DETERMINATION OF THE POPULATION GENETIC STRUCTURE OF *Miniopterus schreibersii* IN THE ANATOLIAN TRANSITION ZONE

by

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ABSTRACT

Populations and species boundaries of *Miniopterus schreibersii* were investigated in Thrace and central Anatolia, using the sequenced segments of a mitochondrial DNA control region. Analyses confirmed the presence of two distinct forms of *M. schreibersii*, namely *M. s. schreibersii* and *M. s. pallidus*. The results showed that the forms separated approximately 750,000 years before present and underwent the subsequent expansions of their populations. The percent of genetic variance among clades was very high compared to variances within the clades, indicating a significant mitochondrial separation between forms. Analysis indicated that the range of *M. s. schreibersii* in Turkey included Thrace and all coastal regions, whereas *M. s. pallidus* occupied the inland regions of central Anatolia. The results also pointed out to a significant difference in forearm lengths between *M. s. schreibersii* and *M. s. pallidus*. These forms might be distinct species, rather than subspecies, as they are recognized now.

ÖZET

Bu çalışmada, Trakya ve Orta Anadolu'daki *Miniopterus schreibersii* yarasasının popülasyon ve tür sınırları mitokondrial DNA sekansları kullanılarak incelenmiştir. Analizler, iki farklı *M. schreibersii* türününün (*M. s. schreibersii* ve *M. s. pallidus*) varlığını onayladı. Sonuçlar bu iki farklı formun günümüzden yaklaşık olarak 750,000 yıl önce ayrıldığını ve popülasyonlarının daha sonra genişlediğini gösterdi. İki grup arasındaki genetik değişimin oranı, grupların içindeki değişime göre çok daha yüksekti ki bu da formların mitokondrial olarak birbirinden ayrıldığına işaret etti. Analizler *M. s. schreibersii* alt türünün Trakya ve Anadolu'nun sahil kesimlerimde, *M. s. pallidus* alt türünün ise orta Anadolu'nun iç kesimlerimde bulunduğunu gösterdi. Ayrıca sonuçlar, bu iki alt türün ön kol uzunluklarının arasında istatistiksel olarak anlamlı bir farkın olduğunu ortaya koydu.

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DNA	Deoxy-ribonucleic Acid
mtDNA	Mitocondrial Deoxy-ribonucleic Acid
RNA	Ribonucleic Acid
tRNA	Transfer Ribonucleic Acid
PCR	Polymerase Chain Reaction
dNTP	Deoxy-ribonucleotide Trishosphate
G + C	Guanine + Cytosine
bp	Base Pairs
kb	1000 Base Pairs
pg	10^{-9} Kg
µL	10^{-6} L
mM	10^{-3} M
µM	10^{-9} M
U	Units

1. INTRODUCTION

The Chiroptera (bats) is the second biggest order of mammals after the Rodentia (rodents). There are 963 bat species in 18 chiropteran families that are distributed all over the world. Bats fill unique ecological roles within natural systems and agricultural areas. For example, fruit eating bats are main pollinators and seed dispersers for many tropical plants (Race and Swift, 1995) and insectivore bats are vital predators of agricultural pests (Pierson, 1998). Consequently, bats are of both ecological and economic importance. They display a wide variety of behavioral and physiological adaptations that are unique to their taxa. However, the low fecundity of bats combined with the destruction of their habitats and increased usage of pesticides, put them under a constant risk of extinction. Therefore, understanding of the ecology and the social structure of bats is essential both for establishing effective conservation programs, and for outlining the details of their evolutionary history.

Bats ability of flight and nocturnality confounded by their longevity, complicated life cycles that may involve hibernation and/or migration, and their gregarious nature, make them a particularly challenging group of animals for biological observation. Frequently, the use of conventional methods to monitor bat populations, such as direct observation of individuals or capture-recapture techniques, is greatly restricted. Here, the introduction of the molecular genetic techniques provided an invaluable new source of information. Following the improvements in molecular applications that utilize polymerase chain reaction (PCR) and do not require destructive sampling, molecular genetics has been a common practice in population studies.

PCR based applications can amplify a specific region of DNA that fits to particular research objectives. To compare the affects of biogeographical factors on populations, such as isolation and climatic changes, the amplified DNA regions have to be highly polymorphic and neutral to the natural selection. Markers that satisfy these requirements are mitochondrial DNA (mtDNA) and microsatellites. MtDNA, which is uniparentally inherited, is used to

analyze the phylogenetic relations between the extant populations and can estimate the historical factors that affected the spatial dynamics of populations. On the other hand, highly polymorphic and biparentally inherited microsatellites are used to investigate current factors related to breeding structure and gene flow between and within subpopulations (Beebee and Rowe, 2004). The molecular genetic studies of bats deal with three main subjects: identification of species boundaries, population genetic structure, and social organization. This study focuses on to the first two concepts to investigate the populations of *Miniopterus schreibersii* in central Anatolia.

1.1. Identification of Species Boundaries

There are many debates on the chiropteran systematics. The debates are usually focused on the subspecific nominations of taxa with wide distributions. Frequently, morphological traits alone cannot clearly define taxonomical status of the bat species and in such cases, molecular genetics become an essential tool for identification of species boundaries. This problem is well exemplified in the European pipistrelle bat (*Pipistrellus pipistrellus*). For many years, pipistrelle bat had been recognized as a single species. Only after the identification of two types of phonic echolocation (Jones and van Parijs, 1993) the unity of the single species had been questioned. In 1997 mtDNA analysis confirmed that there were indeed two distinct lineages, which later acquired different taxonomical status (Barrat et al., 1997).

Identification of species boundaries can also help to clarify the taxonomical status and the classification of lineages that are particularly worthy of conservation. Determination of species distributions is also important for setting conservation priorities and assessing the hybridization effects of on species biology (Soltis and Gitzendanner, 1999).

1.2. Population Genetic Structure

A population can be defined as a set of individuals that are separated in time and space from other populations in regards to mating and dispersal patterns. The differentiations in such patterns are the results of the populations' evolutionary histories. Historical patterns of

colonization, dispersal habits, and migratory behaviors of populations define populations' genetic layout.

Considering bats' ability to fly, it might be expected that the genetic variation of bat populations will be low because of the existing opportunity for the constant gene flow. Population genetic studies, however, found that some bat species have a clearly pronounced genetic structure. The genetic structure of the extant populations is a function of many factors, such as migratory and mating behavior, existence of physical barriers, and past colonization patterns.

1.2.1. Seasonal Migration

The genetic differentiation of two populations is related to the gene flow and migration rates between them. If the gene flow between two populations is high, then their genetic layouts will eventually become identical. On the other hand, two isolated populations will exhibit distinct genetic patterns. As many bat species within the temperate regions migrate between the summer and winter roosting sites, it might be expected that they will exhibit low levels of genetic structure. Indeed, almost all of the genetic studies on migratory bat species support these predictions (Burland and Wilmer, 2001).

The patterns of genetic structure in sedentary bat species are more complicated. Many bat species exhibit significant correlation between geographic and genetic distances that resembles restricted gene flow among subpopulations. In some cases, however, the extensive gene flow between adjacent populations can overcome the limited dispersal capacity of the species. For example, the genetic analyses of brown long-eared bats (*Plecotus auritus*) in North-east Scotland populations identified no genetic differentiation, which suggests that the *P. auritus* populations in that region are continuously mixed via a 'stepping stone' model (Burland, 1998). This also suggests that continuously distributed populations can more efficiently homogenize the genetic structure than those in which the individuals' based gene exchange is the main driving force.

In summary, it can be concluded that migratory species usually have homogenized genetic distributions, whereas sedentary species have more structured genetic layouts. However, factors such as mating patterns and physical barriers can considerably change this conclusion.

1.2.2. Physical Barriers to Gene Flow

Physical features such as mountain ranges, water bodies, and the availability of suitable habitats may act as an effective barrier to the gene flow between the populations. Geographical factors directly limit the dispersal of animals, and hence the genes transfer between the populations. Bats' mobility, however, can notably decrease their affects. A study, which analyzed the mtDNA lineages of *Artibeus jamaicensis* populations in Barbados, Jamaica and St.Vincents revealed that the most common haplotype was distributed in all of the islands with a range of about 1400 km (Pumo et al., 1988).

1.2.3. Past Processes

In addition to the migratory behavior and physical barriers, past processes can also affect the genetic structure of the extant populations. Past population fragmentations caused by the natural historical events, such as climatic changes, land movements, and past colonization patterns, may cause temporary isolations and hence genetic differentiation of populations. Therefore, the current distribution of the populations and their genetic layouts are directly related to the past processes. A study examining the island populations of two sympatric species of Philippine fruit bat (*Cyonopterus brachyotis* and *Haplonycteris fischeri*), did not find any significant structuring in either of them (Peterson and Heaney, 1993). However, when a further analysis included the effect of Pleistocene geography, the high correlation between genetic and geographical distances was found for populations from the connected-in-the-past populations. In a similar study, the effect of Pleistocene was found on to the genetic structure of *Nyctalus noctula* populations in Europe; all extant populations were direct descendants of individuals originated from three different refugia in Southern Europe (Petit et al., 1999).

1.3. *Miniopterus schreibersii*

In Turkey, there are 36 recorded bat species, belonging to five families. Except for the Vespertilionidae family, which has nine genera, the rest of the families are represented by a single genus (MacDonald and Barrett, 1998). *Miniopterus schreibersii* (Kuhl, 1817), the bent-wing bat, is one of the most abundant bat species in Turkey (Figure 1.1). It usually inhabits cave habitats and is known to be a moderate migratory species.

Figure 1.1. A sampled *M. schreibersii*

Although it is generally accepted that there are two subspecies of *M. schreibersii* in Turkey, namely *M. s. schreibersii* and *M. s. pallidus*, there are many debates regarding their distribution. According to some taxonomists, western Turkey is inhabited by the nominate form *M. s. schreibersii* (Benda and Horacek, 1998; Corbet, 1978), and *M. s. pallidus* is present in eastern Turkey (Deblase, 1980; Koopman, 1994). On the other hand, Albayrak and Coşkun (2000) suggested that *M. s. schreibersii* was present only in Thrace and the rest of the Anatolia was inhabited by *M. s. pallidus*. Karataş and Sözen (2004) proposed a similar distribution, but

with the addition of a transition zone between Thrace and eastern Anatolia (Figure 1.2). The differentiation of the subspecies is mainly based on fur coloration; the nominate form having darker fur on the ventral side than *M. s. pallidus* (Steiner and Gaisler, 1994; Albayrak and Coşkun, 2000).

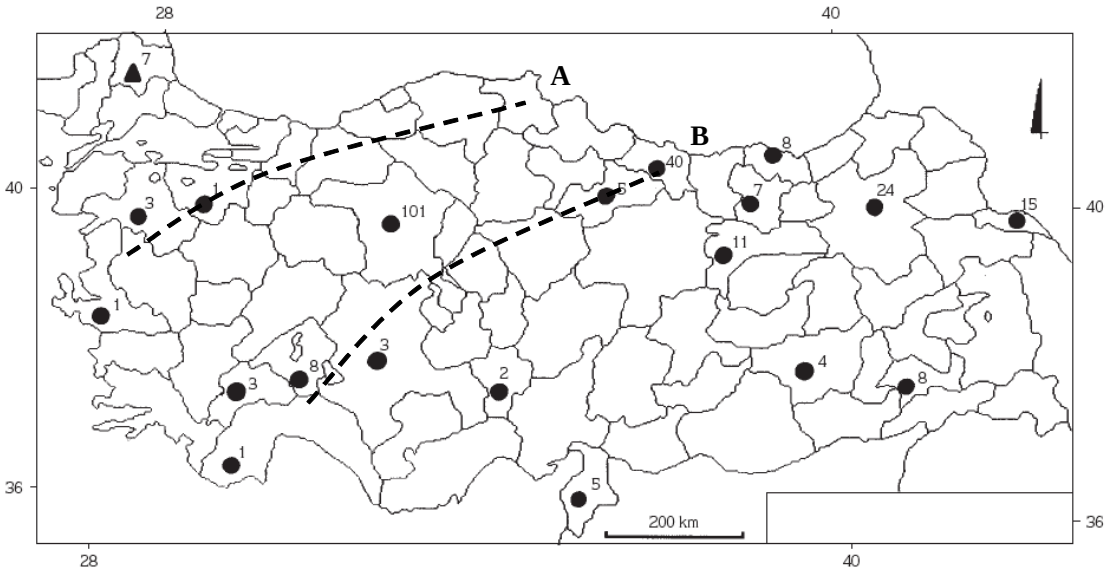

Figure 1.2. Distribution of *M. s. pallidus* and *M. s. schreibersii* in Turkey. According to Albayrak and Coşkun (2000): the presence of *M. s. pallidus* is marked by circles and *M. s. schreibersii* by triangles. According to Karataş and Sözen (2004): *M. s. pallidus* is found below the line B and *M. s. schreibersii* above the line A. The area between A and B is a transition zone.

A recent molecular genetic study investigating *M. schreibersii* in the Eurasian transition zone confirmed the presence of two genetically distinct lineages (Bilgin et al., 2006). Two individuals from eastern Anatolia classified as *M. s. pallidus* were genetically different from 65 individuals of *M. schreibersii* from Bulgaria, Greece, Thrace, and western Anatolia.

1.4. Mitochondrial DNA

MtDNA is a tool of choice for many researchers who aim to study phylogenetic relationships between and within species. It evolves quickly and is maternally inherited, allowing easy interpretations of maternal gene flow. Accordingly, many differences can be found in sequences of even closely related populations. MtDNA of most animals ranges in size from 16 to 18 kb and contains 13 protein genes, 22 transfer RNAs, two ribosomal RNAs, and a regulatory region known as the control region or the displacement loop (Wilkinson and Chapman, 1991). Although it is possible to amplify almost any segment of mtDNA genome (Kocher et al., 1989), the control region (Figure 1.3) is of a particular interest to molecular ecologists studying population related problems.

The control region is the only major noncoding segment of animal mtDNA and it is the most rapidly evolving and polymorphic region of the mtDNA genome (Vigilant et al., 1991). Its general organization is similar in all mammals and includes a central conserved region surrounded by two variable domains. These domains frequently contain arrays of tandem repeats of species-specific sizes (Fumagalli et al., 1996). R1 repeated sequences, located in the left domain of the control region near the tRNA-Pro, are about 80 bp in length. R2 repeats, located on the right domain of the control region have typically involve variable numbers of short 6 to 30 bp units, which often contain the 4 bp motif GTAC, and exhibit considerable length variation. The segment of mtDNA investigated in this study includes about 100 bp of cytochrome b and extends towards conserved sequence blocks. It comprises the R1 region, which is restrained in *M. schreibersii* to only one repeated sequence (Wilkinson et al., 1997).

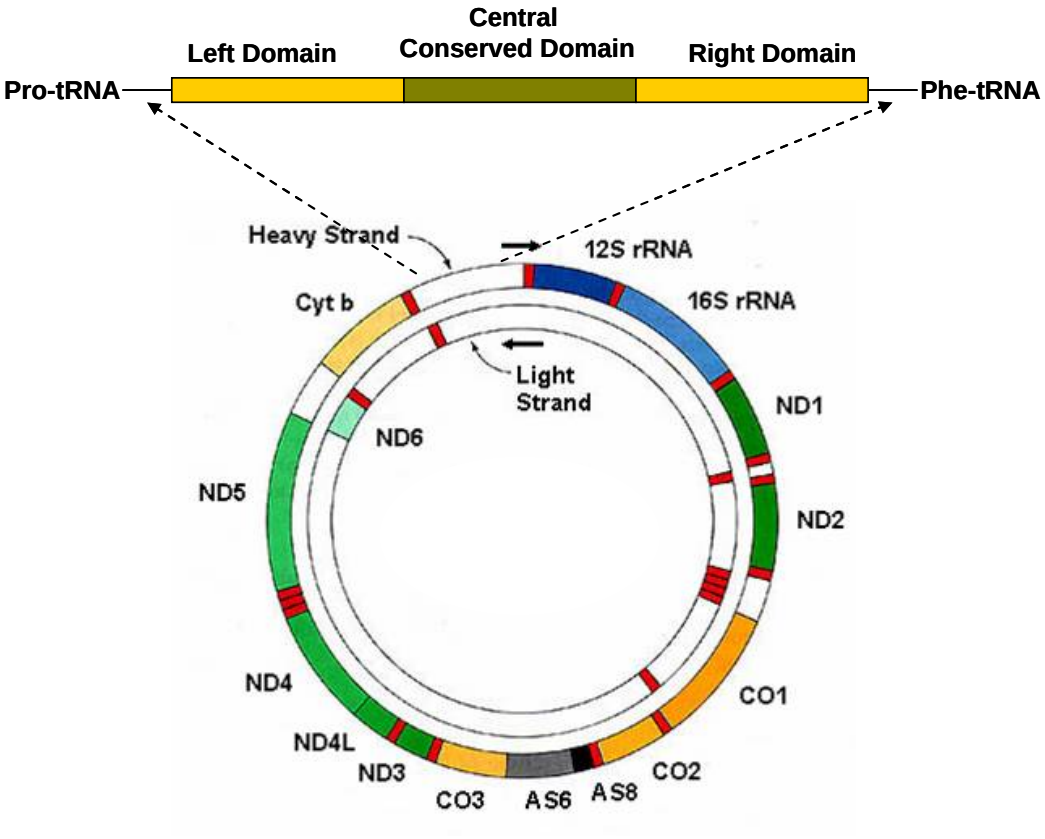

Figure 1.3. Vertebrate mitochondrial genome with amplified control region.

2. THESIS OBJECTIVE

This study aims to investigate the population genetic structure and the species boundaries of the bent-winged bat, *Miniopterus schreibersii* in the Anatolian transition zone. Recently, Bilgin et al. (2006) found two genetically distinct lineages of *M. schreibersii* in Turkey. Accordingly, the objective of this thesis is to focus on the suture zone to identify the distribution borders of these lineages. MtDNA sequences will be used to investigate the phylogenetic structure of the lineages, their demographical features, genetic differentiation, divergence time, and past population changes. Forearm length measurements will be analyzed for morphological differentiation between the groups.

3. MATERIALS AND METHODS

3.1. Fieldwork

The fieldwork was done in August and September, before the maternity roosts disband. The study area included 30 sites in the central Anatolia region and three sites in Thrace (Figure 3.1, Table 3.1). Samples from Georgia and Armenia were collected by other bat researchers.

