Systematic analysis of leisler’s bat *Nyctalus leisleri* (Kuhl, 1817) captured from FATA region, Pakistan

I. Hussain, S. A. Mehmood, S. Ahmed, M. Salim, A. Hussain, S. Noureene, D. Ahmed, M. Israr, F. Akbar, A. Rasool, H. Jabeen, K. Saeedi, A. Alam, Sanaullah, K. Usman, N. Saeed, W. Khan and M. Shah

Department of Zoology, University of Swat, Centre for Animal Science & Fisheries, Saidu Sharif, Khyber Pakhtunkhwa, Pakistan
Department of Forensic Sciences, University of Swat, Khyber Pakhtunkhwa, Pakistan
Centre for Biotechnology & Microbiology, University of Swat, Khyber Pakhtunkhwa, Pakistan
Department of Zoology, University of Malakand, Chakdara, Khyber Pakhtunkhwa, Pakistan
Department of Zoology, Hazara University, Mansehra, Khyber Pakhtunkhwa, Pakistan
Department of Forestry and Wildlife Management, University of Haripur, Khyber Pakhtunkhwa, Pakistan
Department of Ecology & Wildlife Management, University of Swat, Khyber Pakhtunkhwa, Pakistan
Centre for Animal Science & Fisheries, University of Swat, Khyber Pakhtunkhwa, Pakistan

**Abstract**

Extensive field surveys were carried out to explore the distribution of Leisler’s Bat *Nyctalus leisleri* (Kuhl, 1819) in selected area of FATA regions, Pakistan. Specimens of Leisler’s Bat *Nyctalus leisleri* (Kuhl, 1819) (*n* = 5) were collected from Kurrum Agency (Shublan) (N33.8229788 E70.1634414) at elevation 1427m and Khyber Agency (Landi Kotel) (N34.0908899 E71.1457517) at elevation 1091m for two years survey extending from May 2013 through August 2015. The mean head and body length, hind foot length, ear length and tail length *Nyctalus leisleri* specimens captured from the study area was 65.08 ± 1.58 mm, 44.06 ± 0.52 mm, 8.38 ± 0.60 mm, 13.20 ± 0.99 mm and 39.46 ± 1.46 mm, respectively. For molecular analysis the sequences of COI gene were obtained and analyzed. The mean intraspecific divergences of *Nyctalus leisleri* was 0.04%. The mean interspecific divergences of *Nyctalus noctula* and *Nyctalus leisleri* was 0.2%. The mean concentration of each nucleotides was A = (26.3%), T = (32.8%), G = (15.9%) and C = (25.0%). The mean A+T contents were 59.2% and C+G were 40.9%. In the phylogenetic tree *Nyctalus leisleri* and *Nyctalus noctula* clustered with significant bootstrap support value.

**Keywords:** *Nyctalus leisleri*, morphology, Kurrum Agency, Khyber agency, Pakistan.

**1. Introduction**

The genus *Nyctalus* (Bowdich, 1825) includes seven species *N. noctula*, *N. leisleri*, *N. lasiopterus*, *N. planci*, *N. azoreum*, *N. montanus* and *N. velutinus*. In the Indian Subcontinent it is represented by a single genus and three species...
including *Nyctalus noctula* (Schreber, 1774), *Nyctalus leisleri* (Kuhl, 1819) and *Nyctalus montanus* (Barrett-Hamilton, 1906) (Roberts, 1997; Bates and Harrison, 1997). In Pakistan only two species have been reported till now. The first one was reported from Charial in Murree Hills at 2385 m while the second from Kululai, Yakh Tangai at Swat Kohistan with height of 1850 m in 1972 by Dr Walton, (Walton, 1974). The same species ranges from low elevation plains (Ruczyński and Ruczynska, 2000) up to mountain forests in Europe (Hruz et al., 2000; Spitzerenberger and Bauer, 2001).

