	37.94	34.72	F	67.9	10.5	T03	*MmE*
LM402	37.94	34.72	F	67.6	10.1	T13	*MmE*
LM403	37.94	34.72	M	62.4	10.6	T03	*MmE*
LM404	37.94	34.72	F	64.9	10.3	H35	*MmE*
LM405	37.94	34.72	F	64.2	9.8	T15	*MmE*
LM406	37.94	34.72	M	66.1	10.4	T03	*MmE*
LM407	37.94	34.72	F	61.1	9.9	T15	*MmE*
LM408	37.94	34.72	M	63.9	10	T15	*MmE*
LM409	37.94	34.72	F	66.6	10.3	T03	*MmE*
LM410	37.94	34.72	F	66.4	10.2	T03	*MmE*
LM411	41.50	27.92	F	65.3	9.4	T01	*MmE*
LM412	41.50	27.92	M	59.6	9.4	T03	*MmE*
LM413	41.50	27.92	F	62.2	9.4	T03	*MmW*
LM414	41.50	27.92	F	61.4	8.7	H35	*Mb*
LM415	41.50	27.92	F	65.2	9.5	T03	*MmE*
LM416	41.50	27.92	M	62.2	9.8	T03	*MmE*
LM417	41.50	27.92	M	60.5	9.9	T03	*Mm?*
LM418	41.50	27.92	M	59.9	9.2	T03	*MmW*
LM419	41.50	27.92	M	57.5	7.4	H35	*Mb*
LM420	41.50	27.92	M	56	8.3	T02	*Mb*
LM421	41.50	27.92	F	58.2	7.8	T06	*Mb*
LM422	37.94	34.72	F	67.2	10.5	T15	*MmE*
LM423	41.29	28.32	F	58.6	8.5	H35	*Mb*
LM424	41.29	28.32	M	57.7	8.2	H35	*Mb*
LM425	41.29	28.32	F	55.9	7.6	H35	*Mb*
LM426	37.94	34.72	F	66.3	10.4	T03	*MmE*
LM427	41.29	28.32	M	56.7	7.5	T05	*Mb*

LM428	41.29	28.32	M	59.8	8.9	T05	*Mb*
LM429	41.29	28.32	F	59.8	8.8	H35	*Mb*
LM430	37.94	34.72	F	63.6	10.5	T03	*MmE*
LM431	41.84	27.56	F	57.8	N/A	T06	*Mb*
LM432	41.84	27.56	F	59.5	N/A	T45	*Mb*
LM433	39.35	27.81	M	59.2	N/A	H35	*Mb*
LM434	39.35	27.81	F	60.5	N/A	H35	*Mb*
LM435	39.35	27.81	F	60.4	N/A	T03	*Mb*
LM436	39.35	27.81	M	57.4	N/A	H35	*Mb*
LM437	41.08	37.30	F	65.7	10.6	L37	*MmE*
LM438	41.08	37.30	M	64.7	10.7	T15	*MmE*
LM439	41.08	37.30	M	62.8	10.6	T15	*MmE*
LM440	41.08	37.30	M	62.4	10.7	N15	*MmE*
LM441	41.08	37.30	F	65.8	10.5	L54	*MmE*
LM442	41.08	37.30	M	64.9	10.5	L37	*MmE*
LM443	41.08	37.30	F	66.7	10.4	L37	*MmE*
LM444	41.08	37.30	M	63.7	10.4	T15	*MmE*
LM445	41.08	37.30	M	64.2	10.4	T15	*MmE*
LM446	41.08	37.30	F	68.7	10.2	T15	*MmE*
LM447	41.08	37.30	F	66.7	10.7	T15	*MmE*
LM448	41.08	37.30	F	65.8	10.1	T15	*MmE*
LM449	41.08	37.30	F	65.7	10.2	T15	*MmE*
LM450	41.08	37.30	M	64.9	10.2	L54	*MmE*
LM451	41.08	37.30	M	63.6	10.4	T15	*MmE*
LM452	41.08	37.30	F	65	10.5	L37	*MmE*
LM453	41.08	37.30	F	66.3	10.6	L37	*MmE*
LM454	46.13	25.32	M	58.4	9.2	H01	*MmW*
LM455	46.13	25.32	F	64.3	9.4	H01	*MmW*
LM456	46.13	25.32	F	62.7	10.4	H01	*MmW*
LM457	46.13	25.32	F	63.1	9.9	H01	*MmW*
LM458	46.13	25.32	M	60.4	10.2	H01	*MmW*
LM459	46.13	25.32	M	61.4	10.7	H01	*Mm?*

