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Morphometric and phylogenetic analysis of *miniopterus fuliginosus (Schreibersii)* captured from fata regions, Pakistan

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Key words: Cranial measurements, *Minopterus fuliginosus*, morphometric, COI gene.

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Abstract

The present study was conducted to explore the bats of Khyber and Bajur Agencies, Pakistan. During the present study 6 specimen of short-nosed fruit bat *Minopterus fuliginosus* were collected through the mist nets from June 2014 to October 2016. They were identified on the basis of morphological and cranial measurements. Cranial features and morphometry of the captured specimens were matched with the literature. Arms and cranial morphometric is one of the distinct features for identification, while *Minopterus fuliginosus* is reported for the first time from the current study area. Phylogenetic relationships of said species with inter and intra specific divergence on the bases of COI gene sequences were also described.

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Introduction

Miniopterus fuliginosus is reported up to now from Pakistan nut Gaisler (1970) that the current specie is likely to be found in Afghanistan and Iran which is close to the western borders and from India which is at the eastern borders of Pakistan (Roberts, 1997).

The present situation of the species is unknown in Pakistan. IUCN 2008-NT. Bates and Harrison (1997) captured specimens of *M. schreibersii* from India, Sri Lanka and Nepal. Round about head and body length were 54.5 mm (46.0-65.0 mm), hind foot length was 8.5 mm (6.0 – 11.0 mm), tibia length was 18.2 mm (16.7-21.5 mm), tail length was 54.2 mm (43.0-61.0 mm), wing span was 326.0 mm (321.0-327.0 mm), 4th metacarpal was 42.9 mm (41.5-45.2 mm), fore arm length was 48.0 mm (43.7–48.6 mm), 3rd metacarpal was 42.7 mm (40.1-47.4 mm) 5th metacarpal was 37.4 mm (36.0-41.4 mm) and ear length was 12.2 mm (9.7 – 13.0). C. Srinivasulu, Paul A. Racey & Shahroukh Mistry 2011 captured species of *M. schreibersii* from Asia. Their head and body length were (46.0-64.0 mm), tail (45.0-62.0 mm) ear length was (9.7-11.0 mm), hind foot length was (8.0-13.0mm) and fore arm length was (45.7-48.6 mm). Cranial parameter of *M. schreibersii* caught from India, SriLanka and Nepal.

The skull length was 14.7 mm (14.3 – 15.4 mm), mandible length was 12.3 mm (11.7 – 12.8 mm), width of the braincase was 8.9 mm (8.5 – 9.3 mm), postorbital constriction was 4.9 mm (4.8 – 5.1 mm), posterior palatal width was 5.6 mm (7.4-7.9 mm), rostral width was 6.4 mm (6.1-7.7mm), maxillary tooth row length was 7.1 mm (6.8-7.3 mm) condyle canine length was 15.1 mm (14.6 – 15.8 mm), zygomatic breadth was 7.8 mm (7.5 – 8.1 mm), and average mandibular tooth row length was recorded as 7.5 mm (7.3-8.8 mm (Bates and Harrison, 1997).

The Cranial measures of the *M. schreibersii* specimens caught from South Asia by C. Srinivasulu, Paul A. Racey & Shahroukh Mistry 2010. The condyle canine length was (13.6 – 14.8 mm), zygomatic breadth was (8.5– 9.1 mm), mandible length was (10.7– 11.8 mm), maxillary tooth row length was (5.8-6.3 mm) and mandibular tooth row length was recorded as (6.3-6.8 mm).

Materials and methods

Study area

The study was carried out in selected areas (Bajur agency and Khyber Agency) in FATA. The Landscape of FATA has varieties including plain and mountain. It is situated in northern side of KP Pakistan.

In crops, wheat and Rice are abundantly cultivated on large scales. The average maximum and minimum temperatures are observed as 35 ^{o}C and 16^{o}C, respectively. Mainly the whole area is covered with mountainous ranges and Kohi- Safid is the most popular having rich wild fauna of collared pika (*Ochotona rufescens*) (Hussain, 2007).

The study was conducted from June 2014 to October 2016 in all reachable areas of FATA regions. It was a first attempt for identification of bat fauna of the same area. Bats samples were collected from (a) Upper (b) Central (c) Lower of both agencies and adjacent areas in order to identify bat fauna of these areas up to species level through morphological features (Dobson, 1876; Blase, 1981).

Strategy for sampling

Different visits were made possible to locate exact bat roost in the study area. Possible hidden bat roosts such as ruins, old and undisturbed buildings, abandoned wells, tree groves, farm houses, and forest plantations were properly searched. People belonging to same area were also interviewed for gaining fruitful information about the location of bat roosts.