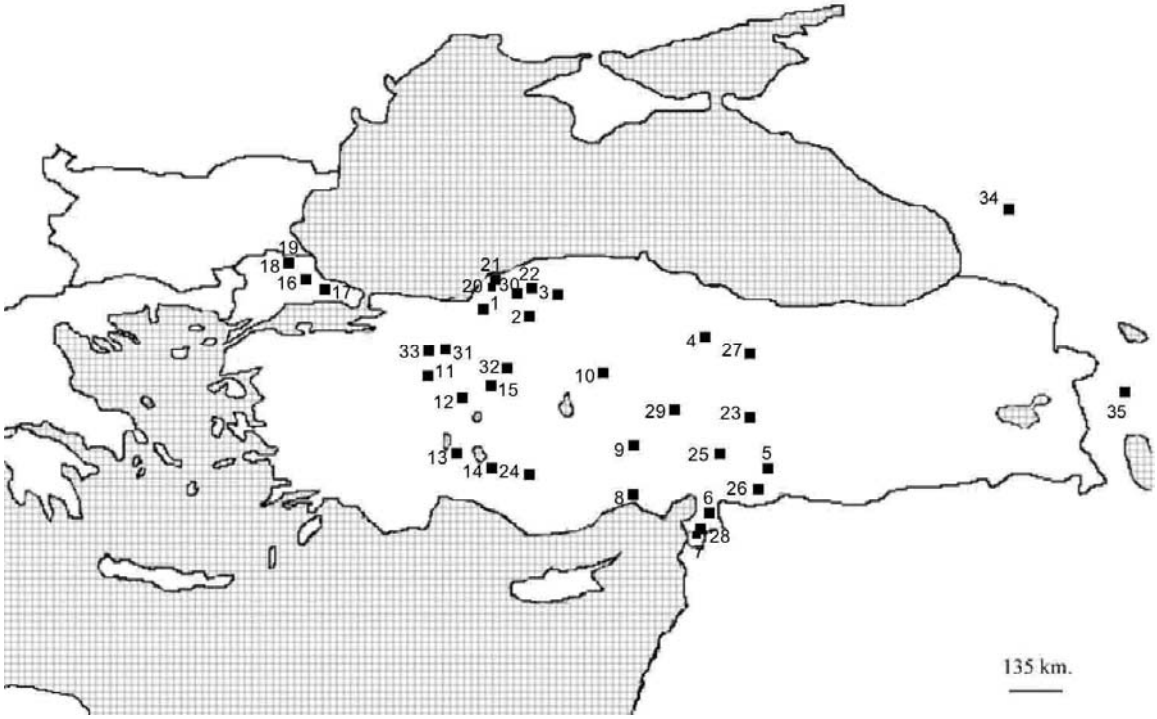

Figure 3.1. Study area and the locations of the caves.

Bats were caught by a hand net in the caves during daytime; they were released approximately one hour after the completion of the sampling protocol. Tissue samples were taken from the membranes of both wings with a 3 mm biopsy-puncher (Worthington Wilmer

and Barratt, 1996) and kept in 90% ethanol solution (Figure 3.2). The bats were sexed and their forearm lengths measured with a caliper (Figure 3.3).

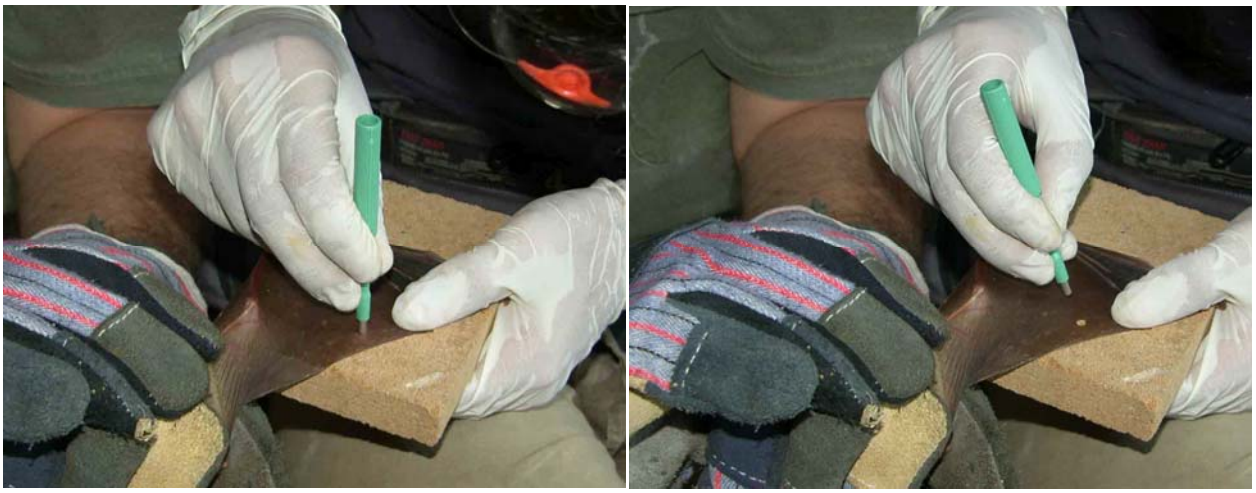

Figure 3.2. Punching of a bat.

Figure 3.3. Measuring bat's forearm length.

Table 3.1 Geographical locations of the caves

Cave Label	Name	Latitude	Longitude	Site
1	Cumayanı	N41.491501	E31.896200	Cave
2	Kelemen	N41.202202	E32.351398	Cave
3	Hızar	N41.316002	E32.659500	Cave
4	Ballıca	N40.280300	E36.298302	Cave
5	Küllü	N37.491501	E37.616199	Cave
6	Sütlü	N36.558800	E36.387100	Tomb
7	Karanlık	N36.222401	E36.202400	Cave
8	Delikli	N36.945202	E34.786800	Cave
9	Epcik	N37.977600	E34.800301	Cave
10	İnlimurat	N39.508202	E34.158298	Underground City
11	Ulubük	N40.006401	E30.845301	Cave
12	Dereköy	N38.979801	E31.202101	Cave
13	Zindan	N37.811901	E31.084499	Cave
14	Çaltepe	N37.499500	E31.820400	Cave
15	Yelini	N39.237499	E31.806000	Cave
16	Horataşı	N41.497299	E27.917700	Cave
17	Kocakuyu	N41.286800	E28.317200	Cave
18	Dupnisa	N41.841400	E27.557199	Cave
19	Kuru	N41.841301	E27.557100	Cave
20	Gökgöl	N41.441110	E31.832150	Cave
21	İnağzı	N41.479290	E31.822120	Cave
22	Mencilis	N41.280870	E32.635100	Cave
23	Şuğul	N38.749430	E37.233600	Cave
24	Güvercinlik 2	N37.499480	E31.820410	Cave
25	Döngel	N37.861650	E36.639100	Cave
26	Dülük	N37.158360	E37.365870	Cave
27	Mağara	N40.060850	E36.497150	Cave
28	Harbiye	N36.145290	E36.143870	Cave
29	Gesi	N38.775020	E35.642090	Underground City
30	Nallıhan	N41.479290	E31.822120	Cave
31	Ulubük	N40.006390	E30.845300	Cave
32	Yelinüstü	N39.295070	E31.798980	Cave
33	Sarıcakaya	N40.029010	E30.593980	Cave
34	Ghliana	N42,223750	E42.358540	Cave
35	Azokh	N39,371490	E46.593140	Cave

3.2. Molecular Analysis

3.2.1. DNA Extraction

Genomic DNA was extracted from one biopsy punch of each individual. Roche High Pure PCR Template Preparation Kit was used, following the manufacturer's protocol with two modifications. First modification was in the first step of the protocol in which the incubation time of sample tissue with Lysis Buffer and Proteinase K was increased up to 72 hours, to enhance the lyses of the cell membranes. The second modification was at the last step of washing procedure, where the amount of the Elution Buffer was lowered to 80 µL to increase the concentration of the total genomic DNA.

3.2.2. PCR Amplification of the Control Region in Mitochondrial DNA

For the mitochondrial DNA analysis, the hyper-variable region I of control region (D-loop), tRNA proline, and tRNA threonine genes of mtDNA was amplified (Figure 3.4). The amplification of this region was done with primers C and E (Wilkinson and Chapman, 1991).

Forward primer C 5'-TGAATTGGAGGACAACCAGT-3'
Reverse primer E 5'-CCTGAAGTAGGAACCAGATG-3'

The PCR amplifications were done in 25 µL reactions containing 2.5 µL of 10X PCR buffer, 2.5 µL of $MgCl_2$ (25 mM), 0.5 µL of dNTP mixture (10 mM), 0.5 µL of each primer, 1 µL of template DNA, and 0.5 µL Fermentas High Fidelity *Taq* Polymerase (5 units/µL). Samples were subjected to an initial denaturation step of 95 ºC for 90 seconds, followed by 35 cycles of 1 minute of denaturation at 95 ºC, 90 seconds of annealing at 58 ºC, 2 minutes of extension at 72 ºC, followed by a final extension step at 72 ºC for 7 minutes. The PCR products were purified by using Genemark PCR cleanup kit.

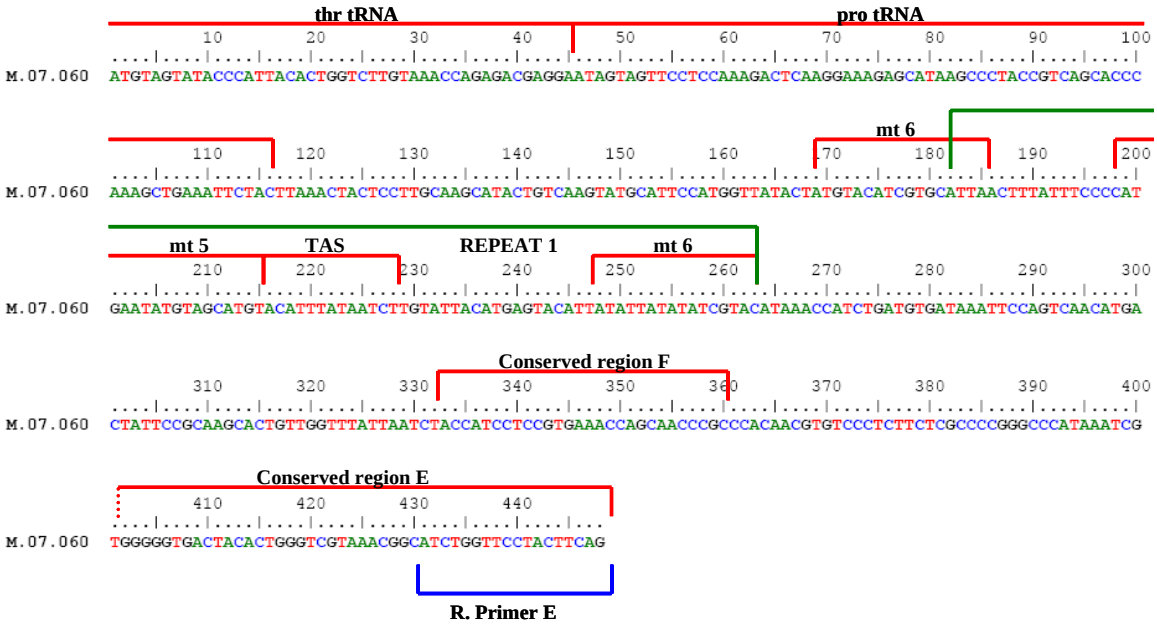

Figure 3.4. The segment of mtDNA control region amplified and analyzed in this study.

3.2.3. Gel Electrophoresis

The PCR products were checked on 2% agarose gels, prepared in 1X TBE (Tris base, boric acid, EDTA) buffer with ethidium bromide. For each reaction, 3 µL of the PCR product was loaded on the gel, mixed with 3 µL of 2X loading dye (Fermentas). Samples were run at 95 V for 45 minutes and finally, the band images were taken under ultraviolet light by Biorad Gel Doc Imaging System.

3.2.4. Sequencing of the PCR Products

Sequencing was done in Düzen Laboratories. The PCR products were first put into cycle sequencing, both in 5' and 3' directions, using the primers C and E, respectively. This involved 40 cycles in 10 µl reactions, which were composed of 1 µl of PCR product, 5.1 µl of H_20, 0.3 µl of primer (20 µM), 1,5 µl of florescent dye (ABI Big Dye) and 2 µl of 5X buffer. The cycle sequencing parameters for each cycle were 30 seconds of denaturation at 94 ^oC, 30

seconds of annealing at 58 ^{o}C and 30 seconds of extension at 72 ^{o}C, followed by a final extension of 10 minutes at 72 ^{o}C.

3.3. Analytical Methods

Sequence polymorphisms of the populations were analyzed by calculating the haplotype diversity (h), nucleotide diversity (P_i) and number of polymorphic sites (Graur and Li, 2000; Nei and Tajima, 1981) with the software DnaSP 4.10 (Rozas et al., 2003). Tajima's D statistic, which is based on the difference between the number of mutation sites and the average number of pairwise differences, was used to confirm the neutrality of the DNA polymorphism data.

Molecular phylogenies were estimated by Bayesian inference using MrBayes 3.b4 (Huelsenbeck and Ronquist, 2001, 2003). Bayesian analysis was done with four search chains for 1,000,000 generations, sampling trees every 100 generations. The first 25% of trees were discarded as burn-in and a consensus tree with posterior probabilities was generated. Parameter stability was assessed by plotting log-likelihood values against the generation time. The choice of a model was determined by Akaike Information Criterion (Akaike, 1973) and a hierarchical likelihood ratio test (Posada and Crandall, 1998), both calculated in MrModeltest (Nylander, 2004). MrModeltest indicated that the HKY+G model was the best fit for the data.

The genetical structure of the populations was investigated by the analysis of molecular variance (AMOVA) (Excoffier et al., 1992). The program Arlequin 3.0 (Schneider et al., 2000) was used to calculate the percent variation within and in between the populations. As a measure of the differentiation, the fixation index ϕ_{st} was calculated and its significance was tested by 1000 permutations.

Associations between haplotypes were determined by constructing a statistical parsimony network with the software TCS (Clement et al., 2000). Statistical parsimony network establishes the most parsimonious connections between the existing haplotypes. Correlations between the genetic and geographical distances were analyzed by spatial autocorrelation

analysis (Smouse and Peakall, 1999) with the software Genalex 6.3 (Peakall and Smouse, 2005). Spatial autocorrelation analysis calculates the autocorrelation coefficient by random shuffling of all individuals among the geographic locations. Confidence intervals are calculated from 1000 random permutations; if the calculated r-value falls outside the 95% confidence range, then the significant spatial genetic structure is inferred.

The demographic changes in the populations were examined by analyzing the mismatch distributions. Mismatch distribution analysis compares the observed frequencies of pairwise nucleotide differences with the expected values calculated by a constant-size and a range expansion model. Fu's Fs (Fu, 1997) and R_2 (Ramos-Onsins and Rozas, 2002) statistics were used to test the population growths. Both of the tests are based on the probability of having a number of haplotypes greater or equal to the observed number in sample drawn from a stationary population. These statistics and their significances were calculated with the software DnaSP 4.0 (Rozas et al., 2003). Bayesian Skyline plots (Drummond et al., 2005) were used to date the beginning of the expansions. The divergence time of reciprocally monophyletic clades were estimated by a Bayesian approach with the software BEAST 1.3 (Drummond and Rambaut, 2003).

Finally, the forearm measurements of the bats were analyzed by two-way ANOVA. Binary logistic regression was used to assess the probability of correct predictions of clade memberships from the forearm lengths.

4. RESULTS AND DISCUSSION

For the mtDNA analysis, 56 samples collected from 11 sites were sequenced. The sequencing of the PCR products yielded approximately 340 base pairs for each sample. Even though, the first PCR products had extra bands with low density (Figure 4.1), the purification process decreased the background noise in the subsequent sequencing (Figure 4.2).

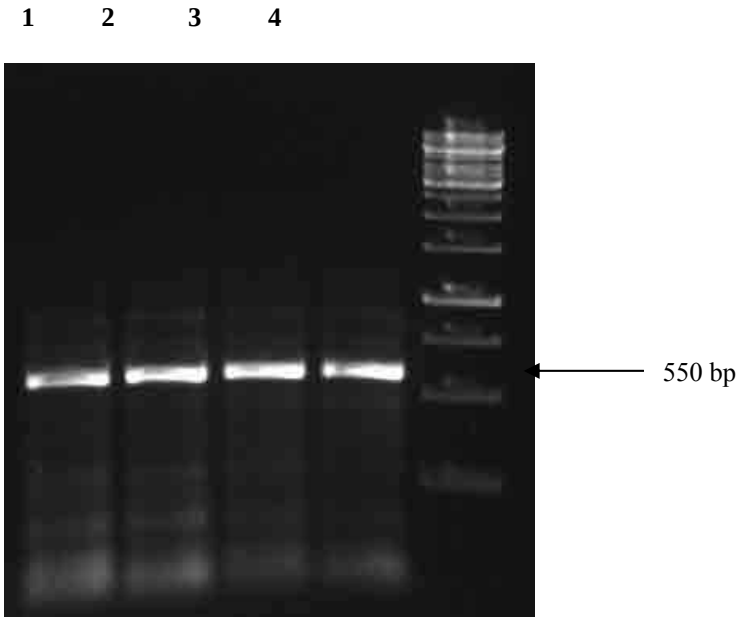

Figure 4.1. The gel electrophoresis of PCR products of four of the samples.

There were 35 polymorphic sites in the amplified region, 30 of which were parsimony informative. The average G + C content of the sequences was 0.382. Eighteen unique haplotypes were identified with a haplotype diversity (h) of 0.910 ± 0.018. The nucleotide diversity (P_i) was found as 0.03316 ± 0.00397. The Tajima's D test for the neutrality was not significant, confirming that the sequences were not subjected to the natural selection.

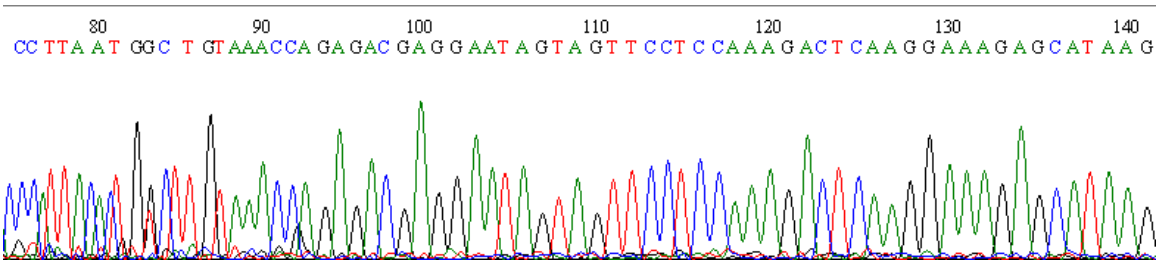

Figure 4.2. A section of the sequencing chromatogram of a sample.

4.1. Phylogenetic Analysis

Phylogenetic trees were used to analyze the relationship between haplotypes. Bayesian analysis was done with four search chains for 1,000,000 generations. In the analysis, HKY+G (Hasegawa et al., 1985) nucleotide evolution model was used. Samples of *Rhinolophus ferrumequinum* and *Rhinolophus euryale* obtained from GenBank were used as outgroups. Bayesian analysis identified two monophyletic clades, X and Y, with 100 percent bootstrap support (Figure 4.3 and Figure 4.4).