LM460	46.13	25.32	M	60.7	10.4	H01	*MmW*
LM461	46.13	25.32	M	59.8	9.9	H01	*MmW*
LM462	46.13	25.32	M	57.4	10.2	H01	*MmW*
LM463	46.13	25.32	F	61.9	10.3	H01	*MmW*
LM464	46.13	25.32	F	61.2	9.6	H01	*MmW*
LM465	46.13	25.32	M	57.6	9.2	H01	*MmW*
LM466	46.13	25.32	F	62.5	9.9	H01	*MmW*
LM467	46.13	25.32	F	64.7	10.4	H01	*MmW*
LM468	46.13	25.32	F	65.9	10.4	H01	*MmW*
LM469	46.13	25.32	F	63.6	10.2	H01	*MmW*
LM470	46.13	25.32	F	60.9	10	H01	*MmW*
LM471	46.13	25.32	F	59.8	9.9	H01	*MmW*
LM472	46.13	25.32	F	61.3	10.4	H01	*MmW*
LM473	46.13	25.32	M	58.8	10.2	H01	*MmW*
LM474	46.13	25.32	F	61.9	9.9	H01	*MmW*
LM475	46.13	25.32	M	58.5	9.9	H03	*MmW*
LM476	46.13	25.32	F	60.4	9.5	H01	*MmW*
LM477	46.13	25.32	F	62.4	9.8	H01	*MmW*
LM478	46.13	25.32	M	62.6	10.3	H01	*MmW*
LM479	46.13	25.32	M	59.4	9.7	H01	*MmW*
LM480	46.13	25.32	M	58.6	10.2	H01	*MmW*
LM481	46.13	25.32	F	62.2	8.8	H01	*MmW*
LM482	46.13	25.32	F	63.5	9.8	H01	*MmW*
LM483	46.13	25.32	M	58.4	10.9	H01	*MmW*
LM484	46.13	25.32	M	58.4	9.8	H01	*MmW*
LM485	46.13	25.32	F	61.9	9.5	H01	*MmW*
LM486	46.13	25.32	F	63.7	10.5	H01	*MmW*
LM487	46.13	25.32	F	63.4	9.7	H01	*MmW*
LM488	46.13	25.32	F	60.8	9.9	H01	*MmW*
LM489	46.13	25.32	F	60.4	9.6	H01	*MmW*

APPENDIX B: ACCESSION NUMBERS OF THE MITOCHONDRIAL HVII HAPLOTYPES USED IN THIS STUDY

HVII Haplotype	Clade	Genbank Accession Number	Reference
H01	A	AF368763	Castella et. al., 2001
H03	A	AF368764	Castella et. al., 2001
H10	A	AF368762	Castella et. al., 2001
H11	B	AF368774	Castella et. al., 2001
H12	B	AF368775	Castella et. al., 2001
H15	C/E	AF368771	Castella et. al., 2001
H17	A	EU374587	Ruedi et. al., 2008
H19	A	EU374589	Ruedi et. al., 2008
H20	A	EU374590	Ruedi et. al., 2008
H22	A	EU374592	Ruedi et. al., 2008
H24	A	EU374594	Ruedi et. al., 2008
H27	A	EU374597	Ruedi et. al., 2008
H29	A	EU374599	Ruedi et. al., 2008
H30	A	EU374600	Ruedi et. al., 2008
H33	A	EU374603	Ruedi et. al., 2008
H34	D	EU374604	Ruedi et. al., 2008
H35	D	EU374605	Ruedi et. al., 2008
H36	D	EU374606	Ruedi et. al., 2008
H37	C/E	EU374607	Ruedi et. al., 2008
H42	F	EU374612	Ruedi et. al., 2008
H48	B	EU374618	Ruedi et. al., 2008
H49	B	EU374619	Ruedi et. al., 2008
H51	B	EU374621	Ruedi et. al., 2008
H52	B	EU374622	Ruedi et. al., 2008
H55	F	EU374625	Ruedi et. al., 2008
H59	D	N/A	Berthier et. al., 2006
H60	F	N/A	Berthier et. al., 2006

