

## Genetic diversity and phylogeography of the greater mouse-tailed bat *Rhinopoma microphyllum* (Brünnich, 1782) in the Levant

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The greater mouse-tailed bat (*Rhinopoma microphyllum*) possesses a large geographical range, covering most of the arid and warm areas of the Old World. We studied the genetic variability of this species using two mitochondrial markers (the cytochrome *b* gene and the control region), from several Israeli colonies and from over most of the species' range. Our results show that the cytochrome *b* sequences, unlike those of the control region, are too conserved to separate among *R. microphyllum* populations. Based on the control region sequences, a high level of sequence similarity was found within the Israeli population. Three clades were observed over the species' range: Oriental, Intermediate and Palaearctic. This division supports most of the traditional taxonomy of the species. The Israeli population, which belongs to the Palaearctic clade, was found to be isolated from the Oriental and Intermediate clades. We suggest that the colonization of the greater mouse-tailed bat in the Levant occurred from African populations during the late Pleistocene, when many Saharan plants and animals penetrated the northern part of the Great Rift Valley.

*Key words:* genetic diversity, mtDNA, *Rhinopoma microphyllum*, Levant

### INTRODUCTION

The greater mouse-tailed bat (*Rhinopoma microphyllum*, also known as the rat-tailed bat) is a medium-sized bat (average body mass 25 g) inhabiting arid and subtropical regions of the Old World, covering about 12,000 km, from Sumatra and India in the east, through Arabia, to north-western Africa (Schlitter and Qumsiyeh, 1996; Simmons, 2005). During the summer, the species can be found in the north of Israel, which is the northern edge of its range. The greater mouse-tailed bat belongs to the monotypic family Rhinopomatidae that includes three other species — *Rhinopoma harwickii*, *R. muscatellum*, and *R. macinnesi* (Van Cakenberghe and De Vree, 1994; Simmons, 2005). *Rhinopoma microphyllum* is easily distinguished from these three species by its larger body size (Van Cakenberghe and De Vree, 1994; Hulva *et al.*, 2007). Traditionally, *R. microphyllum* is morphologically divided into 4–6 different subspecies along its distribution range (Hill, 1977; Van Cakenberghe and De Vree, 1994; Schlitter and Qumsiyeh, 1996). All classifications agree with the presence of the subspecies *R. m. sumatrae* in Sumatra, *R. m. asirensis* in

the southern part of the Arabian Peninsula, *R. m. kinneari* in the Indian subcontinent, and *R. m. microphyllum* in North Africa and the Levant. Schlitter and DeBlase (1974) described an additional subspecies: *R. m. harrisoni* from southern Iran, while Kock (1969) distinguished *R. m. tropicalis* from southern Sudan, Senegal, Mauritania, and central Nigeria. Classifications dividing *R. microphyllum* into six subspecies were presented by Schlitter and Qumsiyeh (1996). Conversely, Van Cakenberghe and De Vree (1994) synonymised *R. m. harrisoni* and *R. m. tropicalis* subspecies with *R. m. microphyllum*.

Almost nothing is known about the genetic variability of this species. The only existing data are based on partial cytochrome *b* sequences of two individuals: one from Jordan and one from India, and they show a low level of sequence variability (Hulva *et al.*, 2007). Hulva *et al.* (2007) suggested that in spite of the 3,400 km separating between the Levant and Indian populations, a gene flow nonetheless exists, thus contradicting all classical morphological divisions of this species.

Here we present the first molecular study of a bat in Israel, showing a high sequence similarity

within the Israeli population of *R. microphyllum*. In addition we studied the genetic variability over most the species' range, and showed that, unlike the cytochrome *b* sequence, the control region sequence supports most of the traditional taxonomy of Hill (1977) for this species.