A deep black, high quality ultra violet stable mist net was used to capture bats. Mist was in shape of either in “V” or “L” with strategic positions at a pair of 4 m long bamboo pole which has last shelf remained two feet above the ground. Mist was ready for operation before half an hour of sunset. All mists were opened at once before sunset, depending on condition of weather.

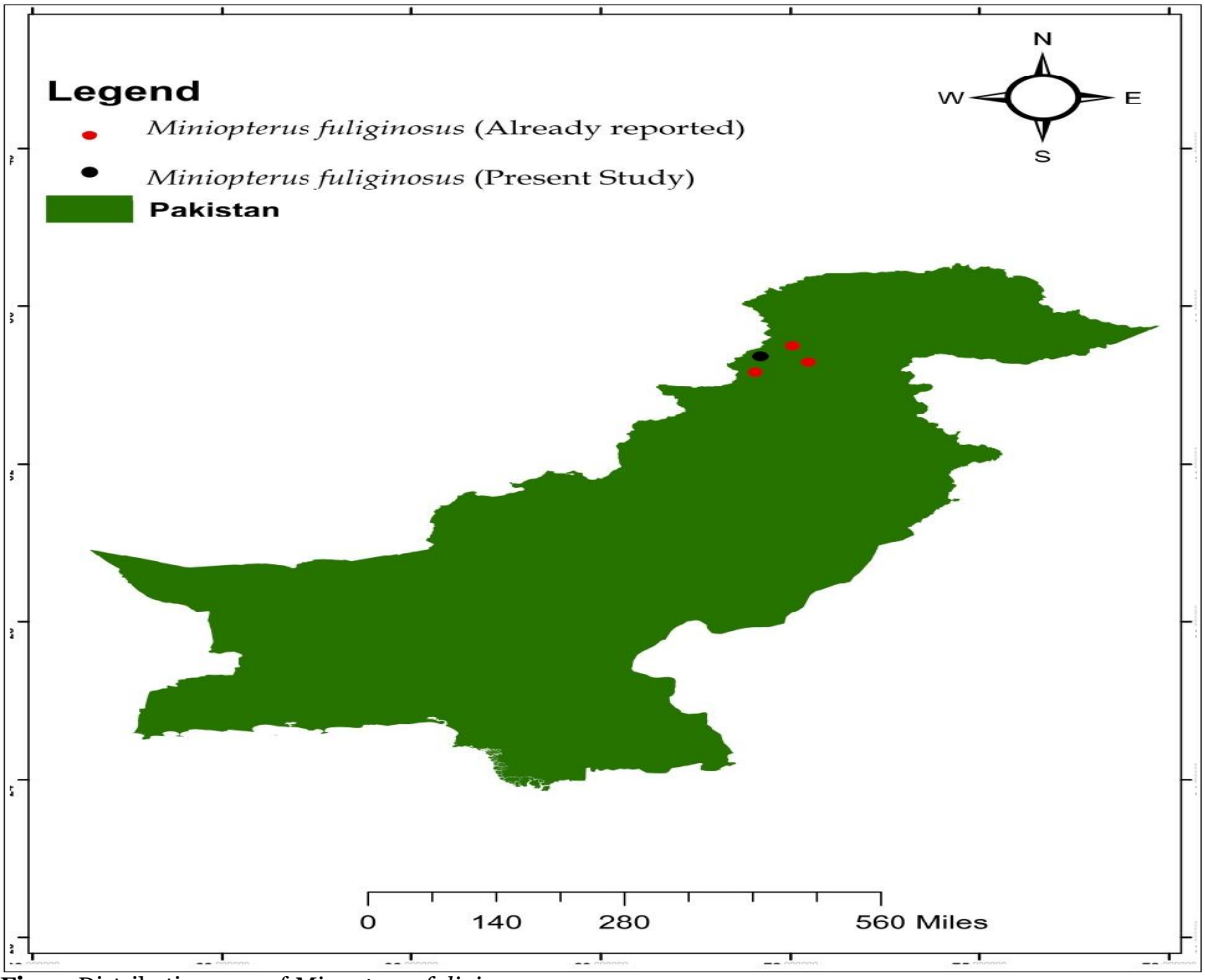

Fig. 1. Distribution map of *Minopterus fuliginosus*.

External Morphology

The capture bats were put in bags of cloth containing cotton and brought immediately for different observation in laboratory. Each bats weighted, sex and age was also determined (Ruprecht, 1988).

