Journal of Applied Biological Sciences 10 (1): 50-54, 2016
ISSN: 1307-1130, E-ISSN: 2146-0108, www.nobel.gen.tr

Revisiting the taxonomic status of *Rhinolophus ferrumequinum* (Schreber, 1774) (Chiroptera: Rhinolophidae) in Turkey

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Received: January 22, 2016
Accepted: February 29, 2016

Abstract

The greater horseshoe bat, *Rhinolophus ferrumequinum* (Schreber, 1774), is one of the most distributed bat species in Turkey. This study is based on statistical evaluation of the cranial measurements of 165 specimens for determining whether a clinal increase in the species throughout the country. In addition, conventional stained karyotype along with C- and Ag-NOR banded chromosomes of the specimens were studied. No clinal increase in size is encountered from west to east in the greater horseshoe bat. On the contrary, Southeastern Anatolia, Eastern Anatolia, Central Anatolia, western and central Black Sea regions form a separate cluster, while the Mediterranean, Aegean and Marmara regions form another one with regard to cranial measurements. In addition, the populations do not differ in terms of their karyotype and banded chromosomes.

Keywords: Greater Horseshoe bat, geographical variation, karyology, nucleolus organizer regions, Turkey

INTRODUCTION

The Greater horseshoe bat, *Rhinolophus ferrumequinum* (Schreber, 1774), has a wide distribution from Europe and North Africa, east to Asia and Japan [1-5]. Unfortunately, a dramatic population decline resulting in the loss or isolation of many maternity colonies has been recorded from Britain [6]. The IUCN conservation status of the species is "decrasing" and it is already extinct in Belgium and the Netherlands [7]. However, the species is still one of the most commonly encountered bat species in Turkey [8]. The Greater horseshoe bat is encountered in large caves, stables, animal enclosures, crevices, tunnels and cellars of castles, inns, abandoned caravansaries and water wells in Turkey and it lives sympatrically with *Myotis myotis/blythii* and is represented by the nominate form [9, 10].

The taxonomic status of the greater horseshoe bat is still controversial in the Palaearctic region [5]. Krystufek [11] stated *R. f. insulanus* and *R. f. obscurus* as the West European taxa and *R. f. martinoi* and *R. f. creticum* as the southeastern taxa in addition to the nominative form. Csorba et al., [2] recognized R. f. ferrumequinum (in Europe and nortwest Africa), *R. f. creticum* (in Crete), *R. f. irani* (in Iran, Iraq, Turkmenistan), *R. f. proximus* (in Afghanistan, Uzbekistan, Kashmir), *R. f. tragatus* (in Northern India to eastern China), *R. f. korai* (in Korea) and *R. f. nippon* (in Japan, eastern China), however, these subspecies are defined by morphological comparison. According to De Paz [1] *R. ferrumequinum* increased in size with regard to external, cranial and dental measurements from west to east in its distribution range while Simmons [3] accepted *insularis*, *martinoi*, *korai*, *obscurus*, *creticum*, *irani* and *nippon* as synonyms.

In recent years, most researchers prefer molecular rather than morphological data for resolving the taxonomic status of many taxa [12]. Rossiter et al., [4] examined the status of the Greater horseshoe bat, using microsatellite variability in its distribution range. Later, Flanders et al., [13] used mtDNA ND2 gene and microsatellite analyses and stated five major lineages of the species; Europe and Africa, Western Asia, Central China, Eastern China and Japan. Recently, Bilgin et al., [14] and Bilgin [15] determined western and eastern mtDNA clades, which differed during the Pleistocene or Pliocene, with high levels of nuclear differentiation in *Rhinolophus ferrumequinum* in Turkey and stated that the two mitochondrial clades should be treated as different conservation units. In addition, Bilgin et al., [14] emphasized the morphological differentiation between the two mitochondrial clades, suggesting them as two separate biological species with the support of mitochondrial and microsatellite data and the parapatry of the clades in Turkey.