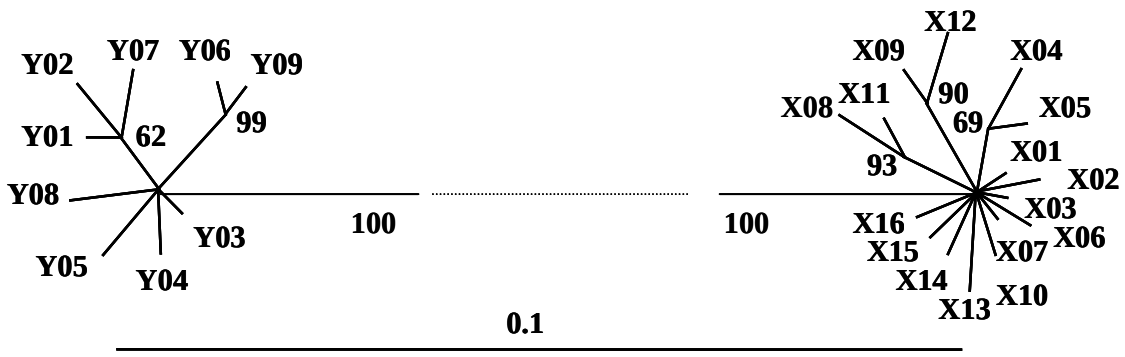

Figure 4.3. Unrooted phylogenetic tree of haplotypes identified in *M. schreibersii* colonies. Bayesian inference was used. Numbers refer to bootstrap values.

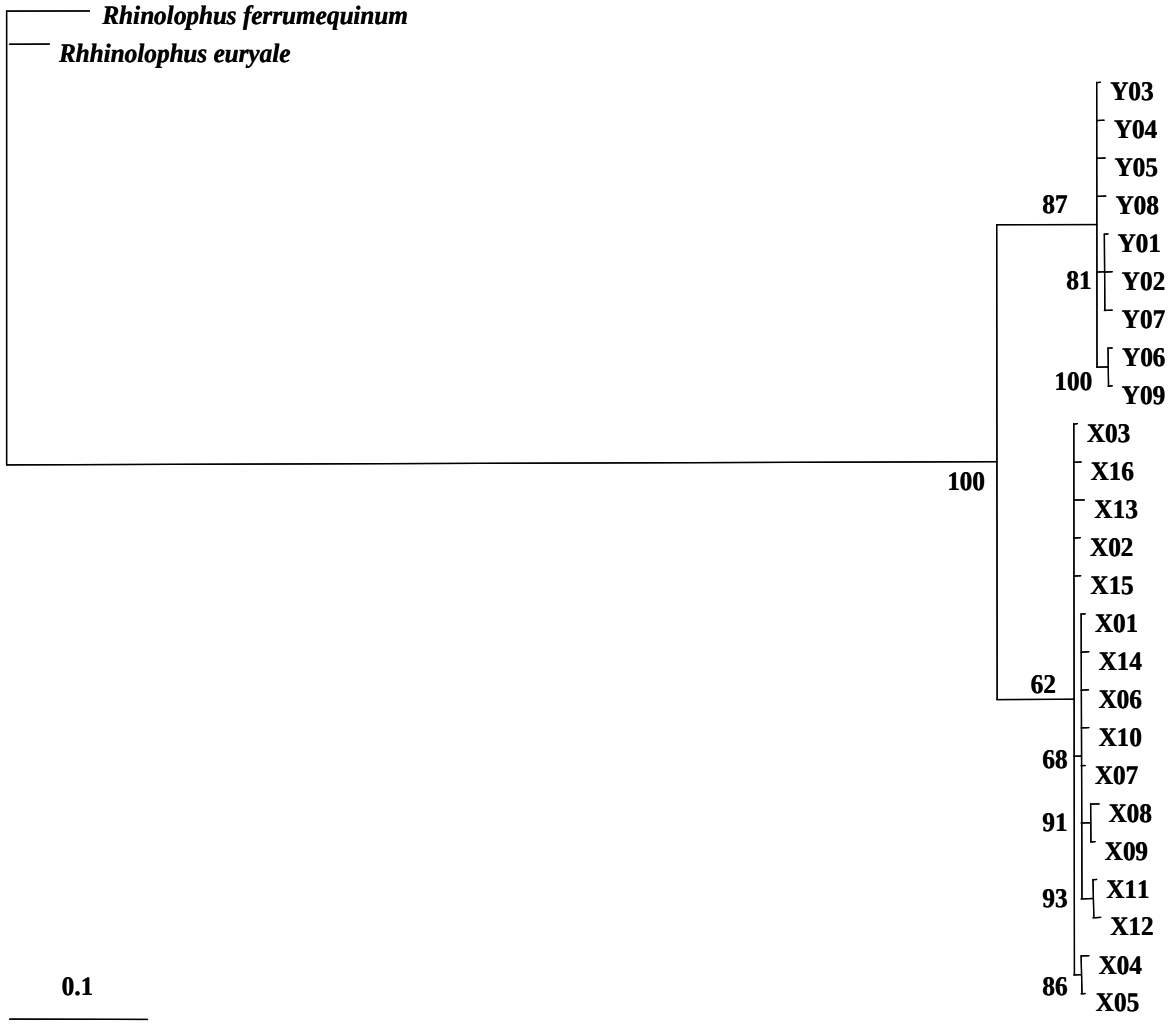

Figure 4.4. Phylogenetic tree of haplotypes identified in colonies. *R. euryale* and *R. ferrumequinum* were used as outgroups. Bayesian inference was used. Numbers refer to bootstrap values.

Table 4.1. Distribution of the 25 sequenced haplotypes among the 11 studied colonies.

	Colony											
	Cum	Hız	Süt	Kar	Zin	Hor	Koc	Kur	Geo	Epc	Arm	Total
HT												
X01	1	2	-	-	-	-	-	-	-	-	-	3
X02	1	-	-	-	-	-	-	-	-	-	-	1
X03	-	2	-	-	-	5	-	3	1	-	-	11
X04	-	1	-	-	-	-	-	-	-	-	-	1
X05	-	3	-	-	-	-	-	-	1	-	-	4
X06	-	-	1	4	-	-	-	-	-	-	-	5
X07	-	-	-	3	-	-	-	-	-	-	-	3
X08	-	-	-	2	-	-	-	-	-	-	-	2
X09	-	-	-	1	-	-	-	-	-	-	-	1
X10	-	-	-	1	-	-	-	-	-	-	-	1
X11	-	-	-	-	4	-	-	-	-	-	-	4
X12	-	-	-	-	1	-	-	-	-	-	-	1
X13	-	-	-	-	-	-	1	-	-	-	-	1
X14	-	-	-	-	-	-	-	1	-	-	-	1
X15	-	-	-	-	-	-	-	1	-	-	-	1
X16	-	-	-	-	-	-	-	-	1	-	-	1
Y01	-	-	-	-	-	-	-	-	-	4	1	5
Y02	-	-	-	-	-	-	-	-	-	1	-	1
Y03	-	-	-	-	-	-	-	-	-	1	-	1
Y04	-	-	-	-	-	-	-	-	-	-	2	2
Y05	-	-	-	-	-	-	-	-	-	-	1	1
Y06	-	-	-	-	-	-	-	-	-	-	1	1
Y07	-	-	-	-	-	-	-	-	-	-	1	1
Y08	-	-	-	-	-	-	-	-	-	-	2	2
Y09	-	-	-	-	-	-	-	-	-	-	1	1
Total	2	8	1	11	5	5	1	5	3	6	9	56

HT, haplotype; Cum, Cumayanı; Hız, Hızar; Süt, Sütlü; Kar, Karanlık; Zin, Zindan; Hor, Horataşı; Koc, Kocakuyu; Kur, Kuru; Geo, Georgia; Epc, Epcik; Arm, Armenia.

The haplotypes identified in this study were compared with the relevant GenBank sequences of *M. schreibersii* from Erzincan, Kayseri, Thrace, Greece, Bulgaria, and Indonesia (Figure 4.5). The Bayesian output was concordant with the results of the study of Bilgin et al. (2006).

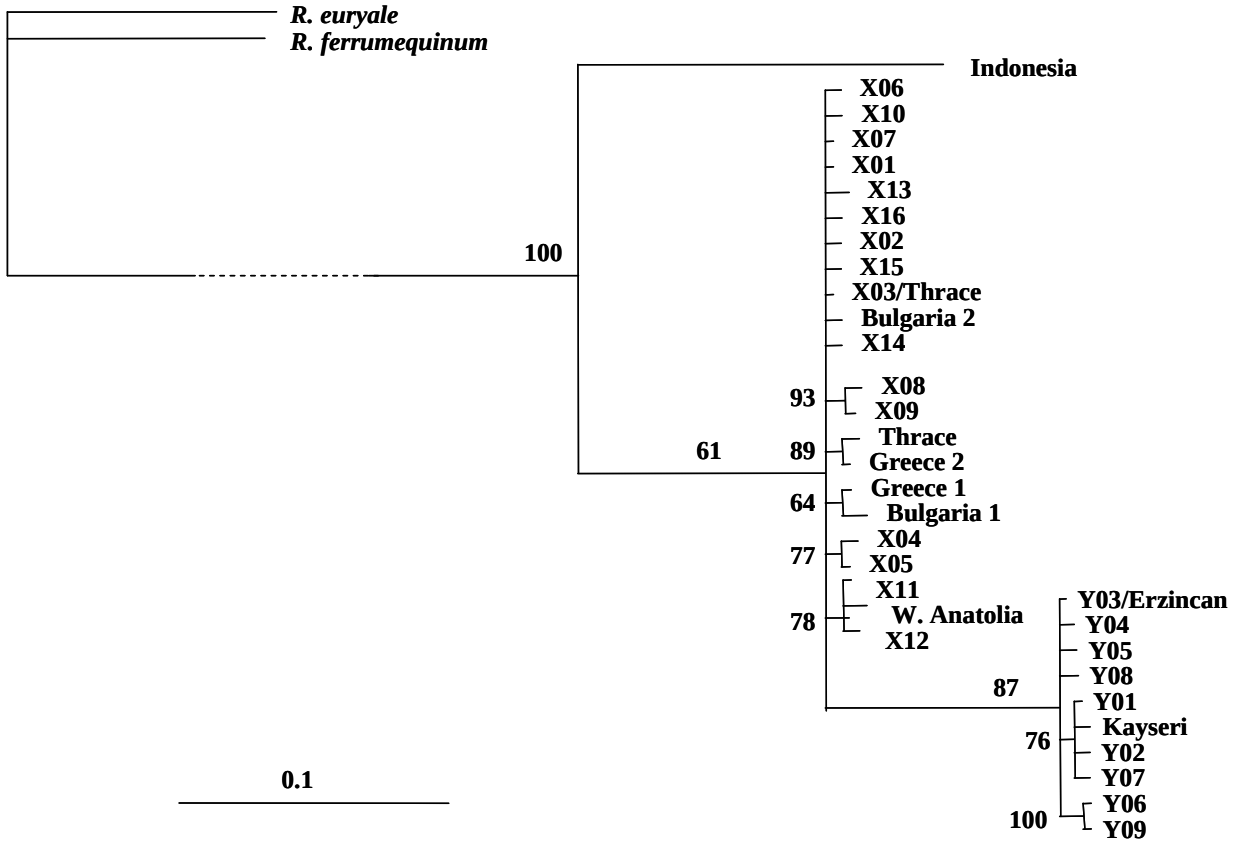

Figure 4.5. Phylogenetic tree of haplotypes identified in this study and the relevant GenBank sequences of *M. schreibersii* from Erzincan, Kayseri, Thrace, Greece, Bulgaria, and Indonesia. *R. euryale* and *R. ferrumequinum* were used as outgroups. Bayesian inference was used. Numbers refer to bootstrap values.

4.2. Genetic Differentiation of the Lineages

The amount of differentiation between the clades X and Y was measured by AMOVA (Excoffier *et al.*, 1992). The pairwise Φ_{st} was 0.95688 showing that the molecular variance between the groups was statistically significant. The percent of variance attributable to differences between clades was 91.88% and that within clades was 3.81%. The result indicated a considerable genetic break between the clades (Figure 4.6, Table 4.2).

Table 4.2. Analysis of Molecular Variance

Source of variation	d.f.	Sum of squares	Variance components	Percent of variation
Among groups	1	268.793	12.04156 Va	91.88
Among populations within groups	9	25.779	0.49873 Vb	3.81
Within Populations	45	25.427	0.56505 Vc	4.31
Total	55	320.000	13.10535	100.00

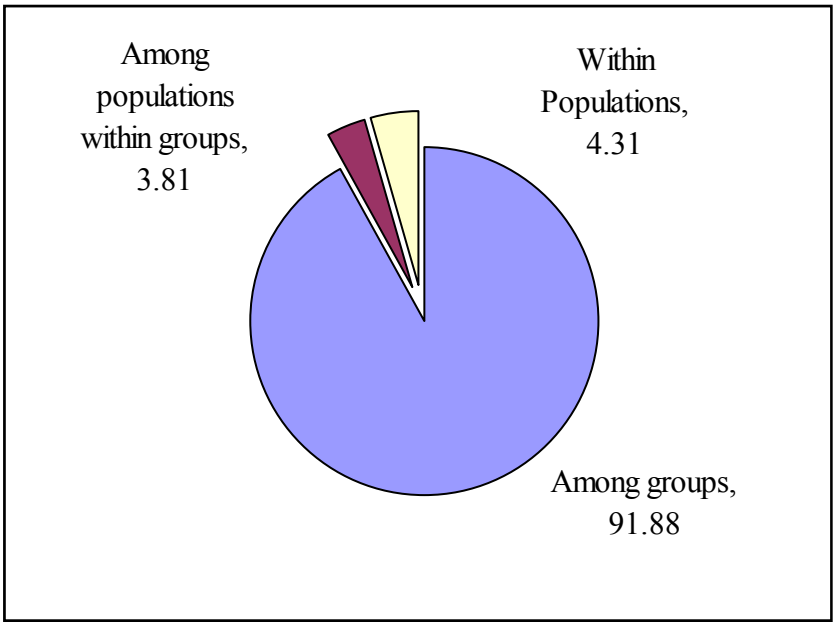

Figure 4.6. The percent distribution of variation of individuals in clade X and Y.

Statistical parsimony network analysis was used to investigate the geographical distribution of the clades. Clade Y was found in the eastern part of Anatolia (on the inland zone), whereas clade X occupied exclusively the coastal zones (Figure 4.7). The most

ancestral haplotype in the clade X was distributed along the entire Black Sea coast. Similarly, one haplotype in clade Y was also present in both of the colonies of that lineage. The geographic distribution of the haplotypes did not show any effects of the physical barriers such as Marmara Sea or Taurus Mountains. For instance, members of the X clade were found in both sides of these geographic barriers.

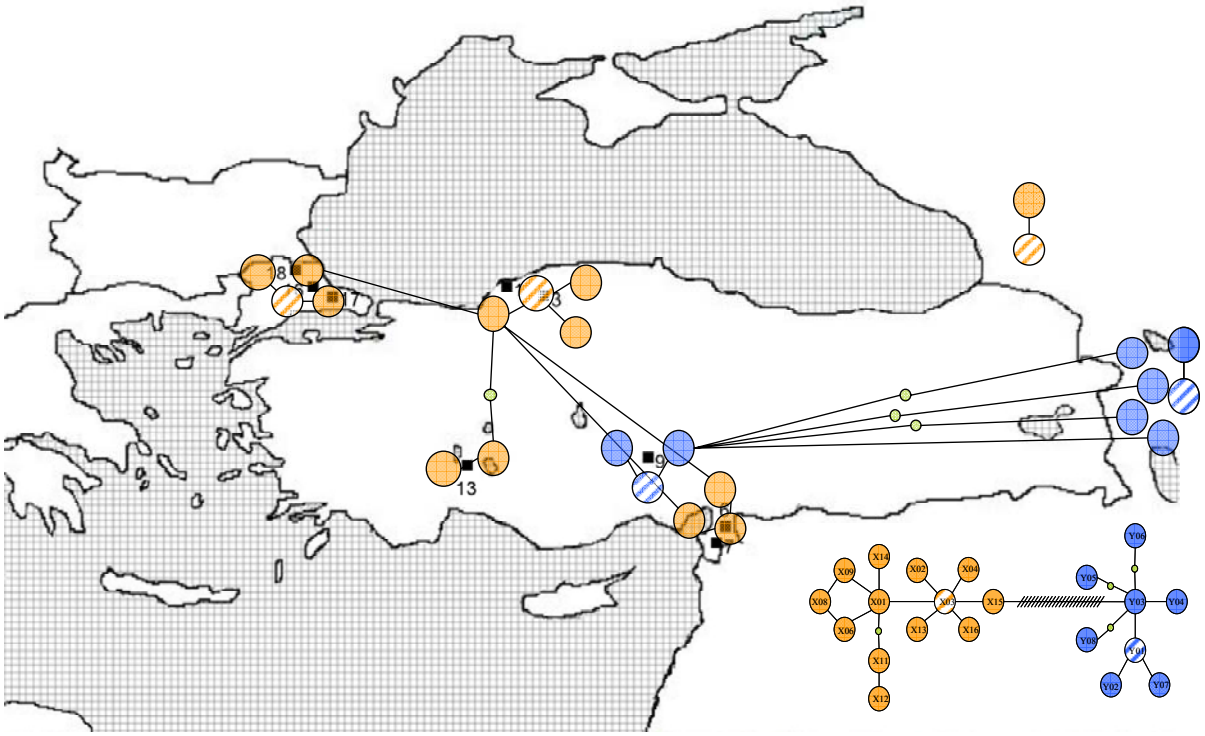

Figure 4.7. Statistical parsimony network of haplotypes identified in *M. schreibersii* colonies, superimposed over a map of Turkey.

Nested Clade Analysis (NCA) was applied to the statistical parsimony networks to investigate association between geographic and population expansion patterns (Figure 4.8). Although the robustness of this analysis is low and negatively affected by small sample size, the star-like networks found for both clades were indicative for past population expansions.

There were 16 fixed nucleotide differences between the populations. Ten of the mutations that were monomorphic in clade Y were polymorphic in clade X, and eight of the

monomorphic mutations in clade X were polymorphic in clade Y. The average number of nucleotide differences between the populations was 25.519 and the divergence (D_{xy}) between clades X and Y was 0.07505.

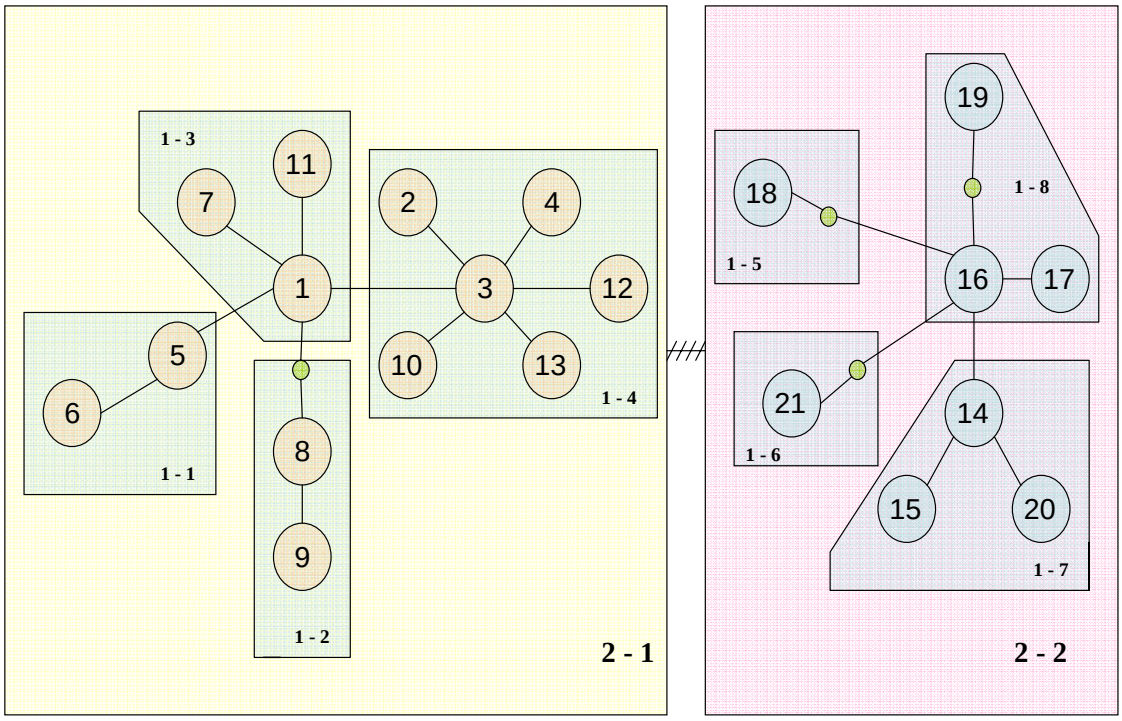

Figure 4.8. The clade formation in nested clade analysis.