It is migratory species in Europe and occurred in northern Himalayan region of Pakistan (Walker et al., 1964). It is not mentioned in Siddiqi’s (1961, 1970) checklists. It is believed that these species migrate to Pakistan in summer, although specimens were collected from the Murree Hills as late as 8th November. The District Swat Kohistan species were collected on 1st September, hunting in open area however Walton did not find its roost.

It was also collected from Palgham in Indian Kashmir from Kumaon and Simla further east, so it have fairly widely distributed in the better forested regions of Himalayans. In Pakistan, it appears to have associated with possible mesic conditions in the Himalayan, moist temperate forest zone. It is very numerous and probably does not appear to be highly gregarious.

Only two species have been reported from two provinces of Afghanistan i.e. Paktya province (Meyer-Oehme, 1965) and Jalalabad (Gaisler, 1970). It is very rare in Afghanistan while not reported from Iran yet. On 18th March a male was reported near Jalalabad which are very active sexually (Gaisler, 1970).

This medium sized insectivorous bat specializes with aerial for fast hawking and their body (forearm length 40–47 mm, body mass 9–20g was reviewed by Bogdanowicz and Ruprecht, 2004). This species emerges in early to hunt, like many of the *Pipistrellus* species and often active before sunset. They have strong and superb flight and feed from small to medium-sized insects (Nematocera, Trichoptera, Coleoptera, and Lepidoptera (Nowak, 1991; Beck, 1995; Vaughan, 1997; Shiel et al., 1998; Waters et al., 1999; Fuhrmann et al., 2002). Exclusively they built roosts in tree-hollows (Ruczyński and Bogdanowicz, 2005), except Ireland, where nursery colonies occupy roof attics (Shiel et al., 1999). The purpose of present study is to broaden the scope to understanding about the morphology and distribution of *N. leisleri* in Pakistan.

### 2. Materials and Methods

#### 2.1. Study area

The study was conducted in selected areas (Kurram agency and Khyber Agency) of FATA region. It is among agencies in Federally Administered Tribal Area (FATA). The Kurram gency head quarter is Parachinar, while that of Khyber is Jamrud. The agency is bordered in the north and west by Afghanistan (province of Paktiya and Ningarhar respectively), Orakzai and Khyber agencies in the East, Kohat to the South and North Waziristan to the West (Figure 1). The Kurram Agency is divided into mainly three administrative units Upper Kurram, Lower Kurram and Central Kurram while Khyber Agency is divided into Bara, Jamrud, Landi kotel and Torkham. Kurram Agencies is surrounded by series of mountains on about all sides. Most importing striking physical feature is the Koh-E-Sufid covered by snow for almost whole the year. Main mountain range in the study area is the Koh-E-Sufid with peak of Sikaram about 4,628 m high meeting a boundary with Afghanistan. It remains about cover with snow throughout the year (Hussain, 2007).

#### 2.2. Method

The study was conducted from May 2013 to August 2015 in all possible and accessible areas of FATA regions. This was a pioneering attempt to identify the poorly known bat fauna of this area. Bats samples were collected from (a) Upper (b) Central (c) Lower Kurram Agency and adjacent areas in order to identify bat fauna of these areas up to species level through morphological features, which were used throughout the world (Dobson, 1876; DeBlase, 1980; Bates and Harrison, 1997; Roberts, 1997; Dietz and Von-Helversen, 2004).

#### 2.3. Sampling strategy

Exploratory visits were made for locating as many bat roosts in all the sub-areas as possible. Potential bat roosts such as old and ruined buildings, abandoned wells, farmhouses, tree groves and forest plantations were searched properly. Local people were interviewed for gaining possible information about the exact territory of various bat roosts.

For collection of bats a fine quality, black, UV strong mist nets were used. On a pair of 3 m long bamboo poles the mist nest were erected either in “L” or “V” shape about one foot above the ground. The nets were ready to operate about half an hour before the sunset and opened simultaneously before sunset and continued to operate, depending on the weather conditions, for two hours. Nets were checked regularly to disentangle any captured of bat. The sampling effort throughout the study remained the same.