In this study, we examined the cranial and dental measurements of the relatively large number of specimens, as well as the C- and Ag-NOR banded chromosomes, aiming to make a contribution to the controversial taxonomic status of *Rhinolophus ferrumequinum* in Turkey.

MATERIAL and METHODS

This study is based on 6 cranial dimensions taken from each skull to an accuracy of 0.1 mm using a digital caliper, of 165 specimens collected from 25 provinces along with the C- and Ag-NOR banded chromosomes of ten specimens of *Rhinolophus ferrumequinum* collected from five provinces in Turkey (Fig. 1 and 2).

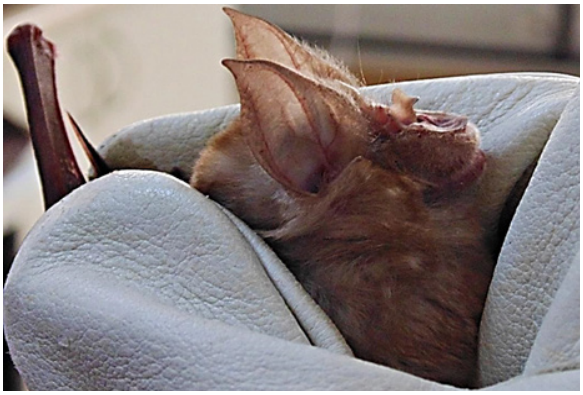

Fig. 1. A male *Rhinolophus ferrumequinum* specimen from Kırıkkale province

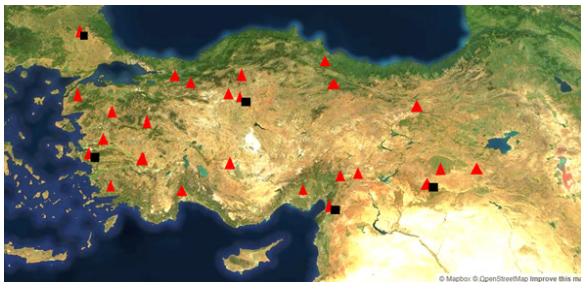

Fig. 2. Collection localities of *Rhinolophus ferrumequinum* in Turkey (Red triangles indicate the localities of specimens statistically evaluated, black squares indicate the localities of karyotyped specimens) (www.mapbox.com)

Males and females were pooled and statistically evaluated together. The specimens were classified according to molar wear pattern and only adult specimens were evaluated in the statistical analyses. Statistical analyses were performed using SPSS 17.0 software. Descriptive statistics of the 6 dimensions were calculated as: M (mean), Maximum (Max), Minimum (Min), and Standard deviation (± SD). The Tukey HSD test was used to determine which populations are different from each other with regard to the cranial characteristics.

Mitotic metaphases were obtained from the bone marrow as described by Lee and Elder [16]. Constitutive heterochromatin distribution was detected by Sumner [17]. The location of Nucleolar organizer regions (NORs) in the chromosomes was determined using the method of Howell and Black [18]. Chromosomes were classified according to Levan et al. [19]. At least 10 well-spread and Ag-NOR banded metaphase plates were photographed in each specimen and arranged to determine the diploid chromosome number (2n), autosomal fundamental number (NFa) and fundamental number (NF). The slides are deposited in Department of Biology, Faculty of Arts and Sciences, University of Kırıkkale.

RESULTS

The descriptive statistics of six measurements of *Rhinolophus ferrumequinum* belonging to 25 populations were given in Table 1.

Table 1. Descriptive statistics of some cranial measurements (mm) of adult males and females of *Rhinolophus ferrumequinum* in Turkey.