Measurements of Cranial

In cranial measurements the tongue, eye balls and flesh was removed from every skull. The clean skulls was thus kept for a night in a dilute solution of Potassium Hydroxide (KOH) (0.2 %) and pure alcohol for other night and then transferred to acetone for third night (Wołk & Ruprecht, 1988).

Molecular identification and Phylogenetic relationships

Extraction of DNA

For extraction of DNA Scientific Thermo Gene JET Genomic Kit was used. The wings membrane was cut with scissor from the specimens who were preserved in ethanol- and by using mortar grinded in liquid of nitrogen. These samples were gathered into 1.6 mL and by adding 190µL solution of digestion of 20µL proteinase potassium solution. These tubes were again incubated for a night at 58°C. The columns were then centrifuged at 7000 rpm for two minutes and then transferred into another tube. The DNA from these columns with elution of 300 µL of buffer was also added and with incubation of three minutes on room temperature these columns were centrifuged for 2 minutes at 7000rpm with purified DNA was collected at -25 °C. This all work was done in the lab of molecular at the Institute in Genetic Engineering and Biotechnology, The Agriculture University of Peshawar.

COI gene Amplification

Polymerase Chain Reaction performed with final volume of 30 µL containing 20 µL of Dream Taq Green Master Mix, 1 µL with forward, 2 µL reverse

primer, 0.6 µL of Taq polymerase, 2 µL of template with DNA and 5.5 µL free water nuclease. The conditions of amplification were 2 cycles, 94 ºC for 6 minutes, 36 cycles, 95ºC for 40 seconds, 58 ºC for 40 seconds, 74 ºC for 36 s, 1 cycle, 74 ºC for 8 minutes, held at 5 ºC. This confirmed products of PCR was got sequenced from the Bio BGI Solutions, Co, Limited (Hong Kong).

Analysis of DNA sequence

Sequences were being used for identification of specimen with the NCBI search. The divergence of species, genus and also family level were recorded on the K2P model (Kimura, 1980). Specimens with related sequence deposited by other researcher were taken from the Gene Bank using Blast for comparing and analyzing of their genetic distance. Sequences were also aligned in clustal W. The Neighbour-Joining tree was constructed having K2P parameters and 1000 bootstrap. The Maximum likelihood was also constructed by having best substitution model in 1000 bootstrap. The Maximum Parsimony approaches were also used by having Tree-Bisection-Reconnection (TBR) parameters with 1000 bootstrap. All analysis was performed by using MEGA 7 software (Kumar *et al.*, 2016).

Result and discussion

Morphology

Minopterus fuliginosus is a medium sized bat. The interfemoral membrane, tail, and hind limbs are somewhat long. Surface of dorsal is a rich brownish in few members, it is a deep black in other. Surface of ventral is mild paler with a tinge.

Table 1. External body measurements (mm) of *Miniopterus fuliginosus schreibersii* caught from Khyber and Bajur Agencies in Khyber Pakhtunkhwa during the current study.

Body Parameters	Mean±SD (n=6)	Range
BM	8.43±3.15	5.40-11.50
HBL	47.51±6.22	43.00-57.00
E	13.76±1.47	12.12-13.49
TRH	7.35±1.72	5.35-7.06
ATRH	3.05±1.03	2.01-2.08
FA	38.60±6.38	35.07-48.54
THC	6.62±1.44	6.20-7.15
2mt	34.39±5.63	30.55-42.72
1ph2mt	4.23±1.21	2.96-3.56
2ph2mt	10.78±2.67	6.62-11.40
3mt	35.36±5.32	32.13-43.11
1ph3mt	14.48±1.66	12.25-14.04
2ph3mt	16.97±9.36	11.66-32.96
3ph3mt	6.21±2.45	4.10-8.04
4mt	34.28±5.05	30.87-41.42
1ph4mt	10.86±1.38	9.18-10.18
2ph4mt	10.73±3.39	8.72-17.52
5mt	32.92±4.03	30.34-38.04
1ph5mt	10.00±1.93	8.08-10.73
2ph5mt	8.56±1.87	6.66-8.65
WS	26.51±33.50	235.00-320.00
TIB	18.60±2.20	16.70-19.94
CA	12.70±2.72	9.97-13.99
HF	9.87±1.84	7.71-10.19
T	39.88±7.93	33.66-52.22

N - The number of specimens; BM - Body mass; HB - Head and body;E - Ear; FA - Forearm; TH - Thumb; 2mt - 2nd metacarpal; 1ph2mt - 1st Phalanx on 2nd metacarpal; 2ph2mt - 2nd Phalanx on 2nd metacarpal; 3mt - 3rd metacarpal; 1ph3mt - 1st Phalanx on 3rd metacarpal; 2ph3mt - 2nd Phalanx on 3rd metacarpal; 4mt - 4th metacarpal; 1ph4mt - 1st Phalanx on 4thmetacarpal; 2ph4mt - 2nd Phalanx on 4th metacarpal; 5mt - 5th metacarpal; 1ph5mt - 1st phalanx on 5th metacarpal; 2ph5mt - 2nd phalanx on 5th metacarpal; WS - Wing span; TIB – Tibia; CA – Calcar; HF - Hind foot.