Once located the bats roost, global position of each of roost was determined using Garmin etrax H Global Position System (GPS).

#### 2.4. External morphology

The capture bats were collected in cloth bags containing wet cotton and were brought directly to the laboratory. The bats were then weighted, their sex and age was calculated (Wolk and Ruprecht, 1988).

Each bat weighed up to 0.1 g (Pesola balance 10050, Swiss made), Field number, age, Sex, exact location and agency were noted. The external body measurements were taken using a digital Varner caliper (0–150 mm). Age of each captured bat specimen was determined following Dietz (2005).

#### 2.5. Cranial measurements

The tongue, eye balls and excessive flesh was removed from the skull for recording cranial measurement. Skulls were cleaned and kept in a dilute solution (0.2% of Potassium Hydroxide (KOH) overnight and absolute alcohol for other night before being transferred to acetone for the third night (Figure 2).
Systematic analysis of Leisler’s bat *Nyctalus leisleri*

2.6. Molecular identification and Phylogenetic analysis

2.6.1. Extraction of DNA

The Thermo scientific Gene JET Genomic DNA purification Kit was used for the extraction of DNA from wing membrane of the ethanol-preserved specimens. For the amplification of COI gene, the following primers (Table 1) were used (Folmer et al., 1994).

2.6.2. Amplification of COI gene

PCR reaction was performed in a final volume i.e 25 µL containing 10 µL of Thermo scientific Dream Taq Green PCR Master Mix, 1 µL forward primer, 1 µL reverse primer, 0.5 µL Taq polymerase, 1 µL template DNA and 6.5 µL nuclease free water. The amplification conditions were 1 cycle, 95 °C for 5 minutes, 35 cycles, 94 °C for 30 seconds, 48 °C for 30 seconds, 72 °C for 35 s, 1 cycle, 72 °C for 10 minutes, hold at 4 °C.

2.6.3. DNA sequence analysis

The sequences were used for the identification of species through NCBI BLAST search. Divergence at species, generic and family level were employed the K2P model of base substitution. Sequences of congeneric specimens deposited by other workers were also taken from Gene Bank using Blast tool for the comparisons and analysis of genetic distance. The sequences were also aligned in clustalW. Neighbour-Joining tree was constructed using K2P parameter and 1000 bootstrap replicates. The Maximum likelihood was constructed using best substitution model with 1000 bootstrap replicates. Maximum Parsimony approach was employed by using Tree-Bisection-Reconnection (TBR) parameter and 1000 bootstrap replicates. All analysis was performed by using MEGA 7 software.

3. Results and Discussion

After extensive field surveys five Leisler’s Bat *Nyctalus leisleri* (Kuhl, 1817) were collected from study area and compared their external body and cranial features with Roberts (1997) and Bates and Harrison (1997), the best authentic sources on bats of the region. This bat is medium-sized insectivorous with forearm length ranging in 43.10 to 44.60. The pelage is longer and denser than other. The dorsal surface mainly darker while the ventral surface is buffy brown. The wings are sharp and long extend to the ankle of hind foot. The ear is blackish and naked, both anterior and hind margins being convex when viewed from another side. There is a low, broad tragus its anterior margin being sharply concave and posterior margin almost in straight position. Our findings are in line with Bates and Harrison (1997) and Roberts (1997).
Hussain, I. et al.

Brazilian Journal of Biology, 2022, vol. 82, e238337

The mean body mass of five Leisler’s Bat was 14.326 ± 1.93 g while according to Chirichella et al. (2003), Kañuch et al. (2005) and Mathias (1988), the mean body mass were 14.50 ± 0.71 mm, 15.05 ± 0.55 mm, 15.32 ± 1.73 mm and 11-20 mm, respectively (Table 2).