	Greatest lenght of skull	Condylobasal length	Zygomatic breadth	Mastoid breadth	Lenght of upper toothrow	Mandibular tooth row length
n	123	159	165	157	163	164
Mean	23,696	20,391	12,041	10,409	8,611	9,210
Std. Deviation	,4378	,4708	,2707	,2092	,1953	,2571
Variance	,192	,222	,073	,044	,038	,066
Range	2,8	3,5	1,3	1,8	1,0	1,3
Minimum	22,0	19,0	11,4	9,2	8,1	8,5
Maximum	24,8	22,5	12,7	11,0	9,1	9,8

Considering the average value of all the measurements for all regions there were similiarities presented between the length of the upper toothrow, zygomatic breadth, the total length of skull measurements with the greatest length of skull measurements; the length of the upper tooth row, zygomatic breadth with total length of skull measurements; lenght of upper tooth row, total length of the skull with the mastoid breadth measurements; the length of the upper tooth row, zygomatic breadth and total length of the skull with condylobasal length (Fig. 3). Furthermore, a clinal increase in cranial measurements is not encountered from western to eastern Anatolia.

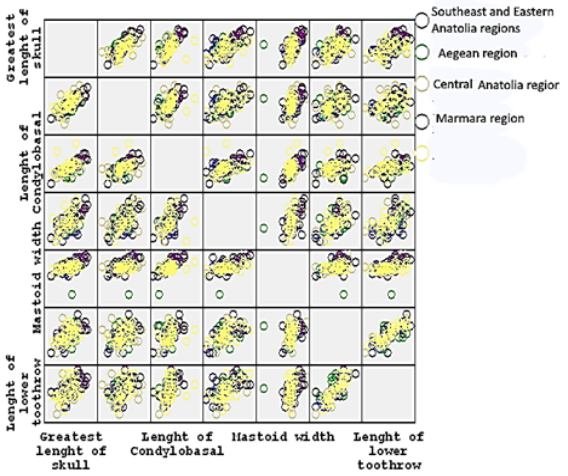

Fig. 3. Graph showing the similar distribution of cranial measurements (mm) of *Rhinolophus ferrumequinum* specimens from different regions of Turkey

However, when we compared some of the cranial measurements (i.e. the greatest length of skull and condylobasal length) with each other in detailed, we clearly detected two clusters; Southeastern Anatolia, Eastern Anatolia, Central Anatolia, western and central Black Sea regions (Adıyaman, Siirt, Diyarbakır, Şanlıurfa, Erzincan, Ankara, Kırıkkale, Konya, Çankırı, Bolu, Tokat and Samsun provinces) form a separate cluster, while the Mediterranean, Aegean and Marmara regions (Antalya, Adana, Hatay, Kahramanmaraş, Denizli, Muğla, Manisa, Kütahya, İzmir, Kırklareli, Çanakkale, Balıkesir and Sakarya provinces) form the other one (Fig. 4 and 5).

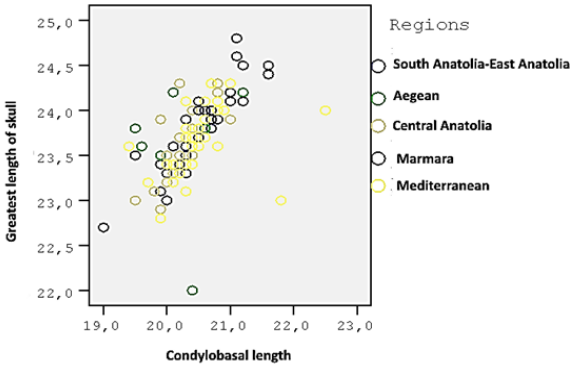

Fig. 4. Graph showing the highest similar distribution of greatest length of skull and condylobasal length of *Rhinolophus ferrumequinum* from the regions in Turkey