## MATERIALS AND METHODS

### DNA Sampling and Sequencing

Sampling in Israel was carried out in 2004 at five summer roosts (two male only colonies: Gonen and Avazim, and three female only colonies: Susita, Bereniki and Cursi) in the northern part of the Jordan Valley (33°11'N, 35°39'E). The bats were trapped using hand nets and samples were collected by biopsy punches (3 mm diameter) from the wing membranes (plagiopatagium). One additional sample was collected from Wadi Darga (31°40'N, 35°26'E) in spring 2006 from a dead male found in a mixed roost of males and females. Captures and samplings in Israel were carried out under license of the Israeli Nature and Parks Authority (NPA), license number 2004/18248. One Jordanian sample was collected in Tabaqat Fahl (32°27'N, 35°37'E) and kindly provided by P. Benda (voucher specimen NMP-47965). Three Moroccan samples were collected from a male colony in the Anti-Atlas Mountains at the edge of the Sahara desert near Agadir-id-Aisha (29°15'N, 09°11'E) and kindly provided by Dr. C. Dietz (voucher specimens CDIS-909, CDIS-910, and CDIS-911). Two Indian sample originated from New Delhi (28°38'N, 77°12'E) and were kindly provided by Dr. P. Bates (voucher specimens HZM-1428204 and HZM-1528205). Two Iranian samples were taken from the mid-Zagros Mountains in summer (no coordinates available) and were kindly provided by Dr. M. Sharifi. *Rhinopoma hardwickii* and *R. muscatellum* (two of the three congeners) were used as outgroups. *Rhinopoma hardwickii* were sampled from the south-eastern part of the Kineret Valley (32°44'N, 35°38'E), Israel, during summer 2005. *Rhinopoma muscatellum* was sampled from the Persian Gulf (27°10'N, 56°15'E); this latter sample was kindly provided by Dr. P. Benda (voucher specimen NMP-48443).

Tissues were homogenized in 100  $\mu$ l of lysis buffer (1% SDS, 10 mM Tris-HCl pH 8, 125 mM NaCl, 5 mM EDTA, and 0.5 mg/ml Proteinase K). Following homogenization, DNA was extracted using a standard phenol-chloroform protocol followed by ethanol/sodium-acetate precipitation (Sambrook *et al.*, 1989). The 5' region of the control region (567 bp) was amplified using the primers D1-Pro 5'-CCACCATCAGCACC-CAAAGC-3' and Dloop-R1 5'-TACCARAGCCATGACAC CACAGTT-3'. The direct primer is a modification of the primer Pro 15975 (Lloyd, 2003) while the reverse primer is located a few nucleotides upstream of the primer H bat 15958r (Lloyd, 2003). Following Hulva *et al.* (2007), the 5' region of the cytochrome *b* gene (402 bp) was amplified using the newly-designed primers MVZ05-new 5'-GACTAATGACATG AAAAAYCACCGT-3' and MVZ04-new 5'-GTTGCTCCT CAGAAAGATATYTG-3'. Amplified fragments were directly sequenced on both strands using PCR primers on an ABI PRISM 3100 (Applied Biosystems) genetic analyzer. Haplotypes were submitted to EMBL under accession numbers AM886153-AM886163.

### Data Analysis

Sequences were aligned using the program MAFFT 2.1.2 (Katoh *et al.*, 2002) using the L-INS-I strategy. Haplotype diversity (*h*) and nucleotide diversity ( $\pi$ ), as well as their sampling variances (Nei, 1987), were computed using the program DnaSP 4.10.9 (Rozas *et al.*, 2003). Two reduced median network (Bandelt *et al.*, 1995) were reconstructed with the program Network 4.2.0.1 (available at <http://www.fluxus-engineering.com>), using either the control region or cytochrome *b* sequences of *R. microphyllum*. Maximum parsimony (MP), and the maximum likelihood (ML) phylogenetic trees were reconstructed based on control region sequences using the program PAUP\* (Swofford, 2000). Phylogenetic trees were rooted using *R. hardwickii* (*R. cystops* sensu Hulva *et al.*, 2007) and *R. muscatellum* sequences. For the MP analysis, an exhaustive tree search was conducted using a branch and bound algorithm. Bootstrap percentages (BP) were computed after 500 replicates. In the ML analysis, the best probabilistic model of sequence evolution was determined using the program MODELTEST 3.07 (Posada and Crandall, 1998) using the Akaike Information Criterion (AIC). The parameters of the model were then determined in an iterative way using PAUP\*. First a heuristic search was conducted using the best parameters identified with MODELTEST. This search was performed starting with a NJ tree and using TBR branch-swapping. The command LSCORES was then used to re-estimate the likelihood and the best parameters of the tree obtained in the previous search. The new parameters were then applied to conduct a new heuristic search. These operations were repeated until convergence. BP were computed using the best parameters found, as indicated above, after 500 replicates, starting with an NJ tree and searching trees with TBR branch-swapping.