H63	A	N/A	Berthier et. al., 2006
H65	A	N/A	Berthier et. al., 2006
H66	C/E	N/A	Berthier et. al., 2006
H68	F	N/A	Berthier et. al., 2006
H77	A	HM117845	Bryja et. al., 2010
H78	A	HM117845	Bryja et. al., 2010
H79	A	HM117845	Bryja et. al., 2010
L01	D	KM235225	Furman, et. al., 2014
L02	D	KM235226	Furman, et. al., 2014
L03	AZOKH	KM235227	Furman, et. al., 2014
L04	D	KM235228	Furman, et. al., 2014
L05	C/E	KM235229	Furman, et. al., 2014
L06	B	KM235230	Furman, et. al., 2014
L07	F	KM235231	Furman, et. al., 2014
L08	F	KM235232	Furman, et. al., 2014
L09	F	KM235233	Furman, et. al., 2014
L10	D	KM235234	Furman, et. al., 2014
L11	AZOKH	KM235235	Furman, et. al., 2014
L13	C/E	KM235237	Furman, et. al., 2014
L14	C/E	KM235238	Furman, et. al., 2014
L15	C/E	KM235239	Furman, et. al., 2014
L16	C/E	KM235240	Furman, et. al., 2014
L16	C/E	KM235241	Furman, et. al., 2014
L17	B	KM235241	Furman, et. al., 2014
L18	B	KM235242	Furman, et. al., 2014
L19	A	KM235243	Furman, et. al., 2014
L20	A	KM235244	Furman, et. al., 2014
L21	A	KM235245	Furman, et. al., 2014
L22	A	KM235246	Furman, et. al., 2014
L23	D	KM235247	Furman, et. al., 2014
L24	D	KM235248	Furman, et. al., 2014

L25	C/E	KM235249	Furman, et. al., 2014
L26	A	KM235250	Furman, et. al., 2014
L27	C/E	KM235251	Furman, et. al., 2014
L28	C/E	KM235252	Furman, et. al., 2014
L33	D	KM235257	Furman, et. al., 2014
L34	F	KM235258	Furman, et. al., 2014
L35	D	KM235259	Furman, et. al., 2014
L36	F	KM235260	Furman, et. al., 2014
L37	D	KM235261	Furman, et. al., 2014
L38	D	KM235262	Furman, et. al., 2014
L39	D	KM235263	Furman, et. al., 2014
L40	D	KM235264	Furman, et. al., 2014
L41	D	KM235265	Furman, et. al., 2014
L44	D	KM235268	Furman, et. al., 2014
L45	F	KM235269	Furman, et. al., 2014
L51	A	KM235275	Furman, et. al., 2014
L52	D	KM235276	Furman, et. al., 2014
L53	D	KM235277	Furman, et. al., 2014
L54	D	KM235278	Furman, et. al., 2014
L55	D	KM235279	Furman, et. al., 2014
L56	D	KM235280	Furman, et. al., 2014
L57	AZOKH	KM235281	Furman, et. al., 2014
L58	AZOKH	KM235282	Furman, et. al., 2014
M06	A	JX442067	Furman, et. al., 2013
M11	D	JX442069	Furman, et. al., 2013
M12	D	GU817340	Bogdanowicz et.al., 2009
M18	D	GU817341	Bogdanowicz et.al., 2009
M21	D	GU817343	Bogdanowicz et.al., 2009
M63	F	GU817350	Bogdanowicz et.al., 2009
New1	A	MF782433	Furman, et. al., 2017
New2	D	MF782437	Furman, et. al., 2017

New9	D	N/A	N/A
New10	D	N/A	N/A
New11	D	MF782434	Furman, et. al., 2017
New15	D	N/A	N/A
New16	D	MF782435	Furman, et. al., 2017
New16	D	N/A	N/A
New17	D	N/A	N/A
New18	D	MF782436	Furman, et. al., 2017
New18	D	N/A	N/A
New19	D	N/A	N/A
New20	D	MF782438	Furman, et. al., 2017
T01	D	JN688979	Furman et. al., 2011
T02	D	JN688980	Furman et. al., 2011
T03	D	JN688981	Furman et. al., 2011
T04	D	JN688982	Furman et. al., 2011
T05	D	JN688983	Furman et. al., 2011
T06	D	JN688984	Furman et. al., 2011
T11	D	JN688989	Furman et. al., 2011
T12	D	JN688990	Furman et. al., 2011
T13	D	JN688991	Furman et. al., 2011
T14	D	JN688992	Furman et. al., 2011
T15	D	JN688993	Furman et. al., 2011
T18	D	JN688996	Furman et. al., 2011
T19	D	JN688997	Furman et. al., 2011
T23	D	JN689001	Furman et. al., 2011
T24	D	JN689002	Furman et. al., 2011
T25	D	JN689003	Furman et. al., 2011
T26	D	JX442115	Furman et. al., 2013
T27	D	JX442116	Furman et. al., 2013
T30	A	JX442119	Furman et. al., 2013
T33	D	JX442122	Furman et. al., 2013

