

## DETERMINATION OF CYTOCHROME *B* GENE OF *ROUSETTUS AEGYPTIACUS* (MAMMALIA: CHIROPTERA) IN TURKEY

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**ABSTRACT:** This study is based on the detection of cytochrome *b* gene of individuals from four population of Egyptian fruit bat, *Rousettus aegyptiacus* distributed four different locations (Harbiye near Hatay Province, Tarsus near Mersin Province, Adana Province and Hassa near Hatay Province) in the Mediterranean Region. Natural habitats of Egyptian fruit bat was investigated and some biological characteristics were recorded. 3 mm ear tissue was used for DNA isolation. Mitochondrial cytochrome *b* gene amplified by PCR and 350 base pair partial sequences were obtained. When the analyses results compared between each other and Genbank records, up to 99% homology was detected.

**KEY WORDS:** Fruit bat, Mediterranean Region, *Rousettus aegyptiacus*, cytochrome *b* gene, Turkey

Classis Mammalia is represented by 29 ordo, 153 families, 1229 genera and 5416 species (Wilson & Reader, 2005). Order Chiroptera is divided into two suborders; Megachiroptera (Big bats) and Microchiroptera (Small bats). Seventy percent of the bats are fed insect (Insectivorous) and fruit (Frugivorous) 23% and the rest are fed with some vertebrate and invertebrate animals (Carnivorous), fish (Psivorous), blood (Sanguivorous), nectar and pollen (Aellen, 1939; Yalden & Morris, 1975; Nowak & Paradiso, 1993).

Pteropodidae, a single family of Megachiroptera, is represented by 42 genera, including *Rousettus* with Ethiopian origin. Of the total 39 species, only one is fruit bat that also exists in Turkey, namely *Rousettus aegyptiacus*, Egyptian fruit bat.

While the studies conducted on mammalian animals have numerously been intensified with taxonomic, systematic, ecological, karyological and zoogeographic, studies at the molecular level have recently gained considerable importance.

In a study carried out in US on the evolution of the cytochrome *b* gene in mammals, 1140 bp 20 in different mammalian animals were studied and the results of phylogenetic analysis and variations on amino acids were compared (Irwin et al., 1991). In a molecular study conducted on the phylogeny of fruit bats in the US, the species belonging to 6 Microchiroptera and 43 Megachiroptera suborders compared and the differences between the populations were evaluated (Giannini & Simmons, 2003).

In Egypt, molecular phylogenetic relationships between *Rousettus aegyptiacus aegyptiacus* and *Rhinopoma hardwickei arabium* subspecies were investigated (Ramadan, 2011). Study of cytochrome *b* gene concerning 17 *Rousettus madagascariensis* from Madagascar in the West Indian Ocean and 8 *Rousettus obliviosus* from Comoros provided a good comparison between their phylogenies and biogeographies (Goodman et al., 2010).

The taxonomical status, distribution, feeding and karyological values of *Rousettus aegyptiacus* living in the Mediterranean region of Turkey were recorded (Albayrak et al., 2008). Some researches were made on rabies viruses in western, central and northeastern Turkey (Albayrak et al., 2011; Ün et al., 2013).

The aim of this work is to contribute to the systematics of fruit bat which is represented by only one species, *Rousettus aegyptiacus* in Turkey, by revealing the cytochrome *b* gene of this species.

### MATERIAL AND METHODS

This research is based on the replication by Polymerase Chain Reaction (PCR) agarose gel electrophoresis imaging and DNA sequence analysis of the cytochrome *b* gene in *Rousettus aegyptiacus*. In order to update the existence of this species in its habitats, field studies were conducted in the provinces of Hatay, Kilis, Kahramanmaraş, Adana, Mersin and Antalya between July 2012 and November 2013. Ear tissue samples of specimens from Hatay Province in 1977 and Mersin Province in 2000 which is preserved in the bat collection and an additional specimen from Adana Province in 2003 during this study were used (Fig. 1).

To explore whether there were different inter and intra-population variations along the distribution area of the species. Ear tissue samples taken from a total of 5 bats belonging to 4 populations were used. Tissue samples from fruit bat specimens were stored at -80 °C until used in the test.