The divergence time between the clades X and Y was calculated by estimating a percent divergence rate. The molecular clock rate was set to 0.2 mutations per million years, based on 20% per million years differentiation of D-loop in the noctule bat (Petit et al., 1999). Bayesian simulation showed that the clades shared their most recent common ancestor approximately 751,000 years before present (95% C.I.: 604,000-747,000 years BP) (Figure 4.9). The timing of the division coincides with the Pleistocene epoch, suggesting that the differentiation of the clades might result from the isolation in separate glacial refugia.

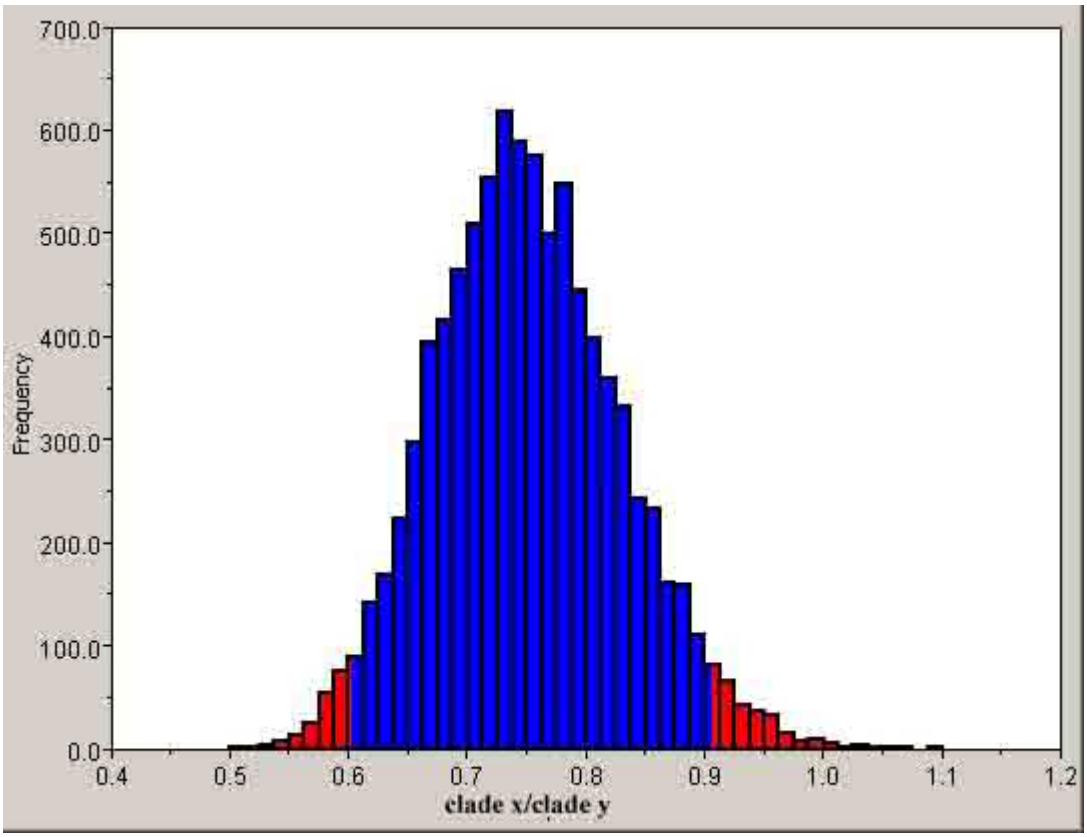

Figure 4.9. Bayesian estimate for the divergence of two clades.

4.3. Subpopulation Genetic Structure

To understand better the structure of the monophyletic clades, the haplotypes of these clades were examined separately. In terms of descriptive statistics, clade X had 11 unique haplotypes compromised of 11 polymorphic sites, six of which were parsimony informative. The haplotype diversity was 0.854 ± 0.028 and the nucleotide diversity was 0.00514 ± 0.00054. The Tajima's D neutrality test was not significant. Clade Y had eight haplotypes with 10 polymorphic sites, six of which were parsimony informative. The haplotype diversity was 0.876 ± 0.067 and the nucleotide diversity was 0.00513 ± 0.00076. The Tajima's D test was also not significant.

The population expansion hypothesis, indicated by the star-like pattern of the maximum parsimony network analysis, was tested by the R_2 and F_s statistics (Ramos-Onsins and Rozas, 2002; Fu, 1997). For clade X, R_2 was 0.0738 and F_s was -3.975, indicating expansion. For clade Y R_2 was 0.1055 and F_s was -2.569. Both statistics were significant. Population size changes were also analyzed by the mismatch distributions of nucleotides. The frequency of pairwise nucleotide differences between haplotypes was plotted against the values expected under a constant-size model and a model of expansion. The results showed that the range expansion model for both clades fits better than constant size population model (Figures 4.10 to 4.13). Bayesian Skyline plots were used to estimate the starting point of population expansions. The graphs confirmed the expansion patterns and estimated the starting points for at least 8,000 and 5000 years ago for clade X and clade Y, respectively (Figure 4.14 and 4.15). This would suggest that both clades started expansion during the Holocene period.

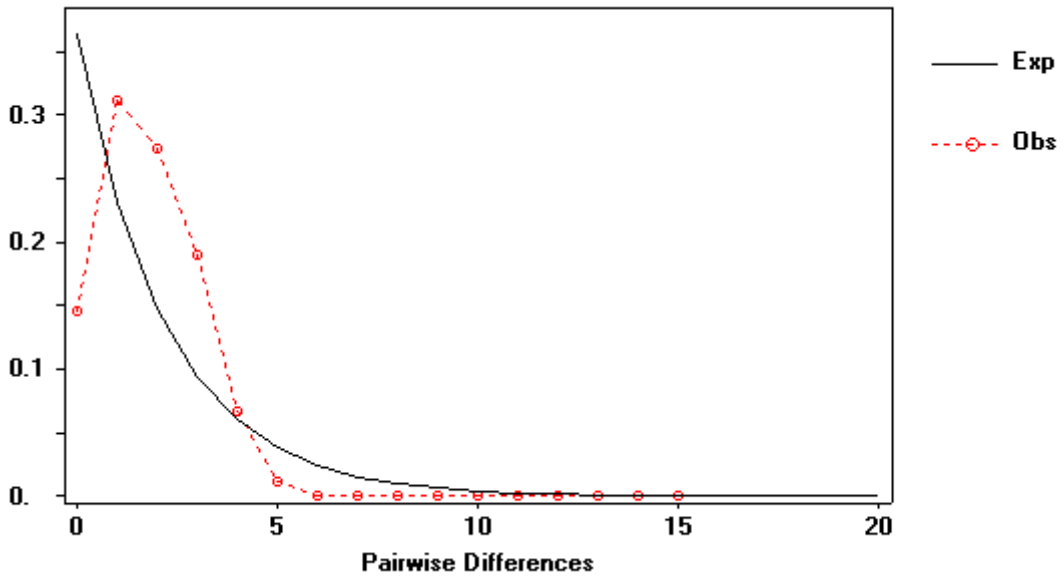

Figure 4.10. The observed and expected mismatch distributions in a constant size population model for clade X.

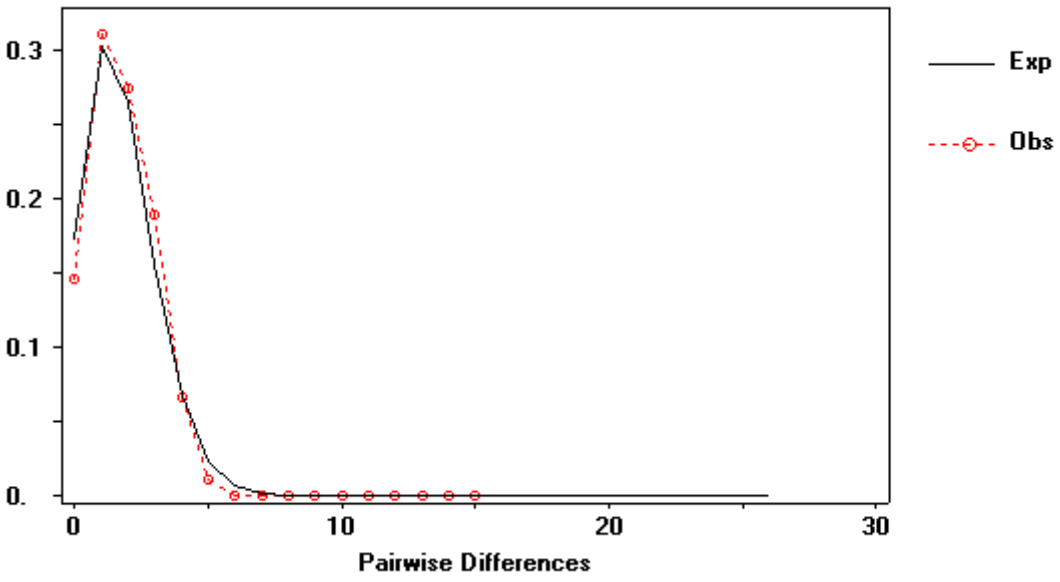

Figure 4.11. The observed and expected mismatch distributions in an expanding population model for clade X.

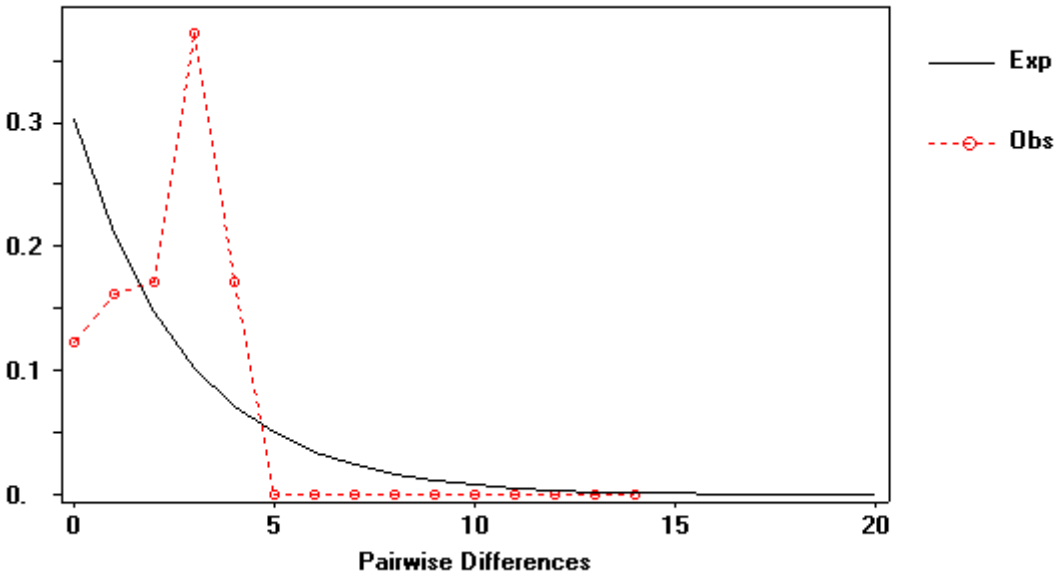

Figure 4.12. The observed and expected mismatch distributions in a constant size population model for clade Y.

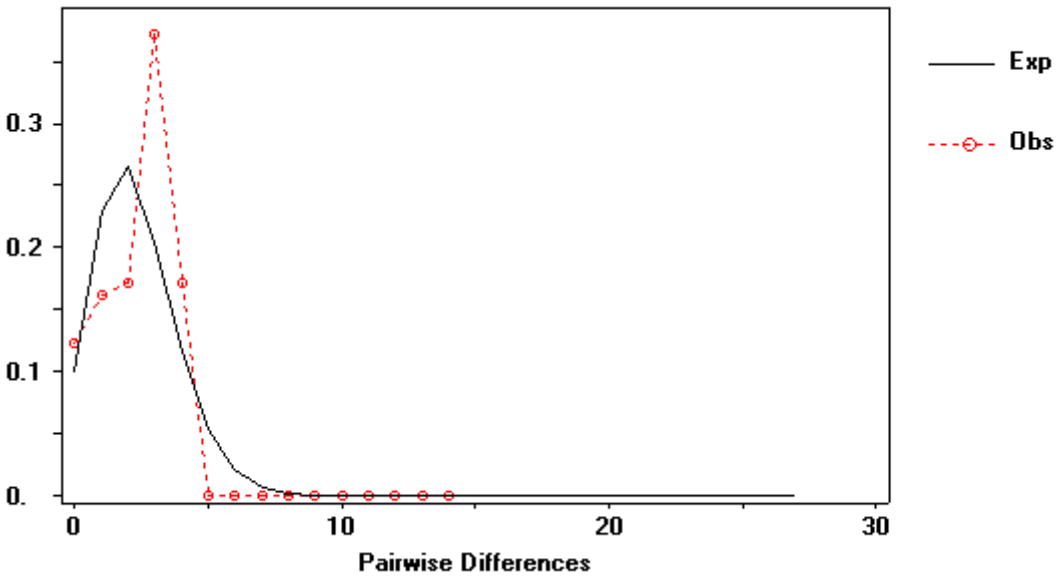

Figure 4.13. The observed and expected mismatch distributions in an expanding population model for clade Y.

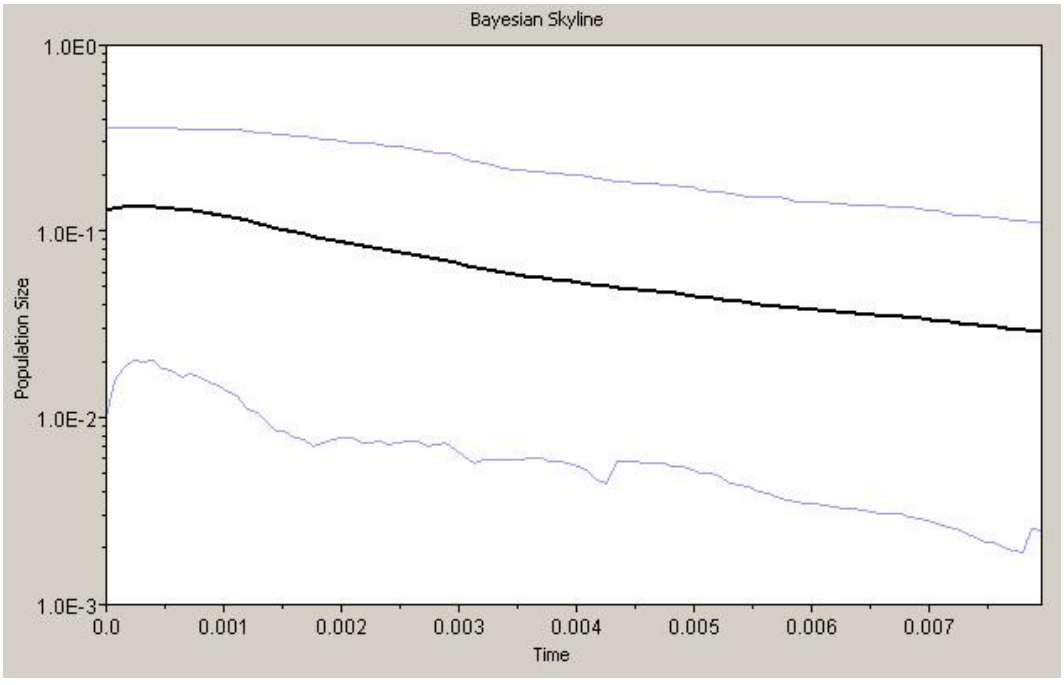

Figure 4.14. Bayesian Skyline plot for Clade X.

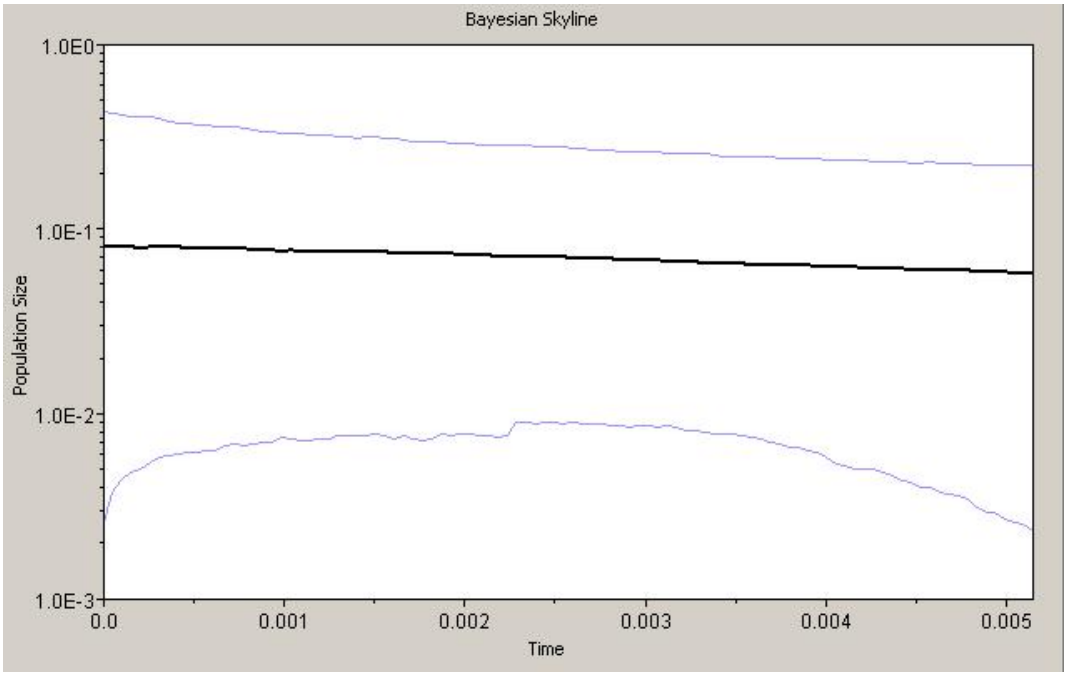

Figure 4.15. Bayesian Skyline plot for Clade Y.