T45	D	JX442134	Furman et. al., 2013
T60	D	JX442149	Furman et. al., 2013
T61	D	JX442150	Furman et. al., 2013
T62	D	JX442151	Furman et. al., 2013
T67	D	JX442156	Furman et. al., 2013
T68	D	JX442157	Furman et. al., 2013
T69	D	JX442158	Furman et. al., 2013
T70	D	JX442159	Furman et. al., 2013
T72	D	JX442161	Furman et. al., 2013
T73	D	JX442162	Furman et. al., 2013
T74	D	JX442163	Furman et. al., 2013

*Mb represents *Myotis blythii s.l.*, MmE represents the eastern lineage of *Myotis myotis* and MmW represents the western lineage of *Myotis myotis*. The species memberships are given according to the STRUCTURE results for K=3.

APPENDIX C: HVII SEQUENCES ADDED TO THE LITERATURE DURING THIS STUDY

N01

TCTCGATGGGATAATTACTAATCACCCCATGCCGCGGCATAACTGTAATGTCATACCCT
TGGTATTTTTTATTTTTAGGGGATGCTTGGACTCAACATTGGCCGTCAAAGGCCCCGAT
CAGACCATAAAGTCAAGCTGGACTTTAAATGTACACCCTAAGCCCGCATAATGAGGAT
GCAGGACTATCGTGTTAATGTTCTAGGGACATAAATATATGGGTCGTGATATCATAAG
AATGGACTTCGTAAATTTATATTACGGCCTATGGTGCTAATAGATTAATGGTTACAG

N02

TCTCGATGGNATAATTACTAATCAGCCCATGCCGCGGCATAACTGTAATGTCATACCCT
TGGTATTTTTTATTTTTAGGGGATGCTTGGACTCAACATTGGCCGTCAAAGGCCCCGAT
CAGACCATAAAGTCAAGCTGGACTTTAAATGTACACCCTGAGCCCGCATAATGAGGAT
GCAGGACTATCATGTTAATGTTCTAAGGACATGAATATATGGGTCGTGATATCATAGG
AATGGACTTCGTAAATTTATATTACGGCCTATGGTGCTAATAGATTAATGGTTACAG

N09

TCTCGATGGGATAATTACTAATCAGCCCATGCCGCGGCATAACTGTAATGTCATACCCT
TGGTATTTTTTATTTTTAGGGGATGCTTGGACTCAACATTGGCCGTCAAAGGCCCCGAT
CAGACCATAGGGTCAAGCTGGACTTTGGATGTACACCCTGAGCCCGCATAATGAGGAT
GCAGGACTATCGTGTTAATGTTCTAAGGACATGAATGTATGGGTCGTGATATCATAGG
AATGGACTTCGTAAATTTATATTACGGCCTATGGTGCTAATAGATTAATGGTTACAG

N10

TCTCGATGGGATAATTACTAATCAGCCCATGCCGCGGCATAACTGTAATGTCATACCCT
TGGTATTTTTTATTTTTAGGGGATGCTTGGACTCAACATTGGCCGTCAAAGGCCCCGAT
CAGACCATAAAGTCAAGCTGGACTTTGGATGTACACCCTGAGCCCGCATAATGAGGAT
GCAGGACTATCGTGTTAATGTTCTAAGGACATGAATGTATGGGTCGTGATATCATAGG
AATGGACTTCGTAAATTTATATTACGGCCTATGGTGCTAATAGATTAATGGTTACAG

N11

TCTCGATGGGATAATTACTAATCAGCCCATGCCGCGGCATAACTGTAATGTCATACCCT
TGGTATTTTTTATTTTTAGGGGATGCTTGGACTCAACATTGGCCGTCAAAGGCCCCGGT
CAGACCATAAAGTCAAGCTGGACTTTAAATGTACACCCTGAGCCCGCATAATGAGGAT
GCAGGACTATCGTGTTAATGTTCTAAGGACATGAATATGTGGGTCGTGATATCATAGG
AATGGACTTCGTAAATTTATATTACGGCCTATGGTGCTAATAGATTAATGGTTACAG