## RESULTS

The partial cytochrome *b* sequences were found to be far less variable than the partial control region sequences. Based on the control region sequences, the main Levant haplotype of *R. microphyllum* differed from the Moroccan haplotype by four nucleotide substitutions and from the Iranian and Indian haplotypes by 15–16 and 17 nucleotide substitutions, respectively (Fig. 1A and Table 1). The Iranian haplotypes are further distinguished by an insertion of a single nucleotide that is absent in all other sequences; this is the single indel of the alignment. Based on cytochrome *b*, the Levant haplotype differed from the Moroccan haplotype by one nucleotide substitution and from the Indian haplotype by two substitutions (Fig. 1B). Unfortunately, the cytochrome *b* sequences could not be obtained for the Iranian samples. Because of the low level of variability in the cytochrome *b* fragments sequenced, only control region fragments were used to determine the genetic diversity of the Levant colonies. The Israeli colonies showed a low level of genetic variation (Table 1). Among the 45 Israeli



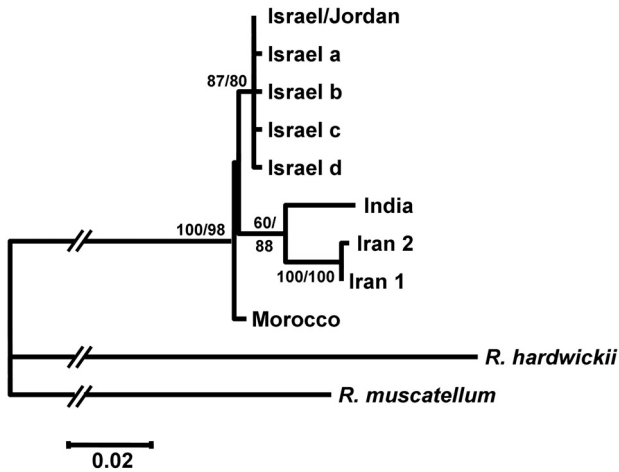


FIG. 2. Best ML tree ( $-\ln$  Likelihood = 1612.626) obtained under the TVM + Gamma model of sequence evolution. For each node, the MP and ML bootstrap percentages above 50 are given to the right and left of the slash, respectively

Contradicting the above observations, Schlitter and Qumsiyeh (1996) drew a continuous species range map for *R. microphyllum* from the Levant, east along the northern Persian gulf to Pakistan. Similarly, based on partial cytochrome *b* sequences analysis, Hulva *et al.* (2007) suggested that a gene flow exists between the Indian and Levant population. The Levant and Indian cytochrome *b* sequences obtained in this work are identical to those published by Hulva *et al.* (2007), supporting their results. Our results, based on partial control region sequences, show the opposite, with substantial differences between Indian and Levant populations, thus supporting the isolation of these populations. Both markers (i.e., the control region and the cytochrome *b* gene) are of mitochondrial origin. In the absence of mitochondrial recombination, these genes can be considered to belong to a single locus. Consequently, they cannot support a different phylogenetic scenario such as two genes from different chromosomes. Our results thus show that the lack of genetic variation in the cytochrome *b* sequences does not reflect the

existence of contact between the Indian and Levant populations; but rather the insufficient variability of this marker to address the population structure of *R. microphyllum*.