The pelage of the forehead is short dense extends to pads. Below the eyes cheeks are hairless; having ears with small in size, each structure widely tip, which mostly projects between pelage of the crown. The pinna is almost half of height of the tragus, somewhat curved inward ward; antitragus is so low and well defined. Usually body is inundated having many ecto-parasites, mostly Nycteribiids and Streblids.

Table 2. Cranial parameter of *Miniopterus fuliginosus schreibersii* caught in Khyber and Bajur Agencies at Khyber Pakhtunkhwa during this study.

Cranial Parameters	Mean±SD (n=6)	Range
CBL	14.76±1.79	13.22-15.30
CCL	14.81±1.75	12.27-14.26
C-M^n	6.33±1.40	5.05-6.09
C-M_n	6.72±1.50	5.34-6.68
GTL	15.49±1.59	13.99-15.61
M	11.55±1.66	9.96-11.75
M^n-M^n	7.09±1.36	5.67-6.66
ZB	9.75±1.28	8.47-9.11
BB	8.28±1.37	6.78-7.86
PC	4.75±1.14	3.57-3.94
C^i-C^i	4.96±1.44	3.66-4.82
RW	4.65±1.43	3.41-5.49

N - The number of specimens; CBL - Condylo-basal length; CCL - Condylo-canine length; CM^n- Maxillary too throw; CM_n - Mandibular toothrow; GTL - Greatest length of skull; M - Mandible length; M^n-M^n- Posterior palatal width; ZB - Zygomatic breadth; BB - Breadth of braincase; IC -Interorbital constriction; C^i-C^i - Anterior palatal width.

External body measurements

The average body mass of six *Minopterus fuliginosus* was 8.43g ± 3.15 SD, (Table 1). Head and body length was 47.51 mm ± 6.22 SD, the ear was 13.76 mm ± 1.47 SD, in length. The forearm and Mean thumb length was 7.62 mm ± 1.44 SD and 38.60 mm ± 6.38 SD. The heights of tragus were 7.35 mm ± 1.72 SD.

The height of anti-tragus was 3.05 mm ± 1.03 SD. The lengths of 2nd metacarpal were 34.39 mm ± 5.63 SD. The length of 1st phalanx on 2nd metacarpal was 4.23 mm ± 1.21 SD. The length of 2nd phalanx on 2nd metacarpal was 10.78 mm ± 2.67 SD. The mean length of 3rd metacarpal was 35.36 mm ± 5.32 SD, however, 1st and 2nd phalanges on 3rd metacarpal were 14.48 mm ±1.66 SD and 16.97 mm ± 9.36 SD respectively,3rd phalanx on 3rd metacarpal were 6.21 mm ± 2.45 SD and length of 4th metacarpal were 34.28 mm ± 5.05 SD. The mean length of 1st and 2nd phalanges on 4th metacarpal was 10.86 mm ± 1.38 SD and 11.73 mm ± 4.39 SD. The mean length of 5th metacarpal was 32.92 mm ± 4.03 SD; however, that of 1st phalanx on 5th metacarpal was 10.0 mm ± 1.93 SD and 2nd phalanx on 5th metacarpal was 8.56 mm ± 1.87 SD. The mean wingspans were 26.51 mm ± 32.50 SD and tibia, calcar and tail were18.60 mm ± 2.20 SD, 12.70 mm ± 2.72 SD and 39.88 mm ± 7.93SD, respectively and the length of hind foot was 9.87 mm ± 1.84SD.

Cranial measurements

The average greatest length of skull of the six specimens were 15.49 mm ± 1.59 SD and width of braincase was 8.22 mm ± 1.37 SD where that of post-orbital constriction were 4.75 mm ± 1.14 SD long and condyle-canine lengths were 13.81 mm ± 1.75 SD and condyle basal length was 14.76 mm ± 1.79 SD.

The anterior and posterior palatal widths measured 4.96 mm ± 1.44 SD and 7.09 mm ± 1.36 SD and maxillary tooth row length was 6.33 mm ± 1.40 SD. Mandibular tooth row length was 6.72 mm ± 1.50 SD and mandible length was measured as 11.55 mm ± 1.66 SD (Table 2).