Average head and body length of 05 specimens collected during present study was 65.08 ± 1.58 mm, forearm length was 44.06 ± 0.52 mm, hind foot length was 8.38 ± 0.60 mm, ear length was 13.20 ± 0.99 mm and the tail was 39.46 ± 1.46 mm long while according to Roberts (1997), the average head and body length, forearm length, hind foot length, ear length and tail length was 67 mm, 39-46 mm, 8 mm, 10.7 mm and 36 m, respectively. All these measurements fall within the ranges given by Roberts (1997) and Bates and Harrison (1997). The mean body mass of five Leisler’s Bat was 14.326 ± 1.93 g while according to Chirichella et al. (2003), Kañuch et al. (2005) and Mathias (1988), the mean body mass were 14.50 ± 0.71 mm, 15.05 ± 0.55 mm, 15.32 ± 1.73 mm and 11-20 mm, respectively (Table 2).

Table 1. Primer pair for COI gene amplification.

| Primer code | Primer sequence          |
|-------------|--------------------------|
| COX-F2      | 5’-GGTCAACAAATCATAAAGATATTGG-3’ |
| COX-R1      | 5’-TAAACTTCAGGGTGACAAAAATCA-3’ |

Figure 2. (A) Nyctalus leisleri; (B) Head; (C) Skull lateral view; (D) Lower jaw dorsal view.
Table 2. Comparison of the body mass (g) and external body measurements (mm) of Nyctalus leisleri captures during present study with (Roberts, 1997; Bates and Harrison, 1997; DeBlase, 1980; Speakman and Webb, 1993; Chirichella et al., 2003; Kaňuch et al., 2005; Mathias, 1988).

| Specimens | Present Study | Roberts (1997) Mean | Bates and Harrison (1997) Mean | DeBlase (1980) Mean | Speakman and Webb (1993) Mean | Chirichella et al. (2003) Mean | Kaňuch et al. (2005) Mean | Mathias (1988) Mean |
|-----------|---------------|---------------------|--------------------------------|---------------------|-------------------------------|-------------------------------|----------------------------|---------------------|
|           | (Mean ± SD) (n=5) |                      | (Mean ± SD) (n=6) | (Mean ± SD) (n=10) | (Mean ± SD) ♂ (n=2) | (Mean ± SD) ♀ (n=17) | (Mean ± SD) ♀ (n=17) | Range |
| BM        | 14.326 ± 1.93 (12.14-17.15) | -                   | -                             | -                   | 14.50 ± 0.71                  | 15.05 ± 0.55 (14.5-15.6) | 15.32 ± 1.73 (10.8-18.0) | 11-20 |
| HB        | 65.08 ± 1.58 (64.00-68.10) | 67                  | 65.5 ± 3.4 (62.0-72.0) | 108.5 ± 4.95 (105-112) | 63.26 ± 2.57 (58-66) | -                           | -                           | 54-64 |
| E         | 13.20 ± 0.99 (12.10-14.40) | 10.7                | 12.2 ± 3.3 (7.0-16.0) | 16.7 ± 0.58 (16-17)  | 20.32 ± 26.17 (9.9-90) | -                           | -                           | 12-13 |
| FA        | 44.06 ± 0.52 (43.10-44.60) | 39-46               | 43.6 ± 1.2 (42.1-45.2) | 44.3 ± 1.29 (42.8-45.2) | 43.20 ± 1.48 (42-45.8) | 43.45 ± 0.35                 | 43.10 ± 1.60 (41.5-44.7) | 44.75 ± 1.22 (41.3-46.8) | 39-47 |
| Th        | 5.23 ± 0.11 (5.11-5.41)    | -                   | -                             | -                   | 10.68 ± 15.93 (5.1-56)    | -                           | -                           | -      |
| 2mt       | 44.02 ± 0.32 (43.50-44.50) | -                   | -                             | -                   | -                            | -                           | -                           | -      |
| 3mt       | 44.46 ± 0.59 (43.40-45.00) | -                   | 43.5 ± 1.3 (42.4-45.7) | -                   | -                            | 76.45 ± 3.04 Finger         | -                           | -      |
| 4mt       | 43.39 ± 0.56 (42.72-44.10) | -                   | 42.7 ± 1.4 (41.6-45.0) | -                   | -                            | -                           | -                           | -      |
| 5mt       | 35.86 ± 0.55 (35.20-36.40) | -                   | 36.2 ± 0.7 (35.4-37.3) | -                   | 32.38 ± 1.17 (30.6-33.9) | 54.15 ± 5.44 Finger         | -                           | -      |
| WS        | -                   | -                   | -                             | -                   | -                            | -                           | -                           | -      |
| TIB       | 17.20 ± 0.08 (17.10-17.29) | -                   | -                             | -                   | 16.96 ± 0.92 (16-18.3)     | -                           | -                           | -      |
| HF        | 8.38 ± 0.60 (7.80-9.10)    | 8                   | 8.3 ± 1.7 (6.0-10.0) | 9.7 ± 0.58 (9-10)    | 8.28 ± 1.26 (6-10)       | -                           | -                           | -      |
| T         | 39.46 ± 1.46 (38.10-42.10) | 36                  | 38.8 ± 5.5 (31.0-45.0) | 45.3 ± 3.21 (43-49)  | 38.67 ± 4.01 (31-45)     | -                           | -                           | -      |