Differences among populations within a species can arise either from divergent selection or from random genetic drift. These two processes are expected to have a stronger impact on peripheral populations that are located in more extreme environmental conditions and harbour a smaller population size than the core population (Lesica and Allendorf, 1995; Mortiz, 2002; Tregenza, 2002). Because of its location at the limit of the species' range, and the mass decline in the number of insectivore bat populations in the Mediterranean region of Israel (Mendelssohn and Yom-Tov, 1999), the Israeli population of *R. microphyllum* was expected to possess a low level of genetic diversity and to be distinct from other populations along the distribution range of this species. Only the first prediction was verified: the 5' region of the control region (i.e., same genomic region amplified in this work) shows a much higher level of genetic variation within other bat species (e.g.,  $h = 0.5-1.0$ ,  $\pi = 0.007-0.056$  — Russell *et al.*, 2005; Chen *et al.*, 2006; Safi *et al.*, 2007). However, the Israeli haplotypes appeared to be very close to the Moroccan haplotypes (only four substitutions). In light of these results, we suggest that the origin of the recent Levant population is from the desert belt of the Sahara. It is likely that the Levant colonization occurred during the late Pleistocene when many Saharan plants and animals penetrated the north of the Great Rift Valley due to climatic changes and desertification (Tchernov and Yom Tov, 1988). The expansion of the bat population into the northern part of the Rift Valley might have been enabled due to a combination of high temperature, availability of water resources in oases and linear landscape elements from the south to the north. One consequence of these special ecological conditions is the presence of this desert species north of its main latitudinal range.

TABLE 2. Uncorrected ('*p*') distance matrix calculated from control region haplotypes

Haplotypes	Israel/Jordan	Israel a	Israel b	Israel c	Israel d	Morocco	India	Iran1	Iran2
Israel/Jordan	–								
Israel a	0.00177	–							
Israel b	0.00177	0.00353	–						
Israel c	0.00177	0.00353	0.00353	–					
Israel d	0.00177	0.00353	0.00353	0.00353	–				
Morocco	0.00707	0.00883	0.00883	0.00883	0.00883	–			
India	0.03004	0.03180	0.03180	0.03180	0.03180	0.02650	–		
Iran1	0.02649	0.02825	0.02825	0.02826	0.02825	0.02649	0.02824	–	
Iran2	0.02825	0.03002	0.03002	0.03003	0.03002	0.02825	0.03000	0.00176	–

TABLE 3. Uncorrected ('p') distance matrix calculated from cytochrome *b* haplotypes

Haplotypes	Israel/Jordan	Morocco	India
Israel/Jordan	–		
Morocco	0.00249	–	
India	0.00498	0.00249	–

The phylogenetic analysis of *R. microphyllum* haplotypes from western and eastern populations revealed three different lineages: an Oriental cluster comprising the Indian bats, an intermediate cluster comprising the Iranian bats and a Palearctic cluster including the Moroccan and Levant populations (Fig. 2). The phylogenetic tree groups the Oriental and intermediate clades (BP = 60–88) and suggests that the Palearctic lineages are paraphyletic (BP < 50). However, it is possible that this result is an artefact created by a wrong positioning of the outgroup (Fig. 1). Indeed, when the outgroup is distantly related to the ingroup (in our case the branches leading to the closest outgroup are  $\approx 10$  time longer than the ingroup branches), the rooting might be unreliable (Anderson and Swofford, 2004; Douzery and Huchon, 2004). The haplotype network (Fig. 1) is in agreement with the classical division of *R. microphyllum* subspecies based on morphological characters (e.g., Schlitter and Qumsiyeh, 1996). The Israeli and Moroccan populations belong to the subspecies *R. m. microphyllum*, while the Indian belong to the subspecies *R. m. kinneari*. However, our results do not support the division suggested by Van Cakenberghe and De Vree (1994) which includes all Iranian specimens in the subspecies *R. m. microphyllum*. It was suggested that the Iranian *R. microphyllum* contained two subspecies — *R. m. harrisoni* and *R. m. microphyllum* (Schlitter and DeBlase, 1974), which can be found in sympatry at the edge of their distribution range. Our sample originated from the Zagros Mountains, a region in which both subspecies can be found. In the absence of morphological measurements for this sample, we can neither confirm nor exclude that it belongs to one of the two subspecies. Additional sampling over the full distribution range of this species is needed in order to establish subpopulation relationships.

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