Albeyrak, 1986 collected specimens from Ankaara of *M. fuliginosus*. The mean body and head length was 66.6(50.0 - 77.0 mm) length of tail 48.5 mm (41.0 - 54.0 mm) hind foot length 12.2 mm (11.0 - 14.0 mm) forearm length 45.0 mm (41.8-46.0 mm) and ear length 11.5 mm (9.0-13.0 mm). Matveev 2004 collected specimens from Cambodia of *M. fuliginosus*. The body mass was (9.0 - 9.1 mm) tail length (48.9 - 51.6 mm) hind foot length (9.5 - 9.7mm) tibia (18.0 - 19.2mm) forearm length (43.8 - 44.6 mm) and ear length (10.6 - 11.7 mm). Srinivasulu *et al.* 2010 collected specimens from South Asia of *M. fuliginosus*. The head and body length was (48.0 - 66.0 mm) hind foot length (8.0 - 11.0 mm) forearm length (45.7 - 48.6 mm) tail (45.0 - 62.0 mm) and ear length (9.7 - 13.0 mm). All the above parameters were found in range with the comparative results. Cranial measurements of the *M. fuliginosus* specimens captured from Sri Lanka, India and Nepal.

Table 3. Percentage of base composition in COI gene of genus *Minopterus*.

Base	A	T	G	C
Mean percentage	23.6	31.7	18.9	25.8

The average length of greatest skull 16.7 mm (16.3 - 17.4 mm) condyle canine length 15.1 mm (14.6 - 15.8 mm) zygomatic breadth 8.8 mm (7.5 - 10.1 mm) breadth of the braincase 8.9 mm (8.5 - 9.3 mm) postorbital constriction 4.9 mm (4.8 - 5.1 mm) mandible length 12.3 mm (11.7 - 12.8 mm) posterior palatal width 7.6 mm (7.4 - 7.9 mm) rostral width 6.4 mm (6.1 - 6.7 mm) maxillary tooth row length 7.1 mm (6.8 - 7.3 mm) and average mandibular tooth row length was noted as 7.5 mm (7.3 - 8.8 mm (Bates and Harrison, 1997). Baker *et al.* (1974) captured from Tunisia with cranial measurements of the *M. fuliginosus* specimens. The average condyle basal length 15.4 mm (15.1 - 15.6 mm), condyle canine length 20.3 mm (20.1 - 21.5 mm) postorbital constriction 4.6 mm (4.5 - 4.7 mm) and maxillary tooth row length 6.7 mm (6.5 - 6.9 mm).

Alabayrak captured from Ankaara 1985 with cranial measurements of the *M. fuliginosus* specimens.

Table 4. Pairwise genetic distance of genus *Minopterus*.

		1	2	3	4	5	6
1	MG299066 *Miniopterus fuliginous*						
2	MG299066 *Miniopterus fuliginous*	0.008					
3	HM540899 *Miniopterus magnate*	0.016	0.018				
4	KP247545 *Miniopterus schreibersii*	0.077	0.079	0.064			
5	JF442470.1_*Miniopterus africanus*	0.088	0.090	0.074	0.016		
6	JF442469.1_*Miniopterus africanus*	0.192	0.194	0.180	0.193	0.179	
7	JF443969.1_*Miniopterus australis*	0.194	0.196	0.182	0.195	0.181	0.002

The mean condyle basal length 16.0 mm (15.6 - 16.3 mm) postorbital constriction 4.7 mm (4.54-5.0 mm) maxillary tooth row length 7.0 mm (6.8 - 7.2 mm) mandibular tooth row length 7.4 mm (7.1 - 7.6 mm) greatest skull length 16.5 mm (16.2 -17.9 mm) mandible length 12.0 mm (11.7 - 12.3 mm) zygomatic breadth 9.6 mm (9.4-9.7 mm) and breadth of braincase 8.9 mm (8.78.1 mm). Matveev captured from Cambodia 2006 with cranial measurements of the *M. fuliginosus* specimens. The average condyle basal length (15.68 - 15.79 mm) condyle canine length (14.88 - 14.94 mm) Maxillary tooth row length (7.09 - 8.19 mm) mandibular tooth row length (7.47 - 8.54 mm) greatest skull length (16.53 - 17.88 mm) posterior palatal width (7.37 - 8.47 mm) and anterior palatal width (5.71 - 6.69 mm). Benda *et al.* 2006 captured from Iran having cranial measurements of the *M. fuliginosus* specimens.