n: The number of specimens; BM: Body mass; HB: Head and body; E: Ear; FA: Forearm; Th: Thumb; 2mt: 2nd metacarpal; 3mt: 3rd metacarpal; 4mt: 4th metacarpal; 5mt: 5th metacarpal; WS: Wing span; TIB: Tibia; HF: Hind foot; T: Tail; SD: standard deviation.
The final aligned data had seven sequences of more than 556 bp length representing seven species. All the barcode gaps were distinct among species. In sequence no overlap deviation was observed. The collected data aligned was shown by 556 characters of which were conserved sites was 486, variable sites 70, parsimony informative sites 68, and singleton sites 2. The increase in the mean K2P deviations was observed. The mean intraspecific deviation of *Nyctalus noctula* was 0.02%. The mean intraspecific divergences of *Nyctalus leisleri* was 0.04%. The mean interspecific divergences of *Nyctalus noctula* and *Nyctalus leisleri* was 0.2% (Table 4).

The compositions of nucleotide sequences of all species were also observed. Nucleotide mean concentration was A = (26.3%), T = (32.8%), G = (15.9%) and C = (25.0%). The mean A+T contents were 59.2% and C+G were 40.9% (Table 5).

| Specimens | Present Study (n=5) (mean ± SD) | Bates and Harrison (1997) (mean ± SD) (n=6) | DeBlase (1980) | Mathias (1988) |
|-----------|--------------------------------|---------------------------------------------|----------------|---------------|
| CBL       | -                              | 15.3 ± 0.42 (15-15.6)                        | 14.50-15.20    |               |
| CM        | 5.60 ± 0.17 (5.40-5.80)         | 5.7 ± 0.1 (5.5-5.9)                          | 5.5 ± 0.28 (5.3-5.7) | -             |
| CM        | 6.18 ± 0.07 (6.10-6.30)         | 6.1 ± 0.2 (5.9-6.3)                          | 6.2 ± 0.00 (6.2-6.2) | -             |
| GTL       | 15.50 ± 0.07 (15.40-15.60)      | 15.6 ± 0.2 (15.3-15.9)                       | 16.0 ± 0.07 (15.9-16) | -             |
| M         | 11.60 ± 0.06 (11.50-11.70)      | 11.6 ± 0.2 (11.4-11.7)                       | 11.7 ± 0.00 (11.7-11.7) | 11.10-11.20 |
| M-M       | 7.28 ± 0.07 (7.20-7.40)         | 7.1 ± 0.2 (6.9-7.4)                          | -              | -             |
| ZB        | 10.38 ± 0.08 (10.30-10.50)      | 10.3 ± 0.4 (9.8-10.7)                        | 10.3 ± 0.00 (10.3-10.3) | -             |
| BB        | 8.42 ± 0.07 (8.30-8.50)         | 8.3 ± 0.1 (8.2-8.5)                          | -              | -             |
| IO        | 4.78 ± 0.07 (4.70-4.90)         | 4.7 ± 0.1 (4.6-4.9)                          | 4.6 ± 0.14 (4.5-4.7) | -             |
| RW        | 6.42 ± 0.19 (6.10-6.70)         | 6.6 ± 0.1 (6.4-6.8)                          | -              | -             |