Total DNA was isolated from fruit bat *Rousettus aegyptiacus* samples using a commercial kit (DNeasy Tissue Kit, Qiagen, Germany), following the 'Purification of Total DNA from Animal Tissue' protocol. For the PCR and sequence application, previously published primers were used (Irwin et al., 1991). L14724 (5'-CGAGATCTGAAAACCATCGTTG-3'), H15915 (5'-GGAATTCATCTCTCCGGTTTACAAGAC-3'), L14841 (5'-AAAAAGCTTCCATCCAACATCTCAGCATGATGAAA-3'), H15149 (5'-AAACTGCAGCCCCCTCAGAATGATATTTGTCTCA-3') primer pairs were used to amplify cytochrome *b* gene. The primer, distilled water, polymerase enzyme, target DNA and buffer solution were prepared into the eppendorf tube and put into PCR Thermalcycler (PTC100 MJ Research, USA). In PCR steps, the temperature, time and cycle numbers specified by Martin and Gerlach (2000) (2 minute at 94 °C, 1 minute at 52 °C, 2 minutes at 72 °C; 40 cycles) were recorded and run by adjusting the thermalcycler. The PCR products were purified on a commercial agarose gel using the commercial kit (QIAquick® Gel Extraction Kit, Qiagen GmbH, Hilden, Germany). Purified samples DNA sequences were obtained using the automated DNA analyzer, ABI PRISM® 310 Genetic Analyzer (Applied Biosystems, Foster City, USA). Sequences were analyzed using the CLC Main Workbench program and the phylogenetic trees were created using Weighbor (Weighted Neighbor Joining: A Likelihood-Based Approach to Distance-Based Phylogeny Reconstruction). Sequences were aligned and compared with Clustal X 1.83 software (Thompson et al., 1997) by using the NCBI basic local alignment search tools BLAST program.

### RESULTS

Samples of 5 *R. aegyptiacus* from four different habitats were evaluated in terms of mitochondrial cytochrome *b* gene.

#### ***Rousettus aegyptiacus* (Geoffroy, 1810) Egyptian Fruit Bat**

1810. *Pteropus egyptiacus* E. Geoffroy, Ann. Mus. Hist. Nat., Paris, 15:96. (misprint) corected to *aegyptiacus* in 1818. Description de l'Égypte Hist. Nat., 2:134.

Type locality: Great Pyramid, Giza, Egypt.

1902. *Rousettus aegyptiacus*, Anderson and de Winton, Zool. Egypt, Mam., London, 84.

**Diagnostic characters:** Premaxilla is well developed and its terminals contacted each other anteriorly, occipital region is narrow, forearm length 87.0-93.6 mm, greatest length of skull 41.3-44.8 mm, condylobasal length 41.2-43.7 mm, zygomatic breadth 26.0-28.7 mm, upper tooth length 16.1-17.1 mm, lower tooth length 16.1-17.1 mm, 17.1-18.8 mm (Albayrak et al., 2008).

**Specimen examined and collection localities:** Hatay Province: Harbiye cave, 1 (♀, 02.05.1977), Demre near Hassa, Karamağara, 1 (♀, 11.07.2006); Adana Province, Cumhuriyet Flour Factory, 2 (♂♂, 14.03.2003; ? 13.11.2013); Mersin Province: Say Village near Tarsus, 1 (♀, 22.04.2000) (Fig. 2).

**Interpretation of PCR Studies Results:** Considering the expected positivity as a result of PCR amplification, it was found that specific bands were formed at about 1000 bp regions for the primer pair H15915 and H15149 of about 100 bp for the L14724 and L14841 primer pair. The results were recorded by taking a polaroid photograph (Fig. 3).

Cytochrome *b* gene sequences of the specimens from four different populations of *Rousettus aegyptiacus* distributed in Mediterranean Region in Turkey showed similarity to some other bat species at different rates. Two primer sets (I. and II.) were used to determine the cytochrome *b* gene of the specimens which were collected from four populations.

The samples from the provinces of Hatay (Harbiye and Hassa) and Mersin (Tarsus) yielded better results with the primer base pairs. When the data were compared with the gene bank, the specimens of the province of Hatay (Harbiye) was similar to *Eumops patagonicus* at 78% of the family Pteropodidae of Megachiroptera; at 77% to *Hipposiderus* sp. and *Emballonura beccarii* of Microchiroptera. Specimens of Tarsus (Mersin) were similar to *Eumops patagonicus* 78%, *Hipposiderus* sp., *Emballonura beccarii* at 77% and to *Pipistrellus pipistrellus* at 72%. Specimens from the province of Hatay (Hassa) were similar to *Pipistrellus kuhlii* and *Platyrrhinus aurarius* and *Vampyrodes caraccidi* belonging to the Microchiroptera at % 83.