N15

TCTCGATGGGATAATTACTAATCAGCCCATGCCGCGGCATAACTGTAATGTCATACCCT
TGGTATTTTTTATTTTTAGGGGATGCTTGGACTCAACATTGGCCGTCAAAGGCCCCGAT
CAGACCATAAGGTCAAGCTGGACTTTGGATGTACACCCTGAGCCCGCATAATGAGGAT
GCAGGACTATCGTGTTAATGTTCTAAGGACATGAATGTATGGGTCGTGATATCATAAG
AATGGACTTCGTAAATTTATATTACGGCCTATGGTGCTAATAGATTAATGGTTACAG

N16

TCTCGATGGGATAATTACTAATCAGCCCATGCCGCGGCATAACTGTAATGTCATACCCT
TGGTATTTTTTATTTTTAGGGGATGCTTGGACTCAACATTGGCCGTCAAAGGCCCCGGT
CAGACCATAAAGTCAAGCTGGACTTTAAATGTACACCCTGAGCCCGCATAATGAGGAT
GCAGGACTATCGTGTTAATGTTCTAGGGACATGAATATATGGGTCGTGATATCATAGG
AATGGACTTCGTAAATTTATATTACGGCCTATGGTGCTAATAGATTAATGGTTACAG

N17

TCTCGATGGGATAATTACTAATCAGCCCATGCCGCGGCATAACTGTAATGTCATACCCT
TGGTATCTTTTATTTTTAGGGGATGCTTGGACTCAACATTGGCCGTCAAAGGCCCCGGT
CAGACCATAAAGTCAAGCTGGACTTTAAATGTACACCCTGAGCCCGCATAATGAGGAT
GCAGGACTATCGTGTTAATGTTCTAAGGACATGAATATATAAGTCGTGATATCATAGG
AATGGACTTCGTAAATTTATATTACAGCCTATGGTGCTAATAGGTTAATGGTTACAG

N18

TCTCGATGGGATAATTACTAATCAGCCCATGCCGCGGCATAACTGTAATGTCATACCCT
TGGTATTTTTTATTTTTAGGGGATGCTTGGACTCAACATTGGCCGTCAAAGGCCCCGGT
CAGACCATAAAGTCAAGCTGGACTTTAAATGTACACCCTGAGCCCGCATAATGAGGAT
GCAGGACTATCGTGTTAATGTTCTAAGGACATGAATATATGGGTCGTGATATCATAGG
AATGGACTTCGTAAATTTATATTACGGCCTATGGTGCTAATAGGTTAATGGTTACAG

N19

TCTCGATGGGATAATTACTAATCAGCCCATGCCGCGGCATAACTGTAATGTCATACCCT
TGGTATTTTTTATTTTTAGGGGATGCTTGGACCCAACATTGGCCGTCAAAGGCCCCGGT
CAGACCATAAGGTCAAGCTGGACTTTAAATGTACACCCTGAGCCCGCATAATGAGGAT
GCAGGACTATCGTGTTAATGTTCTAAGGACATGAATATACGAGTCGTGATATCATAGG
AATGGACTTCGTAAATTTATATTACGGCCTATGGTGCTAATAGATTAATGGTTACAG

N20

TCTCGATGGGATAATTACTAATCAGCCCATGCCGCGGCATAACTGTAATGTCATACCCT
TGGTATTTTTTATTTTTAGGGGATGCTTGGACTCAACATTGGCCGTCAAAGGCCCCGGT
CAGACCATAAAGTCAAGCTGGACTTCAAATGTACACCCTGAGCCCGCATAATGAGGAT
GCAGGACTATCGTGTTAATGTTCTAAGGACATGAATATATGGGTCGTGATATCATAGG
AATGGACTTCGTAAATTTATATTACGGCCTATGGTGCTAATAGATTAATGGTTACAG