The average greatest skull length 16.40 mm (16.10 – 17.76 mm), condyle basal length 16.01 mm (15.67 - 16.32 mm) zygomatic breadth 9.83 mm (9.58 - 10.19

mm) breadth of the braincase 8.90 mm (8.93 - 9.29 mm) interorbital constriction 4.57 mm (4.42-5.79 mm) mandible length 12.02 mm (11.72 - 12.34 mm) anterior palatal width 5.56 mm (5.33-5.81 mm) posterior palatal width 7.50 mm (7.27 – 7.64 mm) maxillary tooth row length 7.05 mm (6.85 - 7.21 mm) and average mandibular tooth row length was noted as 7.46 mm (7.33-7.65 mm). The condyle canine length (14.6 - 15.8 mm) zygomatic breadth (9.5 - 10.1 mm) mandible length (11.7 - 12.8 mm) maxillary tooth row length (6.8 - 7.3 mm) and mandibular tooth row length was noted as (7.3 - 8.8 mm). All the above parameters were found in range with the comparative results.

The sequence of COI gene belonging to genus *Miniopterus*, in the present study was analyzed for nucleotide composition and divergence.

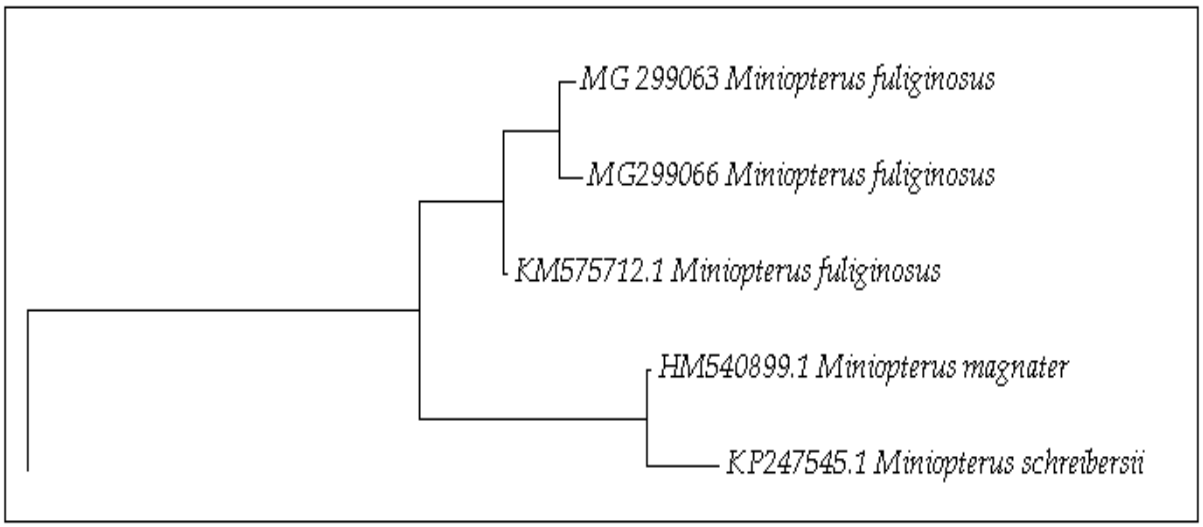

Fig. 2. Phylogenetic tree of genus *Miniopterus* based on COI using Maximum likelihood method. Number indicates the percentage of 1000 bootstrap replicates greater than 50.

The sequence of DNA belonging single genus was procured from Gene Bank for comparison already deposited by other researcher. The final aligned data had nine sequences of more than 685 bp length. Overall gap of barcode between species was distinct. In sequence there was found no overlap in divergence. In aligned data was presented by having 685 genetic characters of which 450 were conserved sites, 218 variable sites and 143 parsimony informative sites, 75singleton sites. In the mean K2P increase divergences across different was observed levels according to taxonomic.

The average intraspecific divergences of *Miniopterus fuliginosus* was 0.018% (Table 4). The compositions of nucleotide in total sequences were analyzed. The mean concentration of each nucleotide was A = (23.6%), T = (31.7%), G = (18.9%) and C = (25.8%) (Table 3). The T+ A contents were 55.3%and C+G were 44.7%. The descending order of contents in A+T were *M.magnater* 56.2%, *M.laustralis* 55.1%, *M. fuliginosis* 54.8%, *M. africanuss* 54.6% and *M. schreibersii* 53.3%. Similarly descending order of contenst in C+G were *M. schreibersii* 45.7%, *M. africanuss* 45.3%, *M.australis* 44.9%, *M. fuliginosis* 44.3% and *M. magnater* 42.0%.