The sample of Adana was renewed and two primer sets were used again and the first primer set was taken into account due to better results attained. The specimen from Adana province showed similarity to *Rousettus aegyptiacus* at 99%, to *Rousettus leschenaultii* at 99% and to *Rousettus madagascariensis* at 93%, which were belonging to the family Pteropodidae of Megachiroptera. The results obtained in this study seem to be compatible with the findings obtained by Irwin et al., (1991) in terms of cytochrome *b* gene.

The results of the research which was carried out with the longest base pair possible reveal more conclusive results concerning the cytochrome *b* gene. This research emphasizes the need for conservation of the Egyptian fruit bat for the biodiversity and the maintenance of this gene resource. The first condition of this is to ensure that the habitats are not destroyed and that this species, which is always considered vulnerable with its large bodies, is protected under national and international regulations.

With the results of this research at the molecular level, the differences inter populations of the species will be better monitored. Thus, protection action plans on the species should be carried out by considering these characteristics.

Note: This study is a part of the Master Thesis of first author, Esra Topaktaş.

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Figure 1. A *Rousettus aegyptiacus* found in an empty hangar.

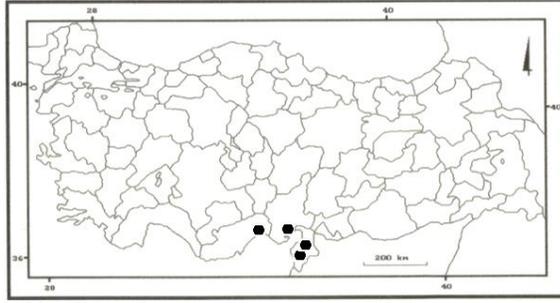


Figure 2. Recorded localities of *Rousettus aegyptiacus* (•).

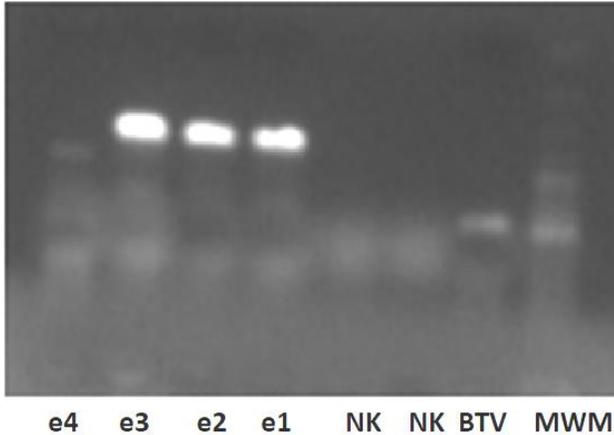


Figure 3. View of the reaction with primer L and Primer H in the agarose gel (e4: *R.aegyptiacus* (Adana), e3: *R.aegyptiacus* (Tarsus), e2: *R.aegyptiacus* Hassa), e1: *R.aegyptiacus* (Harbiye), NK: Negative control MWM: DNA ladder).

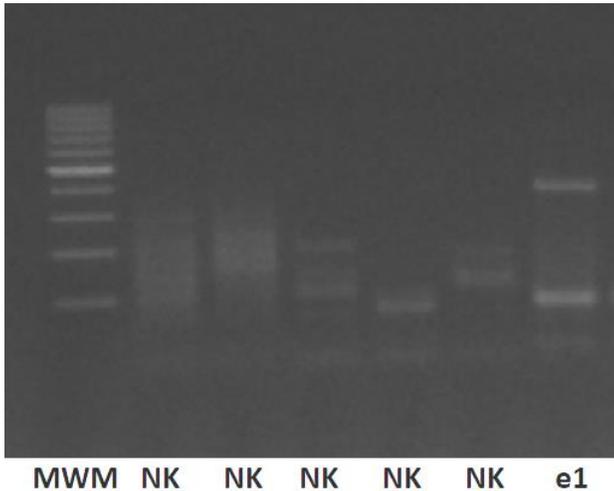


Figure 4. (continued) The reaction image of primer L and Primer H on a agarose gel (MWM: DNA ladder, NK: Negative control, e1: *R.aegyptiacus* (Adana)).