Finally, the correlation between the genetic and the spatial distances was examined by the spatial structure analysis. For the clade X, there was no significant correlation between the genetic and the geographic distances (Figure 4.16). The analysis could not be used for the clade Y, as it had only two sampling sites.

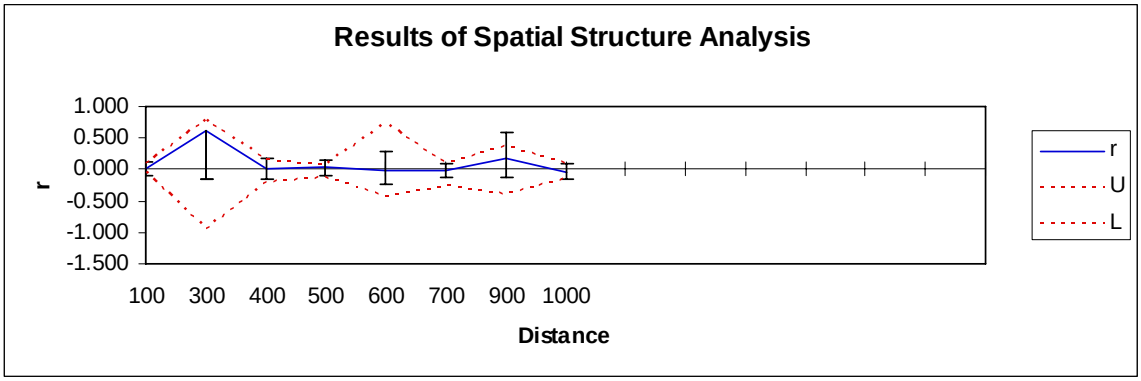

Figure 4.16. Spatial Structure Analysis for Clade X.

4.4. The Analysis of Forearm Measurements

The average length of a forearm in the investigated *M. schreibersii* colonies was 45.8 ± 1.0 mm. The bats from the X clade had a shorter forearm (45.5 ± 0.8 mm) than the bats from the Y clade (46.9 ± 0.7 mm); the difference between clades was significant on 0.001 level (Figure 4.17). Because females of *M. schreibersii* tend to be slightly bigger than males, and gender distribution was different in both clades, two-way ANOVA was used to control for gender when analyzing forearm length differences between clades. Levene's test for homogeneity of error variances showed that variances of the tested groups were approximately equal ($p = .512$). The difference in forearm length between clades was significant ($p < .001$; $\eta_p = .375$) and the gender effect was not ($p = .654$; $\eta_p = .004$) (Figure 4.18). The clade identity explained about 40% of the variance in the length of a forearm. The variations in forearm lengths within each investigated colony were also plotted (Figure 4.19). Epcik and Azokh (Armenia) caves were distinctly different.

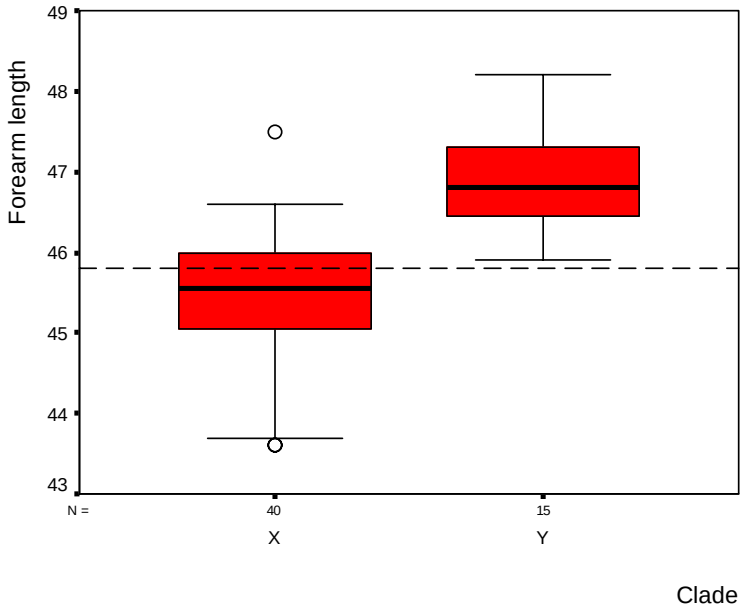

Figure 4.17. Difference in *M. schreibersii* forearm length between clades X and Y. A dashed line marks the forearm length averaged over all individuals.

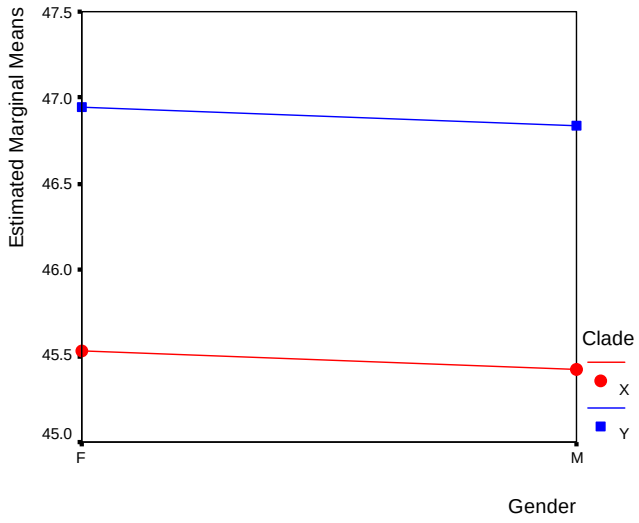

Figure 4.18. The effect of gender on the forearm length difference between clades X and Y of *M. schreibersii*.

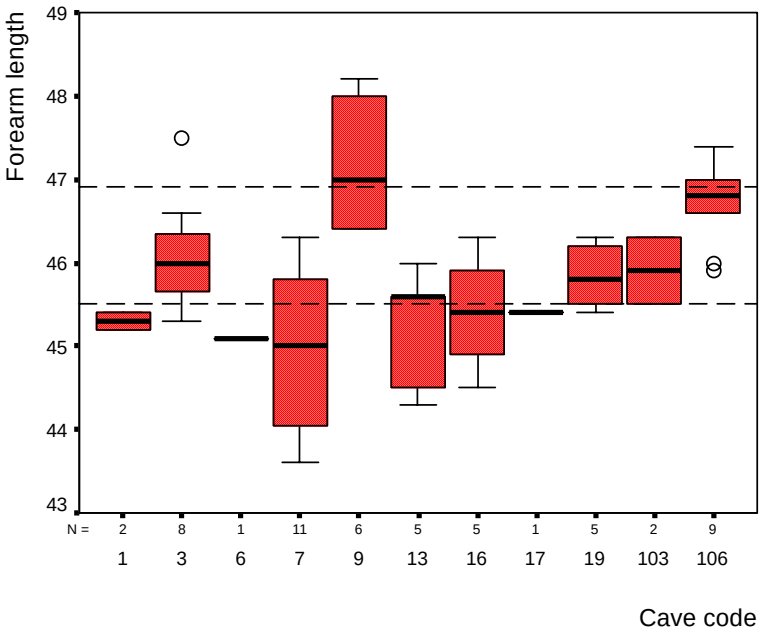

Figure 4.19. Variations in a forearm length within each investigated colony of *M. schreibersii*. Dashed lines mark the average forearm length for X (lower) and Y (upper) clades.

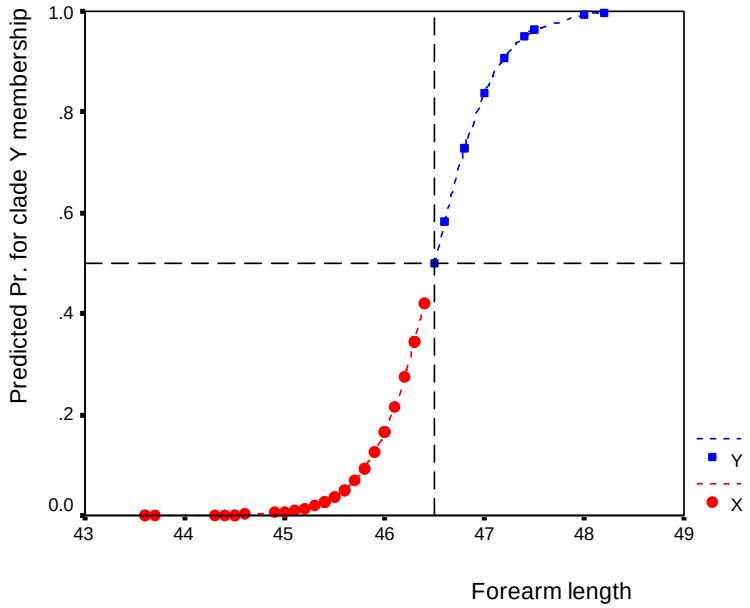

Figure 4.20. The predicted probability of clade Y membership as a function of a forearm length in *M. schreibersii*. The prediction equation is LN (ODDS for clade Y membership) = 3.26*(Forearm length) – 151.56

Finally, binary logistic regression was used to assess probability of a correct prediction of clade membership, when given measure of an individual's forearm length. The model explained between 44% (Cox and Snell R^2) and 64% (Nagelkerke R^2) of the variance and could predict the correct clade membership in 89% of cases. The predicted probability of clade Y membership as a function of a forearm length was plotted on Figure 4.22.

According to Albayrak and Coşkun (2000), and Karataş and Sözen (2004), there were no measurable morphological differences between the two form of *M. schreibersii*. However, the results of this study indicated significant difference in forearm lengths between *M. s. schreibersii* and *M. s. pallidus*. The average forearm length for the clade X corresponded well to the values reported by Karataş and Sözen for *M. s. schreibersii* (as identified by them) from Thrace (45.6 ± 0.8 mm), the western Black Sea (45.2 ± 0.5 mm), and southern Marmara (45.3 ± 1.0 mm). Albayrak and Coşkun reported smaller value of 42.8 ± 1.3 mm, but their sample included only seven individuals. The average forearm length for the clade Y was comparable with the values for *M. s. pallidus* calculated by Karataş and Sözen (as identified by them) from

eastern (46.4 ± 0.6 mm) and central Anatolia (46.6 ± 0.9 mm). Furthermore, the average forearm length given by these authors for the 'intermediate' Aegean region (45.6 ± 0.6 mm) matched the nominate form of *M. s. schreibersii*, and their values for *M. s. pallidus* from Mediterranean region (45.7 ± 2.1 mm) and the eastern Black Sea (45.9 ± 0.9 mm) were already higher and had bigger standard deviation, indicating that the sample might include mixed forms. The average forearm length for *M. s. pallidus* reported by Albayrak and Coşkun (as identified by them) was 46.3 ± 0.4 mm, but this value was strongly biased by their sampling procedure; 100 out of 144 measurements were done on samples collected in the Ankara district.

In summary, the results suggested that there was a morphological difference between the two forms of *M. schreibersii*. Binary logistic regression model based on forearm length could identify correctly the clade membership in about 90% of the cases; only 4 out of 55 bats were misclassified. Misclassification might arise from natural intraclade variations or from a sporadic interbreeding between clades.

5. CONCLUSIONS

Two genetically distinct mitochondrial lineages (X and Y) of *M. schreibersii* were identified. None of the lineages showed any internal genetic structuring. The most common haplotype of the clade X was found in Thrace, western Black Sea, and Georgia. Similarly, the most common haplotype of the clade Y was found in central Anatolia and Armenia. The analyses showed that none of the geographical barriers in Anatolia were responsible for the restricted gene flow. Accordingly, there were no obvious physical barriers that might impede interbreeding between the lineages. Hence, genetic distinctness of the clades might indicate that they might be separate species. The clades probably became separated when occupying separate refugia during the Pleistocene epoch.

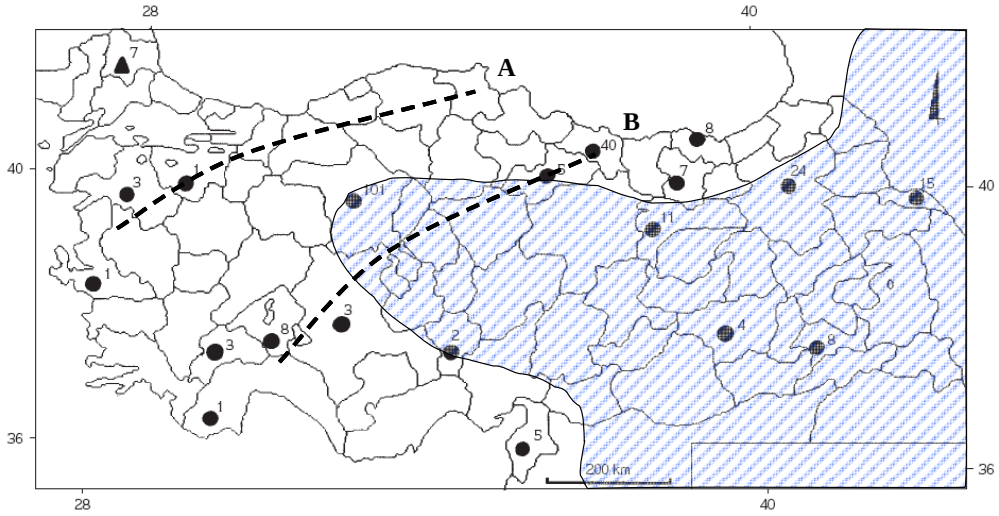

Figure 5.1. Distribution of *M. s. pallidus* and *M. s. schreibersii* In Turkey. According to Albayrak and Coşkun (2000): the presence of *M. s. pallidus* is marked by circles and *M. s. schreibersii* by triangles. According to Karataş and Sözen (2004): *M. s. pallidus* is found below the line B and *M. s. schreibersii* above the line A. The area between A and B is a transition zone. The results of this study suggest that *M. s. pallidus* is distributed within the shaded area and *M. s. schreibersii* occupied the rest of Turkey.

There was a significant difference in forearm lengths between *M. s. schreibersii* and *M. s. pallidus*. The earlier studies did not detect this difference because of the subspecies misidentification and the wrong assumptions about their distribution. The results of this study indicated that both subspecies were present in Anatolia. *M. s. pallidus* was distributed in inland zones of central and eastern Anatolia, whereas *M. s. schreibersii* occupied the rest of Turkey (Figure 5.1). The forearm analyses also showed that none of the sites had populations with intermediate forearm lengths suggesting the absence of any clinal variations. This supported the idea that the lineages did not interbreed and might deserve a status of separate species.

Further studies should focus on increasing the resolution of population genetic structure of clade Y, which could be achieved by more intense sampling of the eastern populations. Furthermore, nuclear markers should be used to clarify the interbreeding status of two lineages.

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Appendix A: Mitochondrial DNA Control Region Sequences

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LOCUS       F-01-002-X01       458 bp

        1 ATGTAGT--A TACCCATTAC ACTGGTCTTG TAAACCAGAG ACGAGGAATA GTAGTTCCTC
       61 CAAAGACTCA AGGAAAGAGC ATAAGCCCTA CCGTCAGCAC CCAAAGCTGA AATTCTACTT
      121 AAACTACTCC TTGCAAGCAT AC-TGTCAAG TATGCAT-TC C-ATGGTTAT ACTATGTACA
      181 TCGTGCATTA ACTTTATTTC CCCATGAATA TGTAGCATGT ACATTTATAA TCTTGTATTA
      241 CATGAGTACA TTATATTATA TATCGTACAT AAACCATCTG ATGTGATAAA TTCCAGTCAA
      301 CATGACTATT CCGCAAGCAC TGTTGGTTTA TTAATCTACC ATCCTCCGTG AAACCAGCAA
      361 CCCGCCCACA ACGTGTCCCT CTTCTCGCCC CGGGCCCATA AATCGTGGGG GTGACTATAC
      421 TGGGTCGTAA ACGGCATCTG GTTCCTACTT CAG-----

LOCUS       M-03-015-X01       458 bp

        1 ATGTAGT--A TACCCATTAC ACTGGTCTTG TAAACCAGAG ACGAGGAATA GTAGTTCCTC
       61 CAAAGACTCA AGGAAAGAGC ATAAGCCCTA CCGTCAGCAC CCAAAGCTGA AATTCTACTT
      121 AAACTACTCC TTGCAAGCAT AC-TGTCAAG TATGCAT-TC C-ATGGTTAT ACTATGTACA
      181 TCGTGCATTA ACTTTATTTC CCCATGAATA TGTAGCATGT ACATTTATAA TCTTGTATTA
      241 CATGAGTACA TTATATTATA TATCGTACAT AAACCATCTG ATGTGATAAA TTCCAGTCAA
      301 CATGACTATT CCGCAAGCAC TGTTGGTTTA TTAATCTACC ATCCTCCGTG AAACCAGCAA
      361 CCCGCCCACA ACGTGTCCCT CTTCTCGCCC CGGGCCCATA AATCGTGGGG GTGACTATAC
      421 TGGGTCGTAA ACGGCATCTG GTTCCTACTT CAG-----

LOCUS       M-03-021-X01       458 bp

        1 ATGTAGT--A TACCCATTAC ACTGGTCTTG TAAACCAGAG ACGAGGAATA GTAGTTCCTC
       61 CAAAGACTCA AGGAAAGAGC ATAAGCCCTA CCGTCAGCAC CCAAAGCTGA AATTCTACTT
      121 AAACTACTCC TTGCAAGCAT AC-TGTCAAG TATGCAT-TC C-ATGGTTAT ACTATGTACA
      181 TCGTGCATTA ACTTTATTTC CCCATGAATA TGTAGCATGT ACATTTATAA TCTTGTATTA
      241 CATGAGTACA TTATATTATA TATCGTACAT AAACCATCTG ATGTGATAAA TTCCAGTCAA
      301 CATGACTATT CCGCAAGCAC TGTTGGTTTA TTAATCTACC ATCCTCCGTG AAACCAGCAA
      361 CCCGCCCACA ACGTGTCCCT CTTCTCGCCC CGGGCCCATA AATCGTGGGG GTGACTATAC
      421 TGGGTCGTAA ACGGCATCTG GTTCCTACTT CAG-----

LOCUS       M-01-005-X02       458 bp

        1 ATGTAGT--A TACCCATTAC ACTGGTCTTG TAAACCAGAG ACGAGGAATA GTAGTTCCTC
       61 CAAAGACTCA AGGAAAGAGC ATAAGCCCTA CCGTCAGCAC CCAAAGCTGA AATTCTACTT
      121 AAACTACTCC TTGCAAGCAT AC-TGTCAAG TATGCAT-TC T-ATGGTTAT ACTATGTACA
      181 TCGTGCATTA ACTTTATTTC CCCATGAATA TGTAGCATGT ACATTTATGA TCTTGTATTA
      241 CATGAGTACA TTATATTATA TATCGTACAT AAACCATCTG ATGTGATAAA TTCCAGTCAA
      301 CATGACTATT CCGCAAGCAC TGTTGGTTTA TTAATCTACC ATCCTCCGTG AAACCAGCAA
      361 CCCGCCCACA ACGTGTCCCT CTTCTCGCCC CGGGCCCATA AATCGTGGGG GTGACTATAC
      421 TGGGTCGTAA ACGGCATCTG GTTCCTACTT CAG-----