CBL: Condylo-basal length; CM: Maxillary toothrow; CM: Mandibular toothrow; GTL: Greatest length of skull; M: Mandible length; M-M: Posterior palatal width; ZB: Zygomatic breadth; BB: Breadth of braincase; IO: Interorbital Width; RW: Rostral width.

Table 4. Pairwise genetic distance of genus *Nyctalus* (Bowdich, 1825).

| JF443048 Nyctalus noctula | FR856759 N. noctula | 0.002 | MK091913 N. leisleri | 0.143 | 0.146 |
|---------------------------|---------------------|-------|----------------------|-------|-------|
| JF443043 N. leisleri      | 0.143               | 0.146 | 0.007                |       |       |
| JF443042 N. leisleri      | 0.148               | 0.151 | 0.007                | 0.004 |       |
| GU707566 N. leisleri      | 0.148               | 0.151 | 0.007                | 0.004 | 0.000 |
| FR856758 N. leisleri      | 0.148               | 0.151 | 0.007                | 0.004 | 0.000 | 0.000 |
| FR856755 N. leisleri      | 0.146               | 0.148 | 0.005                | 0.002 | 0.002 | 0.002 | 0.002 |

Table 5. Composition of base percentage wise in gene COI of genus *Nyctalus leisleri*.

| Base | A   | T   | G   | C   |
|------|-----|-----|-----|-----|
| Mean percentage | 26.3 | 32.8 | 15.9 | 25.0 |
The Neighbor Joining tree was constructed into two clades i.e. clade I and clade II. Clade I comprised upon six sequences, the sequences of Nyctalus leisleri of the present study was among them (Figure 3).

The Maximum Likelihood tree having log likelihood (-2812.83) with same topography to Neighbor Joining tree. Conspecific taxa clumped with 61-100% supported bootstrap (Figure 4).

The Maximum Parsimony tree having length (469), index consistency (0.5419199) index retention (0.714078) and index composite (0.414135) was contracted with same topography to Neighbor Joining tree and Maximum Likelihood tree (Figure 5).

These observations confirm the utility of the COI gene sequence of Nyctalus leisleri, obtained in the current analysis for identification to species level.

Figure 3. Genus Nyctalus peculiar to COI by Neighbour Joining method. Number shows percent of 1000 bootstraps replication above 50. ♦ shows the sequences of species of the present research.

Figure 4. Genus Nyctalus peculiar to COI by Maximum likelihood method. Number shows percent of 1000 bootstraps replication above 50. ♦ shows the sequences of species of the present research.
Figure 5. Peculiar to COI by Maximum parsimony method (K2P model). Numbers show percent of 1000 bootstraps replication above 50. • shows the sequences of species of the present research.

References

BARRETT-HAMILTON, G.E.H., 1906. Description of two new species of Ptyrgistes. *Annals & Magazine of Natural History*, vol. 17, no. 97, pp. 98–100. http://dx.doi.org/10.1080/00222930608562496.

BATES, P.J., and HARRISON, D.L., 1997. Bats of the Indian subcontinent. Sevenoaks, UK: Harrison Zoological Museum, pp. 254–258.

BECK, A., 1995. Fecal analyses of European bat species. *Myotis*, vol. 32, no. 33, pp. 109–119.

BOGDANOWICZ, W., and RUPRECHT, A.L., 2004. *Nyctalus leisleri* Kleinaendsegel. In: F. KRAPP, ed. *Handbuch der Säugetiere Europas. Band 4/II: Fiedertiere (Chiroptera)* II. Wiebelshiem: Aula-Verlag, vol. 10, pp. 582–585.

BOWDICH, T., 1825. Excursions in Madeira and Porto Santo. *Journal of Zoology*, vol. 249, pp. 173–180.