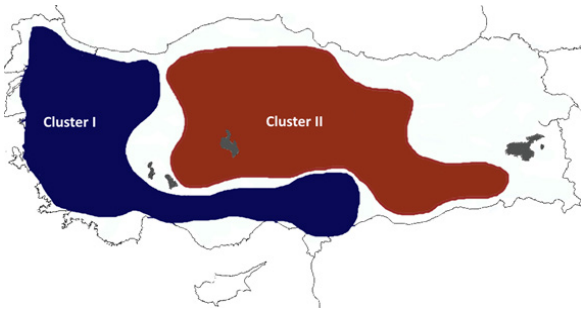

Fig. 5. Cluster I (Mediterranean, Aegean and Marmara regions) and Cluster II (Southeastern Anatolia, Eastern Anatolia, Central Anatolia, Western and central Black Sea regions) described by comparing cranial measurements of *Rhinolophus ferrumequinum* in Turkey.

The karyotype had 2n=58, NF=64 and NFa= 60. The chromosome set consisted of four pairs of metacentric pairs, 52 pairs of acrocentric pairs, gradually decreasing in size. The X chromosome was large sized metacentric while the Y was dot-like acrocentric. In one of the acrocentric pair (no. 14), a secondary constriction which is characteristics to the genus, is encountered (Fig. 6).

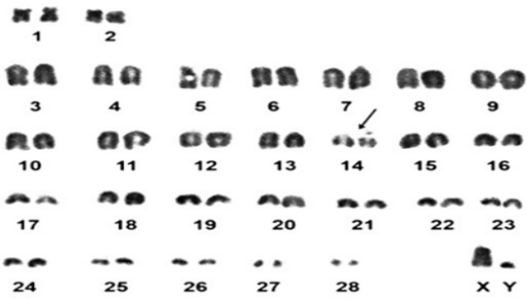

Fig. 6. Conventionally stained karyotype of *Rhinolophus ferrumequinum* from Hatay province, Turkey. Arrow indicates the secondary constriction.

Unfortunately, we could not obtain good quality G-chromosomes prepared from bone marrow.

The dark C-bands were detected in the secondary constriction of the 14th autosome pair and pericentromeric areas of five acrocentric autosomes (nos. 3-5 and 9). However, the Y chromosome was entirely heterochromatic (Fig. 7).

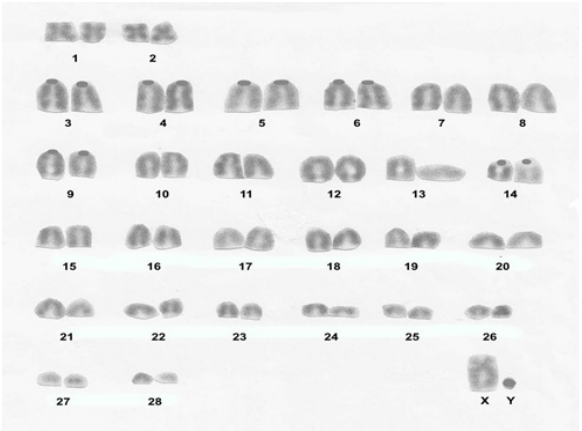

Fig. 7. C-banded karyotype of *Rhinolophus ferrumequinum* from İzmir province, Turkey

Active Ag-NORs were located on the secondary constriction of one of the 14th acrocentric autosome and the telomeric regions of two acrocentrics (Nos. 6 and 12) in the set (Fig. 8).

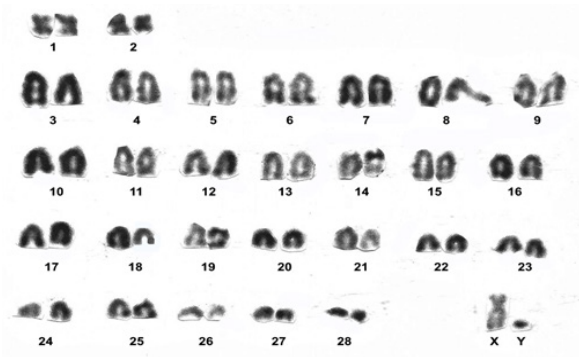

Fig. 8. Ag-NOR stained karyotype of *Rhinolophus ferrumequinum* from Kırıkkale province, Turkey