LOCUS       M-03-016-X03       458 bp

        1 ATGTAGT--A TACCCATTAC ACTGGTCTTG TAAACCAGAG ACGAGGAATA GTAGTTCCTC
       61 CAAAGACTCA AGGAAAGAGC ATAAGCCCTA CCGTCAGCAC CCAAAGCTGA AATTCTACTT
      121 AAACTACTCC TTGCAAGCAT AC-TGTCAAG TATGCAT-TC C-ATGGTTAT ACTATGTACA
      181 TCGTGCATTA ACTTTATTTC CCCATGAATA TGTAGCATGT ACATTTATGA TCTTGTATTA
      241 CATGAGTACA TTATATTATA TATCGTACAT AAACCATCTG ATGTGATAAA TTCCAGTCAA
      301 CATGACTATT CCGCAAGCAC TGTTGGTTTA TTAATCTACC ATCCTCCGTG AAACCAGCAA
      361 CCCGCCCACA ACGTGTCCCT CTTCTCGCCC CGGGCCCATA AATCGTGGGG GTGACTATAC
      421 TGGGTCGTAA ACGGCATCTG GTTCCTACTT CAG-----
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LOCUS       M-03-020-X03       458 bp

        1 ATGTAGT--A TACCCATTAC ACTGGTCTTG TAAACCAGAG ACGAGGAATA GTAGTTCCTC
       61 CAAAGACTCA AGGAAAGAGC ATAAGCCCTA CCGTCAGCAC CCAAAGCTGA AATTCTACTT
      121 AAACTACTCC TTGCAAGCAT AC-TGTCAAG TATGCAT-TC C-ATGGTTAT ACTATGTACA
      181 TCGTGCATTA ACTTTATTTC CCCATGAATA TGTAGCATGT ACATTTATGA TCTTGTATTA
      241 CATGAGTACA TTATATTATA TATCGTACAT AAACCATCTG ATGTGATAAA TTCCAGTCAA
      301 CATGACTATT CCGCAAGCAC TGTTGGTTTA TTAATCTACC ATCCTCCGTG AAACCAGCAA
      361 CCCGCCCACA ACGTGTCCCT CTTCTCGCCC CGGGCCCATA AATCGTGGGG GTGACTATAC
      421 TGGGTCGTAA ACGGCATCTG GTTCCTACTT CAG-----

LOCUS       M-16-111-X03       458 bp

        1 ATGTAGT--A TACCCATTAC ACTGGTCTTG TAAACCAGAG ACGAGGAATA GTAGTTCCTC
       61 CAAAGACTCA AGGAAAGAGC ATAAGCCCTA CCGTCAGCAC CCAAAGCTGA AATTCTACTT
      121 AAACTACTCC TTGCAAGCAT AC-TGTCAAG TATGCAT-TC C-ATGGTTAT ACTATGTACA
      181 TCGTGCATTA ACTTTATTTC CCCATGAATA TGTAGCATGT ACATTTATGA TCTTGTATTA
      241 CATGAGTACA TTATATTATA TATCGTACAT AAACCATCTG ATGTGATAAA TTCCAGTCAA
      301 CATGACTATT CCGCAAGCAC TGTTGGTTTA TTAATCTACC ATCCTCCGTG AAACCAGCAA
      361 CCCGCCCACA ACGTGTCCCT CTTCTCGCCC CGGGCC---- ---------- ----------
      421 ---------- ---------- ---------- --------

LOCUS       M-16-112-X03       458 bp

        1 ATGTAGT--A TACCCATTAC ACTGGTCTTG TAAACCAGAG ACGAGGAATA GTAGTTCCTC
       61 CAAAGACTCA AGGAAAGAGC ATAAGCCCTA CCGTCAGCAC CCAAAGCTGA AATTCTACTT
      121 AAACTACTCC TTGCAAGCAT AC-TGTCAAG TATGCAT-TC C-ATGGTTAT ACTATGTACA
      181 TCGTGCATTA ACTTTATTTC CCCATGAATA TGTAGCATGT ACATTTATGA TCTTGTATTA
      241 CATGAGTACA TTATATTATA TATCGTACAT AAACCATCTG ATGTGATAAA TTCCAGTCAA
      301 CATGACTATT CCGCAAGCAC TGTTGGTTTA TTAATCTACC ATCCTCCGTG AAACCAGCAA
      361 CCCGCCCACA ACGTGTCCCT CTTCTCGCCC CGGGCC---- ---------- ----------
      421 ---------- ---------- ---------- --------

LOCUS       M-16-113-X03       458 bp

        1 ATGTAGT--A TACCCATTAC ACTGGTCTTG TAAACCAGAG ACGAGGAATA GTAGTTCCTC
       61 CAAAGACTCA AGGAAAGAGC ATAAGCCCTA CCGTCAGCAC CCAAAGCTGA AATTCTACTT
      121 AAACTACTCC TTGCAAGCAT AC-TGTCAAG TATGCAT-TC C-ATGGTTAT ACTATGTACA
      181 TCGTGCATTA ACTTTATTTC CCCATGAATA TGTAGCATGT ACATTTATGA TCTTGTATTA
      241 CATGAGTACA TTATATTATA TATCGTACAT AAACCATCTG ATGTGATAAA TTCCAGTCAA
      301 CATGACTATT CCGCAAGCAC TGTTGGTTTA TTAATCTACC ATCCTCCGTG AAACCAGCAA
      361 CCCGCCCACA ACGTGTCCCT CTTCTCGCCC CGGGCC---- ---------- ----------
      421 ---------- ---------- ---------- --------

LOCUS       F-16-116-X03       458 bp

        1 ATGTAGT--A TACCCATTAC ACTGGTCTTG TAAACCAGAG ACGAGGAATA GTAGTTCCTC
       61 CAAAGACTCA AGGAAAGAGC ATAAGCCCTA CCGTCAGCAC CCAAAGCTGA AATTCTACTT
      121 AAACTACTCC TTGCAAGCAT AC-TGTCAAG TATGCAT-TC C-ATGGTTAT ACTATGTACA
      181 TCGTGCATTA ACTTTATTTC CCCATGAATA TGTAGCATGT ACATTTATGA TCTTGTATTA
      241 CATGAGTACA TTATATTATA TATCGTACAT AAACCATCTG ATGTGATAAA TTCCAGTCAA
      301 CATGACTATT CCGCAAGCAC TGTTGGTTTA TTAATCTACC ATCCTCCGTG AAACCAGCAA
      361 CCCGCCCACA ACGTGTCCCT CTTCTCGCCC CGGGCCCATA AATCGTGGGG GTGACTATAC
      421 TGGGTCGTAA ACGGCATCTG GTTCCTACTT CAG-----
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LOCUS       M-16-126-X03       458 bp

        1 ATGTAGT--A TACCCATTAC ACTGGTCTTG TAAACCAGAG ACGAGGAATA GTAGTTCCTC
       61 CAAAGACTCA AGGAAAGAGC ATAAGCCCTA CCGTCAGCAC CCAAAGCTGA AATTCTACTT
      121 AAACTACTCC TTGCAAGCAT AC-TGTCAAG TATGCAT-TC C-ATGGTTAT ACTATGTACA
      181 TCGTGCATTA ACTTTATTTC CCCATGAATA TGTAGCATGT ACATTTATGA TCTTGTATTA
      241 CATGAGTACA TTATATTATA TATCGTACAT AAACCATCTG ATGTGATAAA TTCCAGTCAA
      301 CATGACTATT CCGCAAGCAC TGTTGGTTTA TTAATCTACC ATCCTCCGTG AAACCAGCAA
      361 CCCGCCCACA ACGTGTCCCT CTTCTCGCCC CGGGCCCATA AATCGTGGGG GTGACTATAC
      421 TGGGTCGTAA ACGGCATCTG GTTCCTACTT CAG-----

LOCUS       M-19-206-X03       458 bp

        1 ---------- ---------- ------CTTG TAAACCAGAG ACGAGGAATA GTAGTTCCTC
       61 CAAAGACTCA AGGAAAGAGC ATAAGCCCTA CCGTCAGCAC CCAAAGCTGA AATTCTACTT
      121 AAACTACTCC TTGCAAGCAT AC-TGTCAAG TATGCAT-TC C-ATGGTTAT ACTATGTACA
      181 TCGTGCATTA ACTTTATTTC CCCATGAATA TGTAGCATGT ACATTTATGA TCTTGTATTA
      241 CATGAGTACA TTATATTATA TATCGTACAT AAACCATCTG ATGTGATAAA TTCCAGTCAA
      301 CATGACTATT CCGCAAGCAC TGTTGGTTTA TTAATCTACC ATCCTCCGTG AAACCAGCAA
      361 CCCGCCCACA AC-------- ---------- ---------- ---------- ----------
      421 ---------- ---------- ---------- --------

LOCUS       M-19-207-X03       458 bp

        1 ATGTAGT--A TACCCATTAC ACTGGTCTTG TAAACCAGAG ACGAGGAATA GTAGTTCCTC
       61 CAAAGACTCA AGGAAAGAGC ATAAGCCCTA CCGTCAGCAC CCAAAGCTGA AATTCTACTT
      121 AAACTACTCC TTGCAAGCAT AC-TGTCAAG TATGCAT-TC C-ATGGTTAT ACTATGTACA
      181 TCGTGCATTA ACTTTATTTC CCCATGAATA TGTAGCATGT ACATTTATGA TCTTGTATTA
      241 CATGAGTACA TTATATTATA TATCGTACAT AAACCATCTG ATGTGATAAA TTCCAGTCAA
      301 CATGACTATT CCGCAAGCAC TGTTGGTTTA TTAATCTACC ATCCTCCGTG AAACCAGCAA
      361 CCCGCCCACA ACGTGTCCCT CTTCTCGCCC CGGGCCCATA AATCGTGGGG GTGACTATAC
      421 TGGGTCGTAA ACGGCATCTG GTTCCTACTT CAG-----

LOCUS       F-19-209-X03       458 bp

        1 ATGTAGT--A TACCCATTAC ACTGGTCTTG TAAACCAGAG ACGAGGAATA GTAGTTCCTC
       61 CAAAGACTCA AGGAAAGAGC ATAAGCCCTA CCGTCAGCAC CCAAAGCTGA AATTCTACTT
      121 AAACTACTCC TTGCAAGCAT AC-TGTCAAG TATGCAT-TC C-ATGGTTAT ACTATGTACA
      181 TCGTGCATTA ACTTTATTTC CCCATGAATA TGTAGCATGT ACATTTATGA TCTTGTATTA
      241 CATGAGTACA TTATATTATA TATCGTACAT AAACCATCTG ATGTGATAAA TTCCAGTCAA
      301 CATGACTATT CCGCAAGCAC TGTTGGTTTA TTAATCTACC ATCCTCCGTG AAACCAGCAA
      361 CCCGCCCACA ACGTGTCCCT CTTCTCGCCC CGGGCCCATA AATCGTGGGG GTGACTATAC
      421 TGGGTCGTAA ACGGCATCTG GTTCCTACTT CAG-----

LOCUS       F-G22-X03       458 bp

        1 ATGTAGT--A TACCCATTAC ACTGGTCTTG TAAACCAGAG ACGAGGAATA GTAGTTCCTC
       61 CAAAGACTCA AGGAAAGAGC ATAAGCCCTA CCGTCAGCAC CCAAAGCTGA AATTCTACTT
      121 AAACTACTCC TTGCAAGCAT AC-TGTCAAG TATGCAT-TC C-ATGGTTAT ACTATGTACA
      181 TCGTGCATTA ACTTTATTTC CCCATGAATA TGTAGCATGT ACATTTATGA TCTTGTATTA
      241 CATGAGTACA TTATATTATA TATCGTACAT AAACCATCTG ATGTGATAAA TTCCAGTCAA
      301 CATGACTATT CCGCAAGCAC TGTTGGTTTA TTAATCTACC ATCCTCCGTG AAACCAGCAA
      361 CCCGCCCACA ACGTGTCCCT CTTCTCGCCC CGGGCCCATA AATCGTGGGG GTGACTATAC
      421 TGGGTCGTAA ACGGCATCTG GTTCCTACTT CAGGA---
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LOCUS       M-03-017-X04       458 bp

        1 ATGTAGT--A TACCCATTAC ACTGGTCTTG TAAACCAGAG ACGAGGAATA GTAGTTCCTC
       61 CAAAGACTCA AGGAAAGAGC ATAAGCCCTA CCGTCAGCAC CCAAAGCTGA AATTCTACTT
      121 AAACTACTCC TTGCAAGCAT AC-TGTCAAG TATGCAT-TC C-ATGGTTAT ACTATGTACG
      181 TCGTGCATTA ACTTTATTTC CCCATGAATA TGTAGCATGT ACATTTATGA TCTTGTATTA
      241 CATGAGTACA TTATATTATA TATCGTACAT AAACCATCTG ATGTGATAAA TTCCAGTCAA
      301 CATGACTATT CCGCAAGCAC TGTTGGTTTA TTAATCTACC ATCCTCCGTG AAACCAGCAA
      361 CCCGCCCACA ACGTGTCCCT CTTCTCGCCC CGGGCCCATA AATCGTGGGG GTGACTACAC
      421 TGGGTCGTAA ACGGCATCTG GTTCCTACTT CAG-----

LOCUS       M-03-018-X05       458 bp

        1 ATGTAGT--A TACCCATTAC ACTGGTCTTG TAAACCAGAG ACGAGGAATA GTAGTTCCTC
       61 CAAAGACTCA AGGAAAGAGC ATAAGCCCTA CCGTCAGCAC CCAAAGCTGA AATTCTACTT
      121 AAACTACTCC TTGCAAGCAT AC-TGTCAAG TATGCAT-TC C-ATGGTTAT ACTATGTACG
      181 TCGTGCATTA ACTTTATTTC CCCATGAATA TGTAGCATGT ACATTTATGA TCTTGTATTA
      241 CATGAGTACA TTATATTATA TATCGTACAT AAACCATCTG ATGTGATAAA TTCCAGTCAA
      301 CATGACTATT CCGCAAGCAC TGTTGGTTTA TTAATCTACC ATCCTCCGTG AAACCAGCAA
      361 CCCGCCCACA ACGTGTCCCT CTTCTCGCCC CGGGCCCATA AATCGTGGGG GTGACTATAC
      421 TGGGTCGTAA ACGGCATCTG GTTCCTACTT CAG-----

LOCUS       M-03-019-X05       458 bp

        1 ATGTAGT--A TACCCATTAC ACTGGTCTTG TAAACCAGAG ACGAGGAATA GTAGTTCCTC
       61 CAAAGACTCA AGGAAAGAGC ATAAGCCCTA CCGTCAGCAC CCAAAGCTGA AATTCTACTT
      121 AAACTACTCC TTGCAAGCAT AC-TGTCAAG TATGCAT-TC C-ATGGTTAT ACTATGTACG
      181 TCGTGCATTA ACTTTATTTC CCCATGAATA TGTAGCATGT ACATTTATGA TCTTGTATTA
      241 CATGAGTACA TTATATTATA TATCGTACAT AAACCATCTG ATGTGATAAA TTCCAGTCAA
      301 CATGACTATT CCGCAAGCAC TGTTGGTTTA TTAATCTACC ATCCTCCGTG AAACCAGCAA
      361 CCCGCCCACA ACGTGTCCCT CTTCTCGCCC CGGGCCCATA AATCGTGGGG GTGACTATAC
      421 TGGGTCGTAA ACGGCATCTG GTTCCTACTT CAG-----

LOCUS       M-03-022-X05       458 bp

        1 ATGTAGT--A TACCCATTAC ACTGGTCTTG TAAACCAGAG ACGAGGAATA GTAGTTCCTC
       61 CAAAGACTCA AGGAAAGAGC ATAAGCCCTA CCGTCAGCAC CCAAAGCTGA AATTCTACTT
      121 AAACTACTCC TTGCAAGCAT AC-TGTCAAG TATGCAT-TC C-ATGGTTAT ACTATGTACG
      181 TCGTGCATTA ACTTTATTTC CCCATGAATA TGTAGCATGT ACATTTATGA TCTTGTATTA
      241 CATGAGTACA TTATATTATA TATCGTACAT AAACCATCTG ATGTGATAAA TTCCAGTCAA
      301 CATGACTATT CCGCAAGCAC TGTTGGTTTA TTAATCTACC ATCCTCCGTG AAACCAGCAA
      361 CCCGCCCACA ACGTGTCCCT CTTCTCGCCC CGGGCCCATA AATCGTGGGG GTGACTATAC
      421 TGGGTCGTAA ACGGCATCTG GTTCCTACTT CAG-----

LOCUS       M-G21-X05       458 bp

        1 ATGTAGT--A TACCCATTAC ACTGGTCTTG TAAACCAGAG ACGAGGAATA GTAGTTCCTC
       61 CAAAGACTCA AGGAAAGAGC ATAAGCCCTA CCGTCAGCAC CCAAAGCTGA AATTCTACTT
      121 AAACTACTCC TTGCAAGCAT AC-TGTCAAG TATGCAT-TC C-ATGGTTAT ACTATGTACG
      181 TCGTGCATTA ACTTTATTTC CCCATGAATA TGTAGCATGT ACATTTATGA TCTTGTATTA
      241 CATGAGTACA TTATATTATA TATCGTACAT AAACCATCTG ATGTGATAAA TTCCAGTCAA
      301 CATGACTATT CCGCAAGCAC TGTTGGTTTA TTAATCTACC ATCCTCCGTG AAACCAGCAA
      361 CCCGCCCACA ACGTGTCCCT CTTCTCGCCC CGGGCCCATA AATCGTGGGG GTGACTATAC
      421 TGGGTCGTAA ACGGCATCTG GTTCCTACTT CAGGA---
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LOCUS       M-06-035-X06       458 bp

        1 ATGTAGT--A TACCCATTAC ACTGGTCTTG TAAACCAGAG ACGAGGAATA GTAGTTCCTC
       61 CAAAGACTCA AGGAAAGAGC ATAAGCCCTA CCGTCAGCAC CCAAAGCTGA AATTCTACTT
      121 AAACTACTCC TTGCAAGCAT AC-TGTCAAG TATGCAT-TC C-ATGGTTAT ACTATGTACA
      181 TCGTGCATTA ACTTTATTTC CCCATGAATA TGTAGCATGT ACATTTATAA TCTTATATTA
      241 CATGAGTACA TTATATTATA TATCGTACAT AAACCATCTG ATGTGATAAA TTCCAGTCAA
      301 CATGACTATT CCGCAAGCAC TGTTGGTTTA TTAATCTACC ATCCTCCGTG AAACCAGCAA
      361 CCCGCCCACA ACGTGTCCCT CTTCTCGCCC CGGGCCCATA AATCGTGGGG GTGACTACAC
      421 TGGGTCGTAA ACGGCATCTG GTTCCTACTT CAG-----