CHIRICHELLA, R., MATTIROLI, S., NODARI, M., PREATONI, D.G., WAUTERS, L.A., TOSI, G. and MARTINOLI, A., 2003. The Adamello-Brenta Natural Park bat community (Mammalia, Chiroptera): distribution and population status. *Hystrix the Italian Journal of Mammalogy*, vol. 14, pp. 24–45.

DEBLASE, A.F., 1980. The bats of Iran: systematics, distribution, ecology. *Fieldiana. Zoology*, vol. 4, no. 1, pp. 421–424.

DIETZ, C., and VON-HELVERSEN, O., 2004. Illustrated identification key to the bats of Europe. Germany: Dietz & von Helversen, vol. 1, pp. 1–74.

DIETZ, C., 2005 [viewed 20 May 2020]. Illustrated identification key to the bats of Egypt. Version 1.0 [online]. Germany. Available from: http://www.le-vespere.org/docs/telechargements/Dietz_2005_BatsEgypt.pdf

DOBSON, G.E., 1876. *Monograph of the Asiatic Chiroptera, and catalogue of the species of bats in the collection of the Indian museum*, Calcutta. London.

FOLMER, O., BLACK, M., HOEH, W., LUTZ, R. and VRIJENHOEK, R., 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, vol. 3, no. 5, pp. 294–299. PMID:7881515.

FUHRMANN, M., SCHREIBER, C.H., and TAUCHERT, J., 2002. Telemetric Untersuchungen an Bechsteinfledermäusen ([Myotis bechsteinii] und Kleinen Abendseglern ([Nyctalus leisleri]) im Oberurseler Stadtwald und Umgebung (Hochtaunuskreis). In: A. MESCHEDE, K.-G. HELLER, and P. BOYE, eds. Ökologie, Wanderungen und Genetik von Fledermäusen in Wäldern. Untersuchungen als Grundlage für den Fledermausschutz. Bonn-Bad Godesberg: Bundesamt für Naturschutz, vol. 288. no. 1, pp. 131–140. Schriftenreihe für Landschaftspflege und Naturschutz, no. 71.

GAISLER, J., 1970. The bats (Chiroptera) collected in Afghanistan by the Czechoslovak expeditions of 1965–1967. *Acta Scientiarum Naturalium Academiae Scientiarum Bohemoslovacae Brno*, vol. 4, pp. 1–56.

HÜTZ, V., KRISTÍN, A. and URBAN, P., 2000. Bats of the Po3/4ana Mts. (Central Slovakia). Vesperitilo, vol. 4, pp. 97–104.

HUSSAIN, W., 2007. *File to medicinal study of FATA*. Khyber Pakhtunkhwa: Department of Zoology, Kohat University of Science and Technology, pp. 13–17. M. Phil Thesis in Zoology.

KANUCH, P., KRISTÍN, A. and KRÍSTOFÍK, J., 2005. Phenology, diet, and ectoparasites of Leisler’s bat (*Nyctalus leisleri*) in the Western Carpathians (Slovakia). *Acta Chiropterologica*, vol. 7, no. 2, pp. 249–257. http://dx.doi.org/10.3161/1733-5329(2005)7[249:PD...AEOL2.0.CO;2.

KUHL, H., 1819. Die deutschen Fledermause. *Annalen der Wetterauischen Gesellschaft*, vol. 4, pp. 11–49, 185–215.

MATHIAS, M.L., 1988. An annotated list of the mammals recorded from the Madeira Islands. *Boletim do Museu Municipal do Funchal*, vol. 40, no. 201, pp. 111–137.

MEYER-OEHME, D., 1965. *Die-Säugetiere Afghanistans (Teil III): Chiroptera*. Kabul, Afghanistan: Science, pp. 42–58.

NOWAK, R., 1991. *Order Chiroptera in Walker’s Mammals of the World*. Baltimore: Johns Hopkins University Press, vol. 5, no. 1, pp. 190–194.