In Neighbor Joining tree the sequences in *M. fuliginosis* clustered together with 100% bootstrap support and that of *M. magnater* clustered together with 100% bootstrap support. The relationship between *M. fuliginosis* and *M. australis* was supported by 100% bootstrap value. The sequences of *M. magnater* clustered together with 100% bootstrap support and that of *M. schreibersii* clustered together with 100% bootstrap support. The Maximum Likelihood tree with log likelihood (-2079.87) showed similar topology to that of Neighbor Joining tree. Conspecific taxa clustered together with 100% bootstrap support (Figure 2). The Maximum

Parsimony tree with length (298), consistency index (0.737557) retention index (0.786765) and composite index (0.633636) was also contracted having similar topology to Neighbor Joining tree and Maximum Likelihood tree (Figure 3). These analyses confirm the utility of the COI gene sequence of *M. fuliginosis,* obtained in the present study for species level identification and phylogenetic relationships.

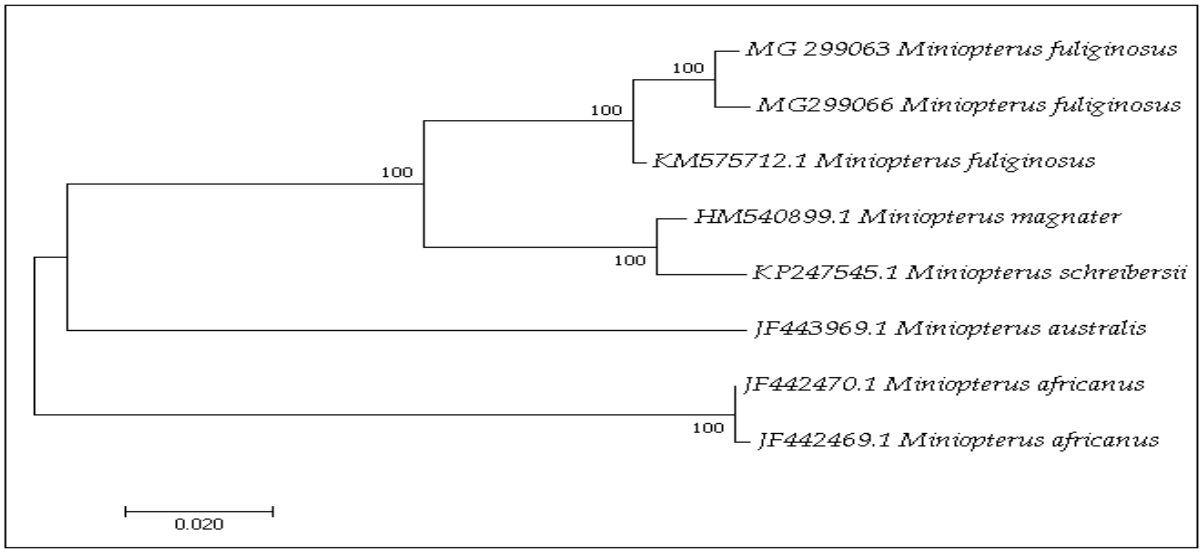

Fig. 3. Phylogenetic tree of genus *Miniopterus* based on COI (K2P model) using Neighbour Joining method. Number indicates the percentage of 1000 bootstrap replicates greater than 50.

The intraspecific divergence of the present study is in agreement with the intraspecific divergence reported in other animal taxa, such as in marine fish 0.27%. Birds, 0.39% (Ward *et al.*, 2005), 0.11% in mayflies (Ball *et al.*, 2005), and 0.06% in bats (Clare *et al.*, 2007).

The interspecific relationships of these taxa from Madagascar, their colonization history, and the evolution of this adaptive radiation have not been sufficiently explored. By sing the mitochondrial cytochrome-b gene, a phylogeny of the Malagasy members of this widespread Old World genus, based on 218 sequences, of which 82 are new and 138 derived from previous studies. Phylogenetic analyses recovered 18 clades, which are divide into five primary lineages: (1) *M. griveaudi*; (2) *M. mahafaliensis*, *M. sororculus* (3) *M. majori*, *M. gleni* ; (4) *M. brachytragos* and (5) *M. manavi* and *M. petersoni* recovered as sister species, which were in the turn linked to a group comprising *M. egeri* and five genetically distinct populations referred to herein as P3, P4, P5, P6 and P7. DNA K2P-distances between recognized taxa ranged from 12.9% to 2.5% and intraspecific variation was less than 1.8%, of the 18 identified clades, Latin binomials are only associated with 11, which indicates much greater differentiation than currently recognized for Malagasy Miniopterus. These data are placed in a context of the dispersal history of this genus on the island and patterns of ecological diversity. (Christidis *et al.*, 2014).