APPENDIX D: PHYSICAL AND GENETIC DISTANCE* MATRIX FOR LARGE MOUSE-EARED BAT POPULATIONS IN THE EXTENDED CONTACT ZONE

	GuraDobrogei	Horataşı	Karanlık	Kocakuyu	Lesu	Yazören	Armutludelik	Epcik	Ilgın	Karanlık	Mantarini	Delikli	Yazkonağı	Devetashka	Lesu	Studnisko	Vargish
GuraDobrogei	0																
Horataşı	347	0															
Karanlık	1131	925	0														
Kocakuyu	367	41	885	0													
Lesu	521	727	1627	765	0												
Yazören	585	239	815	220	932	0											
Armutludelik	900	703	232	662	1396	620	0										
Epcik	900	706	231	665	1398	625	8	0									
Ilgın	744	485	449	445	1205	370	251	257	0								
Karanlık	1131	925	0	885	1627	815	232	231	449	0							
Mantarini	554	296	630	255	1011	251	409	413	195	630	0						
Delikli	997	778	150	737	1485	666	111	115	300	150	482	0					
Yazkonağı	812	784	548	751	1331	828	413	406	551	548	577	508	0				
Devetashka	360	332	1258	373	438	502	1035	1038	815	1258	628	1110	1076	0			
Lesu	521	727	1627	765	0	932	1396	1398	1205	1627	1011	1485	1331	438	0		
Studnisko	870	1154	1999	1188	467	1376	1768	1768	1608	1999	1415	1867	1615	898	467	0	
Vargish	305	555	1426	589	218	780	1194	1195	1017	1426	822	1287	1113	339	218	598	0

	Mb_GuraDobroge	Mb_Horataşı	Mb_Karanlık	Mb_Kocakuyu	Mb_Lesu	Mb_Yazören	MmE_Armutlude	MmE_Epcik	MmE_Ilgın	MmE_Karanlık	MmE_Mantarini	MmE_Delikli	MmE_Yazkonağ	MmW_Devetash	MmW_Lesu	MmW_Studnisko	MmW_Vargish
Mb_Karanlık (n=14)	0.71154	0.59449	0														
Mb_Kocakuyu (n=10)	0.11111	0.0127	0.69123	0													
Mb_Lesu (n=7)	0.44444	0.10069	0.57942	0.39145	0												
Mb_Yazören (n=7)	0.13333	0.0411	0.59153	0.12847	0.35294	0											
MmE_Armutludelik (n=17)	0.36538	0.24578	0.43046	0.34978	0.33526	0.14178	0										
MmE_Epcik (n=23)	0.57143	0.45258	0.54773	0.54951	0.46528	0.43854	0.13799	0									
MmE_Ilgın (n=23)	0.88333	0.65154	0.51879	0.8212	0.68451	0.58667	0.31341	0.50636	0								
MmE_Karanlık (n=14)	1	0.68519	0.70414	0.91919	0.74359	0.60606	0.28261	0.6442	0.88333	0							
MmE_Mantarini (n=14)	0.96296	0.69788	0.56145	0.88814	0.7285	0.64015	0.33133	0.52915	0.05253	0.96296	0						
MmE_Delikli (n=19)	0.80093	0.57837	0.61902	0.75032	0.65682	0.46515	0.23651	0.57408	0.70417	0.2037	0.75766	0					
MmE_Yazkonağı (n=20)	0.71319	0.58929	0.61982	0.68729	0.58006	0.59734	0.30099	0.02816	0.63572	0.78183	0.66395	0.71168	0				
MmW_Devetashka (n=7)	0.56589	0.48227	0.55487	0.55155	0.51942	0.46689	0.38181	0.44609	0.5437	0.60284	0.55889	0.55391	0.51278	0			
MmW_Lesu (n=19)	0.94302	0.82756	0.79096	0.91685	0.83445	0.83875	0.68749	0.74685	0.90416	0.94302	0.93139	0.87022	0.80874	0.15331	0		
MmW_Studnisko (n=18)	1	0.87681	0.82517	0.97133	0.87654	0.89431	0.72951	0.78571	0.95758	1	0.98734	0.92168	0.84715	0.18841	0.04762	0	
MmW_Vargish (n=35)	0.99526	0.87302	0.82268	0.96687	0.87318	0.88997	0.72662	0.78292	0.95324	0.99526	0.98272	0.91759	0.84426	0.18678	0.04419	0	0

* The first matrix indicates the air distance between the caves in kilometers, the secong matrix indicates the genetic distances between western M. myotis (MmW), eastern M. myotis (MmE), and M. blythii s.l. (Mb) as fixation indexes.