ROBERTS, T.J. (1997). *Mammals of Pakistan*. Oxford: Oxford Univ. Press.

RUCZYŃSKI, I., and BOGDANOWICZ, W., 2005. Roost cavity selection by *Nyctalus noctula* and *N. leisleri* (Vespertilionidae, Chiroptera) in Białowieża primeval forest, Eastern Poland. *Journal of Mammalogy*, vol. 86, no. 5, pp. 921–930. http://dx.doi.org/10.1644/1545-1542(2005)86[921:RCSBNJ]2.0.CO;2.

RUCZYŃSKI, I., and RUCZYŃSKA, I., 2000. Roosting sites of Leisler’s bat *Nyctalus leisleri* in Białowieża Forest preliminary results. *Myotis*, vol. 37, pp. 55–60.
Systematic analysis of leisler's bat *Nyctalus leisleri*

SHIEL, C., DUVERGÉ, P., SMIDDY, P. and FAIRLEY, J., 1998. Analysis of the diet of Leisler’s bat (*Nyctalus leisleri*) in Ireland with some comparative analyses from England and Germany. *Journal of Zoology*, vol. 246, no. 4, pp. 417–425. http://dx.doi.org/10.1111/j.1469-7998.1998.tb00173.x.

SHIEL, C.B., SHIEL, R.E. and FAIRLEY, J.S., 1999b. Seasonal changes in the foraging behaviour (*Nyctalus leisleri*) in Ireland as revealed by radio-telemetry. *Journal of Zoology*, vol. 249, no. 3, pp. 347–358. http://dx.doi.org/10.1111/j.1469-7998.1999.tb00770.x.

SIDDIQI, M.S., 1961. Checklist of mammals of Pakistan with particular reference to the mammalian collection in the British Museum (Natural History), London. *Biologia*, vol. 7, pp. 93–225.

SIDDIQI, M.S.U., 1970. On some bats of the Oriental Region. *Records Zoological Survey of Pakistan*, vol. 2, pp. 1–9.

SPEAKMAN, J.R. and WEBB, P.I., 1993. Taxonomy, status and distribution of the Azorean bat (*Nyctalus azoreum*). *Journal of Zoology*, vol. 231, no. 1, pp. 27–38. http://dx.doi.org/10.1111/j.1469-7998.1993.tb05350.x.

SPITZENBERGER, F. and BAUER, K., 2001. Weißrandfledermaus Pipistrellus kuhlii (Kuhl, 1817). In: F. SPITZENBERGER, ed. *Die Säugetierfauna Österreichs*. Graz: Bundesministerium für Land- und Forstwirtschaft Umwelt und Wasserwirtschaft, pp. 245–248.

VAUGHAN, N., 1997. The diets of British bats (Chiroptera). *Mammal Review*, vol. 27, no. 2, pp. 77–94. http://dx.doi.org/10.1111/j.1365-2907.1997.tb00373.x.

WALKER, E.P., WARNICK, F., LANGE, K.L., UIBLE, H.E., HAMLET, S.E., DAVIS, M.A. and WRIGHT, P.E., 1964. *Mammals of the world*. Baltimore: Johns Hopkins Press, i: ixlviii, 1644, figs, 5 tables.

WALTON, D.W., 1974. New records of bats (Chiroptera) from Pakistan. *Journal of the Mammalogical Society of Japan*, vol. 6, no. 2, pp. 43–50.

WATERS, D., JONES, G. and FURLONG, M., 1999. Foraging ecology of Leisler’s bat (*Nyctalus leisleri*) at two sites in southern Britain. *Journal of Zoology*, vol. 249, no. 2, pp. 173–180. http://dx.doi.org/10.1111/j.1469-7998.1999.tb00755.x.

WOLK, E. and RUPRECHT, A.L., 1988. Haematological values in the serotine bat, *Eptesicus serotinus* (Schreber, 1774). *Acta Theriologica*, vol. 33, no. 40, pp. 545–553. http://dx.doi.org/10.4098/AT.arch.88-45.