The phylogenetic relationships of the 3 Neodiplostomum spp. (Digenea: Neodiplostomidae) occurring in Korea (*N. seoulense*, *N. leei*, and *N. boryongense*) were analyzed using the partial mitochondrial cytochrome c oxidase subunit 1 (CO1) gene. The adult flukes were recovered from Sprague-Dawley rats (*N. seoulense*) and newborn chicks (*N. leei* and *N. boryongense*) experimentally infected with the neodiplostomula from the grass snake, Rhabdophis tigrinus tigrinus. The genomic DNA was amplified using specific primers, and the sequence of CO1 was obtained. According to the results, the pairwise similarity was 96.1% between *N. boryongense* and *N. seoulense*, but was 95.0% between *N. boryongense* and *N. leei* and 94.2% between *N. leei* and *N. seoulense*. The results

demonstrated a closer phylogenetic relationship between *N. seoulense* and *N. boryongense*. This high relationship of *N. seoulense* and *N. boryongense* may be related to their similar morphologic features including the limited distribution of vitellaria and the presence of a genital cone. *N. leei* is distinct on the other hand with an extensive distribution of vitellaria and the absence of a genital cone.

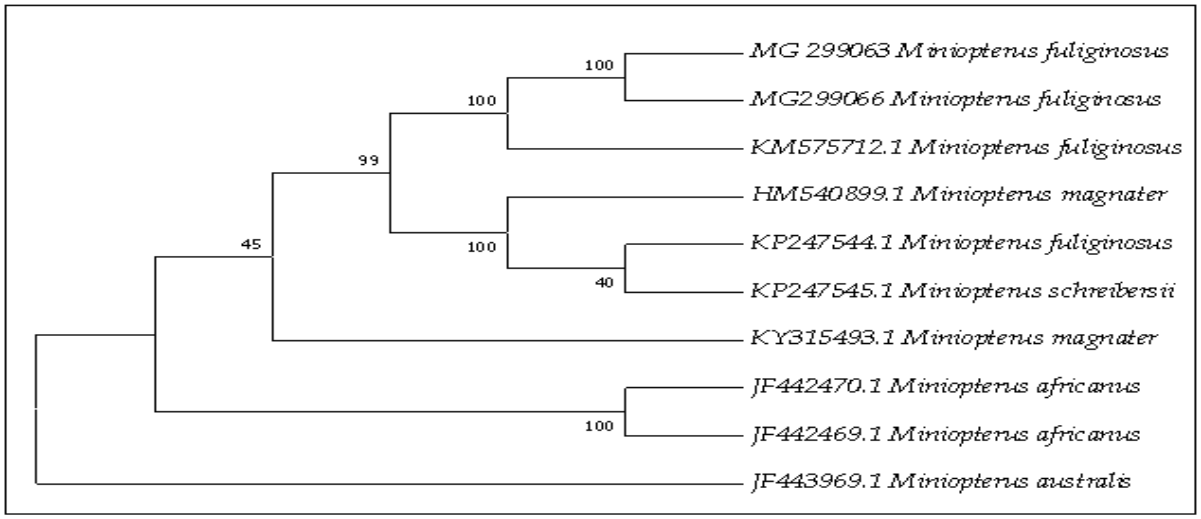

Fig. 4. Phylogenetic tree of genus *Miniopterus* based on COI using Maximum parsimony method. Number indicates the percentage of 1000 bootstrap replicates greater than 50.

To our known knowledge, the present study of bats diversity with DNA barcoding from FATA Region is the first survey. Further confirmation that DNA barcoding is a powerful tool for species identification is provided by our result. The molecular data will be a powerful tool for guiding the systematic research and furthering phylogeographer studies. As from vouchered specimen our sequence is derived the reference database will be valuable tool for validating. When vouchering is impractical and the discrimination of some species requires examination of morphological characters which cannot be evaluated on live specimens (e.g. cranial or dental characters). In addition the molecular tool can help for identify partial remains or trace materials from guano when capture, morphological assessment or tissue acquisition are not possible. In the present study intraspecific divergence is with agreement of the concern divergence (0.06%) mention by (Clare *et al.*, 2007).

Conclusion

The results of the present analysis are congruent with previous studies and morphology based identification. These analyses confirm the utility of COI gene sequence for identification of *Miniopterus* and to elaborate their phylogenetic relationships. Further studies are needed to add more data to the BOLD database for easy and correct identification of *Miniopterus* species.

Recommendations

We propose to use the information generated in this study and available through BOLD to develop appropriate microarrays for identification of bat species of Pakistan. In the future as barcoding information for bat species improves, similar approaches might be considered in other jurisdictions, where the diversity of bat species is much larger.

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