LOCUS       F-07-037-X06       458 bp

        1 ATGTAGT--A TACCCATTAC ACTGGTCTTG TAAACCAGAG ACGAGGAATA GTAGTTCCTC
       61 CAAAGACTCA AGGAAAGAGC ATAAGCCCTA CCGTCAGCAC CCAAAGCTGA AATTCTACTT
      121 AAACTACTCC TTGCAAGCAT AC-TGTCAAG TATGCAT-TC C-ATGGTTAT ACTATGTACA
      181 TCGTGCATTA ACTTTATTTC CCCATGAATA TGTAGCATGT ACATTTATAA TCTTATATTA
      241 CATGAGTACA TTATATTATA TATCGTACAT AAACCATCTG ATGTGATAAA TTCCAGTCAA
      301 CATGACTATT CCGCAAGCAC TGTTGGTTTA TTAATCTACC ATCCTCCGTG AAACCAGCAA
      361 CCCGCCCACA ACGTGTCCCT CTTCTCGCCC CGGGCCCATA AATCGTGGGG GTGACTACAC
      421 TGGGTCGTAA ACGGCATCTG GTTCCTACTT CAG-----

LOCUS       M-07-046-X06       458 bp

        1 ATGTAGT--A TACCCATTAC ACTGGTCTTG TAAACCAGAG ACGAGGAATA GTAGTTCCTC
       61 CAAAGACTCA AGGAAAGAGC ATAAGCCCTA CCGTCAGCAC CCAAAGCTGA AATTCTACTT
      121 AAACTACTCC TTGCAAGCAT AC-TGTCAAG TATGCAT-TC C-ATGGTTAT ACTATGTACA
      181 TCGTGCATTA ACTTTATTTC CCCATGAATA TGTAGCATGT ACATTTATAA TCTTATATTA
      241 CATGAGTACA TTATATTATA TATCGTACAT AAACCATCTG ATGTGATAAA TTCCAGTCAA
      301 CATGACTATT CCGCAAGCAC TGTTGGTTTA TTAATCTACC ATCCTCCGTG AAACCAGCAA
      361 CCCGCCCACA ACGTGTCCCT CTTCTCGCCC CGGGCCCATA AATCGTGGGG GTGACTACAC
      421 TGGGTCGTAA ACGGCATCTG GTTCCTACTT CAG-----

LOCUS       M-07-055-X06       458 bp

        1 ATGTAGT--A TACCCATTAC ACTGGTCTTG TAAACCAGAG ACGAGGAATA GTAGTTCCTC
       61 CAAAGACTCA AGGAAAGAGC ATAAGCCCTA CCGTCAGCAC CCAAAGCTGA AATTCTACTT
      121 AAACTACTCC TTGCAAGCAT AC-TGTCAAG TATGCAT-TC C-ATGGTTAT ACTATGTACA
      181 TCGTGCATTA ACTTTATTTC CCCATGAATA TGTAGCATGT ACATTTATAA TCTTATATTA
      241 CATGAGTACA TTATATTATA TATCGTACAT AAACCATCTG ATGTGATAAA TTCCAGTCAA
      301 CATGACTATT CCGCAAGCAC TGTTGGTTTA TTAATCTACC ATCCTCCGTG AAACCAGCAA
      361 CCCGCCCACA ACGTGTCCCT CTTCTCGCCC CGGGCCCATA AATCGTGGGG GTGACTACAC
      421 TGGGTCGTAA ACGGCATCTG GTTCCTACTT CAG-----

LOCUS       F-07-058-X06       458 bp

        1 ATGTAGT--A TACCCATTAC ACTGGTCTTG TAAACCAGAG ACGAGGAATA GTAGTTCCTC
       61 CAAAGACTCA AGGAAAGAGC ATAAGCCCTA CCGTCAGCAC CCAAAGCTGA AATTCTACTT
      121 AAACTACTCC TTGCAAGCAT AC-TGTCAAG TATGCAT-TC C-ATGGTTAT ACTATGTACA
      181 TCGTGCATTA ACTTTATTTC CCCATGAATA TGTAGCATGT ACATTTATAA TCTTATATTA
      241 CATGAGTACA TTATATTATA TATCGTACAT AAACCATCTG ATGTGATAAA TTCCAGTCAA
      301 CATGACTATT CCGCAAGCAC TGTTGGTTTA TTAATCTACC ATCCTCCGTG AAACCAGCAA
      361 CCCGCCCACA ACGTGTCCCT CTTCTCGCCC CGGGCCCATA AATCGTGGGG GTGACTACAC
      421 TGGGTCGTAA ACGGCATCTG GTTCCTACTT CAG-----
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LOCUS       M-07-050-X07       458 bp

        1 ATGTAGT--A TACCCATTAC ACTGGTCTTG TAAACCAGAG ACGAGGAATA GTAGTTCCTC
       61 CAAAGACTCA AGGAAAGAGC ATAAGCCCTA CCGTCAGCAC CCAAAGCTGA AATTCTACTT
      121 AAACTACTCC TTGCAAGCAT AC-TGTCAAG TATGCAT-TC C-ATGGTTAT ACTATGTACA
      181 TCGTGCATTA ACTTTATTTC CCCATGAATA TGTAGCATGT ACATTTATAA TCTTGTATTA
      241 CATGAGTACA TTATATTATA TATCGTACAT AAACCATCTG ATGTGATAAA TTCCAGTCAA
      301 CATGACTATT CCGCAAGCAC TGTTGGTTTA TTAATCTACC ATCCTCCGTG AAACCAGCAA
      361 CCCGCCCACA ACGTGTCCCT CTTCTCGCCC CGGGCCCATA AATCGTGGGG GTGACTACAC
      421 TGGGTCGTAA ACGGCATCTG GTTCCTACTT CAG-----

LOCUS       M-07-040-X07       458 bp

        1 ATGTAGT--A TACCCATTAC ACTGGTCTTG TAAACCAGAG ACGAGGAATA GTAGTTCCTC
       61 CAAAGACTCA AGGAAAGAGC ATAAGCCCTA CCGTCAGCAC CCAAAGCTGA AATTCTACTT
      121 AAACTACTCC TTGCAAGCAT AC-TGTCAAG TATGCAT-TC C-ATGGTTAT ACTATGTACA
      181 TCGTGCATTA ACTTTATTTC CCCATGAATA TGTAGCATGT ACATTTATAA TCTTGTATTA
      241 CATGAGTACA TTATATTATA TATCGTACAT AAACCATCTG ATGTGATAAA TTCCAGTCAA
      301 CATGACTATT CCGCAAGCAC TGTTGGTTTA TTAATCTACC ATCCTCCGTG AAACCAGCAA
      361 CCCGCCCACA ACGTGTCCCT CTTCTCGCCC CGGGCCCATA AATCGTGGGG GTGACTACAC
      421 TGGGTCGTAA ACGGCATCTG GTTCCTACTT CAG-----

LOCUS       M-07-060-X07       458 bp

        1 ATGTAGT--A TACCCATTAC ACTGGTCTTG TAAACCAGAG ACGAGGAATA GTAGTTCCTC
       61 CAAAGACTCA AGGAAAGAGC ATAAGCCCTA CCGTCAGCAC CCAAAGCTGA AATTCTACTT
      121 AAACTACTCC TTGCAAGCAT AC-TGTCAAG TATGCAT-TC C-ATGGTTAT ACTATGTACA
      181 TCGTGCATTA ACTTTATTTC CCCATGAATA TGTAGCATGT ACATTTATAA TCTTGTATTA
      241 CATGAGTACA TTATATTATA TATCGTACAT AAACCATCTG ATGTGATAAA TTCCAGTCAA
      301 CATGACTATT CCGCAAGCAC TGTTGGTTTA TTAATCTACC ATCCTCCGTG AAACCAGCAA
      361 CCCGCCCACA ACGTGTCCCT CTTCTCGCCC CGGGCCCATA AATCGTGGGG GTGACTACAC
      421 TGGGTCGTAA ACGGCATCTG GTTCCTACTT CAG-----

LOCUS       M-07-041-X08       458 bp

        1 ATGAAGTTGA TAACCATTAC ACTGGtCTTG TAAACCAGAG ACGAGGAATA GTAGTTCCTC
       61 CAAAGACTCA AGGAAAGAGC ATAAGCCCTA CCGTCAGCAC CCAAAGCTGA AATTCTACTT
      121 AAACTACTCC TTGCAAGCAT AC-TGTCAAG TATGCAT-TC C-ATGGTTAT ACTATGTACA
      181 TCGTGCATTA ACTTTATTTC CCCATGAATA TGTAGCATGT ACATTTATAA TCTTATATTA
      241 CATGAGTACA TTATATTATA TATCGTACAT AAACCATCTG ATGTGATAAA TTCCAGTCAA
      301 CATGACTATT CCGCAAGCAC TGTTGGTTTA TTAATCTACC ATCCTCCGTG AAACCAGCAA
      361 CCCGCCCACA ---------- ---------- ---------- ---------- ----------
      421 ---------- ---------- ---------- --------

LOCUS       M-07-043-X08       458 bp

        1 ATGAAGTTGA TAACCATTAC ACTGGtCTTG TAAACCAGAG ACGAGGAATA GTAGTTCCTC
       61 CAAAGACTCA AGGAAAGAGC ATAAGCCCTA CCGTCAGCAC CCAAAGCTGA AATTCTACTT
      121 AAACTACTCC TTGCAAGCAT AC-TGTCAAG TATGCAT-TC C-ATGGTTAT ACTATGTACA
      181 TCGTGCATTA ACTTTATTTC CCCATGAATA TGTAGCATGT ACATTTATAA TCTTATATTA
      241 CATGAGTACA TTATATTATA TATCGTACAT AAACCATCTG ATGTGATAAA TTCCAGTCAA
      301 CATGACTATT CCGCAAGCAC TGTTGGTTTA TTAATCTACC ATCCTCCGTG AAACCAGCAA
      361 CCCGCCCACA ACG------- ---------- ---------- ---------- ----------
      421 ---------- ---------- ---------- --------
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LOCUS       F-07-045-X09       458 bp

        1 ATGAAGTTGA TAACCATTAC ACTGGtCTTG TAAACCAGAG ACGAGGAATA GTAGTTCCTC
       61 CAAAGACTCA AGGAAAGAGC ATAAGCCCTA CCGTCAGCAC CCAAAGCTGA AATTCTACTT
      121 AAACTACTCC TTGCAAGCAT AC-TGTCAAG TATGCAT-TC C-ATGGTTAT ACTATGTACA
      181 TCGTGCATTA ACTTTATTTC CCCATGAATA TGTAGCATGT ACATTTATAA TCTTGTATTA
      241 CATGAGTACA TTATATTATA TATCGTACAT AAACCATCTG ATGTGATAAA TTCCAGTCAA
      301 CATGACTATT CCGCAAGCAC TGTTGGTTTA TTAATCTACC ATCCTCCGTG AAACCAGCAA
      361 CCCGCCCACA AC-------- ---------- ---------- ---------- ----------
      421 ---------- ---------- ---------- --------

LOCUS       F-07-057-X10       458 bp

        1 ATGTAGT--A TACCCATTAC ACTGGTCTTG TAAACCAGAG ACGAGGAATA GTAGTTCCTC
       61 CAAAGACTCA AGGAAAGAGC ATAAGCCCTA CCGTCAGCAC CCAAAGCTGA AATTCTACTT
      121 AAACTACTCC TTGCAAGCAT AC-TGTCAAG TATGCAT-TC C-ATGGTTAT ACTATGTACA
      181 TCGTGCATTA ACTTTATTTC CCCATGAATA TGTAGCATGT ACATTTATAA TCTTGTATTA
      241 CATGAGTACA TTATATTATA TATCGTACAT AAACCATCTG ATGTGATAAA TTCCAGTCAA
      301 CATGACTATT CCGCAAGCAC TGTTGGTTTA TTAATCTACC ATCCTCCGTG AAACCAGCAA
      361 CCCGCCCACA ACGTGTCCCT CTTCTCGCCC CGGGCCCATA AATCGTGGGG GTGACTACAC
      421 TGGGTCGTAA ACGGCATCTG GTTCTTACTT CAG-----

LOCUS       M-13-094-X11       458 bp

        1 ATGTAGT--A TACCCATTAC ACTGGTCTTG TAAACCAGAG ATGAGGAATA GTAGTTCCTC
       61 CAAAGACTCA AGGAAAGAGC ATAAGCCCTA CCGTCAGCAC CCAAAGCTGA AATTCTACTT
      121 AAACTACTCC TTGCAAGCAT AC-TGTCAAG TATGCAT-TC C-ATGGTTAT ACTATGTACA
      181 TCGTGCATTA ACTTTATTTC CCCATGAATA TGTAGCATGT ACATTTATAA TCTTGTATTA
      241 CATGAGTACA TTATATTATA TATCGTACAT AAACCATCTG ATGTGATAAA TTCCAGTCAA
      301 CATGACTATC CCGCAAGCAC TGTTGGTTTA TTAATCTACC ATCCTCCGTG AAACCAGCAA
      361 CCCGCCCACA ACGTGTCCCT CTTCTCGCCC CGGGCCCATA AATCGTGGGG GTGACTATAC
      421 TGGGTCGTAA ACGGCATCTG GTTCCTACTT CAG-----

LOCUS       M-13-095-X11       458 bp

        1 ATGTAGT--A TACCCATTAC ACTGGTCTTG TAAACCAGAG ATGAGGAATA GTAGTTCCTC
       61 CAAAGACTCA AGGAAAGAGC ATAAGCCCTA CCGTCAGCAC CCAAAGCTGA AATTCTACTT
      121 AAACTACTCC TTGCAAGCAT AC-TGTCAAG TATGCAT-TC C-ATGGTTAT ACTATGTACA
      181 TCGTGCATTA ACTTTATTTC CCCATGAATA TGTAGCATGT ACATTTATAA TCTTGTATTA
      241 CATGAGTACA TTATATTATA TATCGTACAT AAACCATCTG ATGTGATAAA TTCCAGTCAA
      301 CATGACTATC CCGCAAGCAC TGTTGGTTTA TTAATCTACC ATCCTCCGTG AAACCAGCAA
      361 CCCGCCCACA ACGTGTCCCT CTTCTCGCCC CGGGCCCATA AATCGTGGGG GTGACTATAC
      421 TGGGTCGTAA ACGGCATCTG GTTCCTACTT CAG-----

LOCUS       M-13-096-X11       458 bp

        1 ATGTAGT--A TACCCATTAC ACTGGTCTTG TAAACCAGAG ATGAGGAATA GTAGTTCCTC
       61 CAAAGACTCA AGGAAAGAGC ATAAGCCCTA CCGTCAGCAC CCAAAGCTGA AATTCTACTT
      121 AAACTACTCC TTGCAAGCAT AC-TGTCAAG TATGCAT-TC C-ATGGTTAT ACTATGTACA
      181 TCGTGCATTA ACTTTATTTC CCCATGAATA TGTAGCATGT ACATTTATAA TCTTGTATTA
      241 CATGAGTACA TTATATTATA TATCGTACAT AAACCATCTG ATGTGATAAA TTCCAGTCAA
      301 CATGACTATC CCGCAAGCAC TGTTGGTTTA TTAATCTACC ATCCTCCGTG AAACCAGCAA
      361 CCCGCCCACA ACGTGTCCCT CTTCTCGCCC CGGGCCCATA AATCGTGGGG GTGACTATAC
      421 TGGGTCGTAA ACGGCATCTG GTTCCTACTT CAG-----
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LOCUS       M-13-097-X11       458 bp

        1 ATGTAGT--A TACCCATTAC ACTGGTCTTG TAAACCAGAG ATGAGGAATA GTAGTTCCTC
       61 CAAAGACTCA AGGAAAGAGC ATAAGCCCTA CCGTCAGCAC CCAAAGCTGA AATTCTACTT
      121 AAACTACTCC TTGCAAGCAT AC-TGTCAAG TATGCAT-TC C-ATGGTTAT ACTATGTACA
      181 TCGTGCATTA ACTTTATTTC CCCATGAATA TGTAGCATGT ACATTTATAA TCTTGTATTA
      241 CATGAGTACA TTATATTATA TATCGTACAT AAACCATCTG ATGTGATAAA TTCCAGTCAA
      301 CATGACTATC CCGCAAGCAC TGTTGGTTTA TTAATCTACC ATCCTCCGTG AAACCAGCAA
      361 CCCGCCCACA ACGTGTCCCT CTTCTCGCCC CGGGCCCATA AATCGTGGGG GTGACTATAC
      421 TGGGTCGTAA ACGGCATCTG GTTCCTACTT CAG-----

LOCUS       F-13-098-X12       458 bp

        1 ATGTAGT--A TACCCATTAC ACTGGTCTTG TAAACCAGAG ATGAGGAATA GTAGTTCCTC
       61 CAAAGACTCA AGGAAAGAGC ATAAGCCCTA CCGTCAGCAC CCAAAGCTGA AATTCTACTT
      121 AAACTACTCC TTGCAAGCAT AC-TGTCAAG TATGCAT-TC C-ATGGTTAT ACTATGTACA
      181 TCGTGCATTA ACTTTATTTC CCCATGAATA TGTAGCATGT ACATTTATAA TCTTGTATTA
      241 CATGAGTACA TTATATTATA TATCGTACAT AAACCATCTG ATGTGATAAA TTCCAGTCAA
      301 CATGGCTATC CCGCAAGCAC TGTTGGTTTA TTAATCTACC ATCCTCCGTG AAACCAGCAA
      361 CCCGCCCACA ACGTGTCCCT CTTCTCGCCC CGGGCC---- ---------- ----------
      421 ---------- ---------- ---------- --------

LOCUS       F-17-130-X13       458 bp

        1 ATGTAGT--A TACCCATTAC ACTGGTCTTG TAAACCAGAG ACGAGGAATA GTAGTTCCTC
       61 CAAAGACTCA AGGAAAGAGC ATAAGCCCTA CCGTCAGCAC CCAAAGCTGA AATTCTACTT
      121 AAACTACTCC TTGCAAGCAT AC-TGTCAAG TATGCAT-TC C-ATGGTTAT ACTATGTACA
      181 TCGTGCATTA ACTTTATTTC CCCATGAATA TGTAGCATGT ACATTTATGA TCTTGTATTA
      241 CATGAGTACA TTATATTATA TATCGTACAT AAACCATCTG ATGTGATAAA TTCCAGTCAA
      301 CATGACTATT CCGCAAGCAC TGTTGGTTTA TTAATCTACC ATCCTCCGTG AAACCAGCAA
      361 CCCACCCACA ACGTGTCCCT CTTCTCGCCC CGGGCCCATA AATCGTGGGG GTAACTATAC
      421 TGGGTCGTAA ACGGCATCTG GTTCCTACTT CAG-----

LOCUS       M-19-203-X14       458 bp

        1 ATGTAGT--A TACCCATTAC ACTGGTCTTG TAAACCAGAG ACGAGGAATA GTAGTTCCTC
       61 CAAAGACTCA AGGAAAGAGC ATAAGCCCTA CCGTCAGCAC CCAAAGCTGA AATTCTACTT
      121 AAACTACTCC TTGCAAGCAT AC-TGTCAAG TATGCAT-TC C-ATGGTTAT ACTATGTACA
      181 TCGTGCATTA ACTTTATTTC CCCATGAATA TGTAGCATGT ACATTTATAA TCTTGTATTA
      241 CATGAGTACA TTATATTATA TATCGTACAT AAACCATCTG ATGTGATAAA TTCCAGTCAA
      301 CATGACTATT CCGCAAGCAC TGTTGATTTA TTAATCTACC ATCCTCCGTG AAACCAGCAA
      361 CCCGCCCACA ACGTGTCCCT CTTCTCGCCC CGGGCCCATA AATCGTGGGG GTGACTATAC
      421 TGGGTCGTAA ACGGCATCTG GTTCCTACTT CAG-----