DISCUSSION

Kumerloeve [20] was the first researcher dealing with the taxonomy of the greater horseshoe bat in Turkey. He stated the nominate subspecies distributed in Turkish Thrace while *R. f. irani* is distributed in Eastern Anatolia without giving any morphometrical data. Later, Felten et al., [21] suggested that the nominate subspecies was distributed in whole Turkey, while DeBlase [22] recorded *R. f. irani* from eastern Turkey. However, Krystufek [11] and De Paz [1] determined a clinal increase in size in south eastern Europe and the western Palaearctic region, including the Mediterranean region and the Western Black Sea region, respectively. The latter author detected an East – Southwest size gradient, and stated that the northern Africa populations of the species are the smallest, while specimens from Turkey are the largest. In addition, Benda and Horacek [23] also described a slight increase in size from the western to the eastern part of Turkey. Albayrak et al., [10] compared the morphometric data of the Turkish *Rhinolophus ferrumequinum* specimens with those from Europe, Kırım and Transcaucasia, Iran, Lebanon, Syria and Israeli and found no statistically significant differences between them. Therefore, they concluded that the species is represented by the nominate form in Turkey. Similarly, Zagorodniuk [24] determined no significant differences among both the western and eastern European greater horseshoe bat, and stated that the East European specimens should be classified as the nominate form. In the present study, we compared specimens from 25 provinces from seven regions and identified two clusters with regard to cranial measurements.

Flanders et al., [13] examined the phylogeography of the greater horseshoe bat without any specimens from Turkey and determined similarities between European and Syrian populations with regard to mtDNA. Rossiter et al., [4] examined the genetic diversity in *Rhinolophus ferrumequinum* specimens from the UK, Europe and the Middle East including specimens from Turkey, and observed a decline in the genetic diversity from the Middle East and southeastern Europe to the Britain. According to the authors, no genetic exchange had occurred between these regions since the Last Glacial Maximum (LGM) as a consequence of the rising the Marmara Sea. However, they stated the Levant region as being a refugial region, because of the high allelic

richness in the Syria populations. Recently, Bilgin et al., [14] and Bilgin [15] examined the phylogeny of *Rhinolophus ferrumequinum* using mitochondrial DNA sequencing in Central Anatolia and Turkey, respectively. Bilgin et al., [14] did not detect any morphological differences between the two mtDNA clades and proposed the distribution of two separate biological species within Central Anatolia. Recently, Bilgin [15] described western and eastern mtDNA clades by evaluating more specimens in Turkey. Border of the both cranial mesurements clusters (Cluster I and II) in the present study, were not completely similar to the data given by the author. Therefore, as a conclusion, more specimens from Central Anatolia, the Mediterranean along with the Black Sea regions are needed for further morphological and molecular studies to elucidate the accurate taxonomic status of the species.

The 2n, NF and NFa values and the chromosome set of *Rhinolophus ferrumequinum* from 5 provinces in Turkey was similar to the previous data summarized in Albayrak et al., [10]. Discrepancies with regard to the number of diploid number, number of autosomal arms and the shape of the autosomes along with the sex chromosomes were detected by Zima [25] from former Czechoslovakia, Ando et al., [26] from Japan and Karataş et al., [27] from Iran. Recently, Arslan and Zima [28] examined the karyotype of specimens from İçel and Hatay provinces and stated a similar karyotype with the present study. However, we did not detect any differences in the karyotype of populations examined in Turkey therefore, we suggested that karyology did not make a contribution to the taxonomy of *R. ferrumequinum*.

Acknowledgement

The karyological part of the study was financially supported by Coordination Unit of Scientific Research Project of University of Kırıkkale (Grant No: 2009/38). We would like to thank Prof. Dr. İrfan Albayrak for providing some of the specimens for the karyological study and using some cranial and dental data of the specimens in his collection.

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