LOCUS       F-19-208-X15       458 bp

        1 ATGTAGT--A TACCCATTAC ACTGGTCTTG TAAACCAGAG ACGAGGAATA GTAGTTCCTC
       61 CAAAGACTCA AGGAAAGAGC ATAAGCCCTA CCGTCAGCAC CCAAAGCTGA AATTCTACTT
      121 AAACTACTCC TTGCAAGCAT AC-TGTCAAG TATGCAT-TC C-ATGGTTAT ACTATGTACA
      181 TCGTGCATTA ACTTTATTTC CCCATGAATA TGTAGCATGT ACATTTATGA TCTTGTATTA
      241 CATGAGTACA TTATATTATA TATCGTACAT AAACCATCTA ATGTGATAAA TTCCAGTCAA
      301 CATGACTATT CCGCAAGCAC TGTTGGTTTA TTAATCTACC ATCCTCCGTG AAACCAGCAA
      361 CCCGCCCACA ACGTGTCCCT CTTCTCGCCC CGGGCCCATA AATCGTGGGG GTGACTATAC
      421 TGGGTCGTAA ACGGCATCTG GTTCCTACTT CAG-----
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LOCUS       M-G23-X16       458 bp

        1 ATGTAGT--A TACCCATTAC ACTGGTCTTG TAAACCAGAG ACGAGGAATA ATAGTTCCTC
       61 CAAAGACTCA AGGAAAGAGC ATAAGCCCTA CCGTCAGCAC CCAAAGCTGA AATTCTACTT
      121 AAACTACTCC TTGCAAGCAT AC-TGTCAAG TATGCAT-TC C-ATGGTTAT ACTATGTACA
      181 TCGTGCATTA ACTTTATTTC CCCATGAATA TGTAGCATGT ACATTTATGA TCTTGTATTA
      241 CATGAGTACA TTATATTATA TATCGTACAT AAACCATCTG ATGTGATAAA TTCCAGTCAA
      301 CATGACTATT CCGCAAGCAC TGTTGGTTTA TTAATCTACC ATCCTCCGTG AAACCAGCAA
      361 CCCGCCCACA ACGTGTCCCT CTTCTCGCCC CGGGCCCATA AATCGTGGGG GTGACTATAC
      421 TGGGTCGTAA ACGGCATCTG GTTCCTACTT CAGGA---

LOCUS       M-09-075-Y01       458 bp

        1 ATGTAGT--A TACCCATTAC ACTGGTCTTG TAAACCAGAG ACGAGGAATA GTAGTTCCTC
       61 CAAAGACTCA AGGAAAGAGC ATAAGCTCTA CCATCAGCAC CCAAAGCTGA AATTCTACTT
      121 AAACTATTCC TTGCAAGCAC A--TATGCTG GACATATATC CTATAGTTAT ACTATGTACA
      181 TCGTGCATTA ACTTTATTTC CCCATGGATA TGTAGCATGT ACATTTATGA TCTTACATTA
      241 CATGAGTACA TTGTATTATA TATCGTACAT AAACCATCTA ATGTGATAAG TTCCAGCCAA
      301 CATGGCTATC CTACAAGTAC TGTTAGTTTA TTAATCTACC ATCCTCCGTG AAACCAGCAA
      361 CCCGCCCACA GCGTGTCCCT CTTCTCGCCC CGGGCCCATA AATCGTGGGG GTAGCTATAC
      421 TGGGTCGTAA ACGGCATCTG GTTCCTACTT CAG-----

LOCUS       F-09-076-Y01       458 bp

        1 ATGTAGT--A TACCCATTAC ACTGGTCTTG TAAACCAGAG ACGAGGAATA GTAGTTCCTC
       61 CAAAGACTCA AGGAAAGAGC ATAAGCTCTA CCATCAGCAC CCAAAGCTGA AATTCTACTT
      121 AAACTATTCC TTGCAAGCAC A--TATGCTG GACATATATC CTATAGTTAT ACTATGTACA
      181 TCGTGCATTA ACTTTATTTC CCCATGGATA TGTAGCATGT ACATTTATGA TCTTACATTA
      241 CATGAGTACA TTGTATTATA TATCGTACAT AAACCATCTA ATGTGATAAG TTCCAGCCAA
      301 CATGGCTATC CTACAAGTAC TGTTAGTTTA TTAATCTACC ATCCTCCGTG AAACCAGCAA
      361 CCCGCCCACA GCGTGTCCCT CTTCTCGCCC CGGGCCCATA AATCGTGGGG GTAGCTATAC
      421 TGGGTCGTAA ACGGCATCTG GTTCCTACTT CAG-----

LOCUS       M-09-077-Y01       458 bp

        1 ATGTAGT--A TACCCATTAC ACTGGTCTTG TAAACCAGAG ACGAGGAATA GTAGTTCCTC
       61 CAAAGACTCA AGGAAAGAGC ATAAGCTCTA CCATCAGCAC CCAAAGCTGA AATTCTACTT
      121 AAACTATTCC TTGCAAGCAC A--TATGCTG GACATATATC CTATAGTTAT ACTATGTACA
      181 TCGTGCATTA ACTTTATTTC CCCATGGATA TGTAGCATGT ACATTTATGA TCTTACATTA
      241 CATGAGTACA TTGTATTATA TATCGTACAT AAACCATCTA ATGTGATAAG TTCCAGCCAA
      301 CATGGCTATC CTACAAGTAC TGTTAGTTTA TTAATCTACC ATCCTCCGTG AAACCAGCAA
      361 CCCGCCCACA GCGTGTCCCT CTTCTCGCCC CGGGCCCATA AATCGTGGGG GTAGCTATAC
      421 TGGGTCGTAA ACGGCATCTG GTTCCTACTT CAG-----

LOCUS       M-09-078-Y01       458 bp

        1 ATGTAGT--A TACCCATTAC ACTGGTCTTG TAAACCAGAG ACGAGGAATA GTAGTTCCTC
       61 CAAAGACTCA AGGAAAGAGC ATAAGCTCTA CCATCAGCAC CCAAAGCTGA AATTCTACTT
      121 AAACTATTCC TTGCAAGCAC A--TATGCTG GACATATATC CTATAGTTAT ACTATGTACA
      181 TCGTGCATTA ACTTTATTTC CCCATGGATA TGTAGCATGT ACATTTATGA TCTTACATTA
      241 CATGAGTACA TTGTATTATA TATCGTACAT AAACCATCTA ATGTGATAAG TTCCAGCCAA
      301 CATGGCTATC CTACAAGTAC TGTTAGTTTA TTAATCTACC ATCCTCCGTG AAACCAGCAA
      361 CCCGCCCACA GCGTGTCCCT CTTCTCGCCC CGGGCCCATA AATCGTGGGG GTAGCTATAC
      421 TGGGTCGTAA ACGGCATCTG GTTCCTACTT CAG-----
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LOCUS       F-A09-Y01       458 bp

        1 ATGTAGT--A TACCCATTAC ACTGGTCTTG TAAACCAGAG ACGAGGAATA GTAGTTCCTC
       61 CAAAGACTCA AGGAAAGAGC ATAAGCTCTA CCATCAGCAC CCAAAGCTGA AATTCTACTT
      121 AAACTATTCC TTGCAAGCAC A--TATGCTG GACATATATC CTATAGTTAT ACTATGTACA
      181 TCGTGCATTA ACTTTATTTC CCCATGGATA TGTAGCATGT ACATTTATGA TCTTACATTA
      241 CATGAGTACA TTGTATTATA TATCGTACAT AAACCATCTA ATGTGATAAG TTCCAGCCAA
      301 CATGGCTATC CTACAAGTAC TGTTAGTTTA TTAATCTACC ATCCTCCGTG AAACCAGCAA
      361 CCCGCCCACA GCGTGTCCCT CTTCTCGCCC CGGGCCCATA AATCGTGGGG GTAGCTATAC
      421 TGGGTCGTAA ACGGCATCTG GTTCCTACTT CAGGAG--

LOCUS       F-09-079-Y02       458 bp

        1 ATGTAGT--A TACCCATTAC ACTGGTCTTG TAAACCAGAG ACGAGGAATA GTAATTCCTC
       61 CAAAGACTCA AGGAAAGAGC ATAAGCTCTA CCATCAGCAC CCAAAGCTGA AATTCTACTT
      121 AAACTATTCC TTGCAAGCAC A--TATGCTG GACATATATC CTATAGTTAT ACTATGTACA
      181 TCGTGCATTA ACTTTATTTC CCCATGGATA TGTAGCATGT ACATTTATGA TCTTACATTA
      241 CATGAGTACA TTGTATTATA TATCGTACAT AAACCATCTA ATGTGATAAG TTCCAGCCAA
      301 CATGGCTATC CTACAAGTAC TGTTAGTTTA TTAATCTACC ATCCTCCGTG AAACCAGCAA
      361 CCCGCCCACA GCGTGTCCCT CTTCTCGCCC CGGGCCCATA AATCGTGGGG GTAGCTATAC
      421 TGGGTCGTAA ACGGCATCTG GTTCCTACTT CAG-----

LOCUS       F-09-080-Y03       458 bp

        1 ATGTAGT--A TACCCATTAC ACTGGTCTTG TAAACCAGAG ACGAGGAATA GTAGTTCCTC
       61 CAAAGACTCA AGGAAAGAGC ATAAGCTCTA CCATCAGCAC CCAAAGCTGA AATTCTACTT
      121 AAACTATTCC TTGCAAGCAC A--TATGCTG GACATATATC CTATAGTTAT ACTATGTACA
      181 TCGTGCATTA ACTTTATTTC CCCATGGATA TGTAGCATGT ACATTTATGA TCTTACATTA
      241 CATGAGTACA TTGTATTATA TATCGTACAT AAACCATCTA ATGTGATAAG TTCCAGTCAA
      301 CATGGCTATC CTACAAGTAC TGTTAGTTTA TTAATCTACC ATCCTCCGTG AAACCAGCAA
      361 CCCGCCCACA GCGTGTCCCT CTTCTCGCCC CGGGCCCATA AATCGTGGGG GTAGCTATAC
      421 TGGGTCGTAA ACGGCATCTG GTTCCTACTT CAG-----

LOCUS       M-A06-Y04       458 bp

        1 ATGTAGT--A TACCCATTAC ACTGGTCTTG TAAACCAGAG ACGAGGAATA GTAGTTCCTC
       61 CAAAGACTCA AGGAAAGAGC ATAAGCTCTA CCATCAGCAC CCAAAGCTGA AATTCTACTT
      121 AAACTATTCC TTGCAAGCAC A--TATGCTG GACATATATC CTATAGTTAT ACTATGTACA
      181 TCGTGCATTA ACTTTATTTC CCCATGGATA TGTAGCATGT ACATTTATGA TCTTATATTA
      241 CATGAGTACA TTGTATTATA TATCGTACAT AAACCATCTA ATGTGATAAG TTCCAGTCAA
      301 CATGGCTATC CTACAAGTAC TGTTAGTTTA TTAATCTACC ATCCTCCGTG AAACCAGCAA
      361 CCCGCCCACA GCGTGTCCCT CTTCTCGCCC CGGGCCCATA AATCGTGGGG GTAGCTATAC
      421 TGGGTCGTAA ACGGCATCTG GTTCCTACTT CAGGAG--

LOCUS       M-A17-Y04       458 bp

        1 ATGTAGT--A TACCCATTAC ACTGGTCTTG TAAACCAGAG ACGAGGAATA GTAGTTCCTC
       61 CAAAGACTCA AGGAAAGAGC ATAAGCTCTA CCATCAGCAC CCAAAGCTGA AATTCTACTT
      121 AAACTATTCC TTGCAAGCAC A--TATGCTG GACATATATC CTATAGTTAT ACTATGTACA
      181 TCGTGCATTA ACTTTATTTC CCCATGGATA TGTAGCATGT ACATTTATGA TCTTATATTA
      241 CATGAGTACA TTGTATTATA TATCGTACAT AAACCATCTA ATGTGATAAG TTCCAGTCAA
      301 CATGGCTATC CTACAAGTAC TGTTAGTTTA TTAATCTACC ATCCTCCGTG AAACCAGCAA
      361 CCCGCCCACA GCGTGTCCCT CTTCTCGCCC CGGGCCCATA AATCGTGGGG GTAGCTATAC
      421 TGGGTCGTAA ACGGCATCTG GTTCCTACTT CAGGAG--
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LOCUS       F-A07-Y05       458 bp

        1 ATGTAGT--A TACCCATTAC ACTGGTCTTG TAAACCAGAG ACGAGGAATA GTAGTTCCTC
       61 CAAAGACTCA AGGAAAGAGC ATAAGCTCTA CCATCAGCAC CCAAAGCTGA AATTCTACTT
      121 AAACTATTCC TTGCAAGCAC A--TATGCTG GACATATATC CTATAGTTAT ACTATGTACA
      181 TCGTGCATTA ACTTTATTTC CCCATGGATA TGTAGCATGT ACATTTATGA TCTTACATTA
      241 CATGAGTACA TTATATTATA TATCGTACAT AAACCATCTA ATGTGATAAG TTCCAGTCAA
      301 CATGGCTATC CTACAAGTAC TGTTGGTTTA TTAATCTACC ATCCTCCGTG AAACCAGCAA
      361 CCCGCCCACA GCGTGTCCCT CTTCTCGCCC CGGGCCCATA AATCGTGGGG GTAGCTATAC
      421 TGGGTCGTAA ACGGCATCTG GTTCCTACTT CAGGAG--

LOCUS       M-A11-Y06       458 bp

        1 ATGTAGT--A TACCCATTAC ACTGGTCTTG TAAACCAGAG ACGAGGAATA GTAGTTCCTC
       61 CAAAGACTCA AGGAAAGAGC ATAAGCTCTA CCATCAGCAC CCAAAGCTGA AATTCTACTT
      121 AAACTATTCC TTGCAAGCAC A--TATGCTG GACATATATC CTATAGTTAT ACTATGTACA
      181 TCGTGCATTA ACTTTATTTC CCCATGGATA TGTAGCATGT ACATTTATGA TCTTACATTA
      241 CATGAGTACA TTGTATTATA TATCGTACAT AAACCATCTA ATGTGATAAG TTCCAGTCAA
      301 CATGACTATC CTACAAGTAC TGTTAGTTTA TTAATCTTCC ATCCTCCGTG AAACCAGCAA
      361 CCCGCCCACA GCGTGTCCCT CTTCTCGCCC CGGGCCCATA AATCGTGGGG GTAGCTATAC
      421 TGGGTCGTAA ACGGCATCTG GTTCCTACTT CAGGAG--

LOCUS       F-A15-Y07       458 bp

        1 ATGTAGT--A TACCCATTAC ACTGGTCTTG TAAACCAGAG ACGAGGAATA GTAGTTCCTC
       61 CAAAGACTCA AGGAAAGAGC ATAAGCTCTA CCATCAGCAC CCAAAGCTGA AATTCTACTT
      121 AAACTATTCC TTGCAAGCAC A--TATGCTG GACATATATC CTATAGTTAT ACTATGTACA
      181 TCGTGCATTA ACTTTATTTC CCCATGGATA TGTAGCATGT ACATTTATGA TCTTACATTA
      241 CATGAGTACA TTGTATTATA TATCGTACAT AAACCATCTA ATGTGATAAG TTCCAGCCAA
      301 CATGGCTATC CTACAAGTAC TGTTAGTTTA TTAATCTACC ATCCTCCGTG AAACCAGCAA
      361 CCCGCCCACA ACGTGTCCCT CTTCTCGCCC CGGGCCCATA AATCGTGGGG GTAGCTATAC
      421 TGGGTCGTAA ACGGCATCTG GTTCCTACTT CAGGAG--

LOCUS       F-A18-Y08       458 bp

        1 ATGTAGT--A TACCCATTAC ACTGGTCTTG TAAACCAGAG ACGAGGAATA GTAGTTCCTC
       61 CAAAGACTCA AGGAAAGAGC ATAAGCCCTA CCATCAGCAC CCAAAGCTGA AATTCTACTT
      121 AAACTATTCC TTGCAAGCAC A--TATGCTG GACATATATC CTATAGTTAT ACTATGTACA
      181 TCGTGCATTA ACTTTATTTC CCCATGAATA TGTAGCATGT ACATTTATGA TCTTACATTA
      241 CATGAGTACA TTGTATTATA TATCGTACAT AAACCATCTA ATGTGATAAG TTCCAGTCAA
      301 CATGGCTATC CTACAAGTAC TGTTAGTTTA TTAATCTACC ATCCTCCGTG AAACCAGCAA
      361 CCCGCCCACA GCGTGTCCCT CTTCTCGCCC CGGGCCCATA AATCGTGGGG GTAGCTATAC
      421 TGGGTCGTAA ACGGCATCTG GTTCCTACTT CAGGAG--

LOCUS       F-A19-Y08       458 bp

        1 ATGTAGT--A TACCCATTAC ACTGGTCTTG TAAACCAGAG ACGAGGAATA GTAGTTCCTC
       61 CAAAGACTCA AGGAAAGAGC ATAAGCCCTA CCATCAGCAC CCAAAGCTGA AATTCTACTT
      121 AAACTATTCC TTGCAAGCAC A--TATGCTG GACATATATC CTATAGTTAT ACTATGTACA
      181 TCGTGCATTA ACTTTATTTC CCCATGAATA TGTAGCATGT ACATTTATGA TCTTACATTA
      241 CATGAGTACA TTGTATTATA TATCGTACAT AAACCATCTA ATGTGATAAG TTCCAGTCAA
      301 CATGGCTATC CTACAAGTAC TGTTAGTTTA TTAATCTACC ATCCTCCGTG AAACCAGCAA
      361 CCCGCCCACA GCGTGTCCCT CTTCTCGCCC CGGGCCCATA AATCGTGGGG GTAGCTATAC
      421 TGGGTCGTAA ACGGCATCTG GTTCCTACTT CAGGA---
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LOCUS       F-A14-Y09       458 bp

        1 ATGTAGT--A TACCCATTAC ACTGGTCTTG TAAACCAGAG ACGAGGAATA GTAGTTCCTC
       61 CAAAGACTCA AGGAAAGAGC ATAAGCTCTA CCATCAGCAC CCAAAGCTGA AATTCTACTT
      121 AAACTATTCC TTGCAAGCAC A--TATGCTG GACATATATC CTATAGTTAT ACTATGTACA
      181 TCGTGCATTA ACTTTATTTC CCCATGGATA TGTAGCATGT ACATTTATGA TCTTACATTA
      241 CATGAGTACA TTGTATTATA TATCGTACAT AAACCATCTA ATGTGATAAG TTCCAGTCAA
      301 CATGACTATC CTACAAGTAC TGTTAGTTTA TTAATCTTCC ATCCTCCGTG AAACCAGCAA
      361 CCCGCCCACA GCGTGTCCCT CTTCTCGCCC CGGGCCCATA AATCGTGGGG GTAGCTATAC
      421 TGGGTCGTAA ACGGCATCTG GTTCCTACTT CAGGAGAT
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