

First record of *Vespertilio murinus* from the Arabian Peninsula

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Abstract. A specimen of *Vespertilio murinus* was captured on 13 May 2014 on the grounds of the Breeding Centre for Endangered Arabian Wildlife, Sharjah, United Arab Emirates. The species was unambiguously identified based on molecular (cytochrome *b* gene) and morphological characters. This represents the first record of *V. murinus* from the Arabian Peninsula. A revised checklist of the Vespertilionidae is presented for the Arabian Peninsula which includes 28 species belonging to 13 genera. A phylogeny for the Arabian vespertilionid species is also presented showing the paraphyly of *Eptesicus* and the position of *Nyctalus* within *Pipistrellus*.

Vespertilio murinus, first record, morphological characters, genetic identification, United Arab Emirates, Arabia

Introduction

The bats of the Arabian Peninsula were last reviewed in Harrison & Bates (1991) who reported 45 species from this region. Since this publication, several species have been added to the list of Arabian bats, including the description of at least three new species (Benda & Vallo 2009, Benda et al. 2009, 2011a, b). In the Arabian Peninsula, the family Vespertilionidae is represented by 12 genera of bats including: *Barbastella*, *Eptesicus*, *Hypsugo*, *Myotis*, *Nyctalus*, *Nycticeinops*, *Neoromicia*, *Otonycteris*, *Pipistrellus*, *Plecotus*, *Rhynoptesicus*, and *Scotophilus* (Harrison & Bates 1991, Benda et al. 2011a).

Another member of the Vespertilionidae is the genus *Vespertilio*, which is represented by two widely distributed species, *V. murinus* Linnaeus, 1758 and *V. sinensis* (Peters, 1880). The former species occurs from West Europe eastwards through Russia and the Balkans to Mongolia and north-eastern China, while the latter species occurs in China, Korea and Japan (Simmons 2005). *Vespertilio murinus* has only been recorded marginally in the Middle East from three localities in Turkey (Benda & Horáček 1998), and seven localities in western and northern Iran (Benda et al. 2012), although it occurs widely to the north (Mitchell-Jones et al. 1999). In contrast, it has not

been recorded to the south in Iraq, Syria and/or Jordan (Benda et al. 2006, 2010, Al-Sheikhly et al. 2015), or in the Arabian Peninsula (Harrison & Bates 1991). This paper reports the first record of *V. murinus* in the Arabian Peninsula.

Methods

A single male adult bat (field number: Sharjah_01) was observed on 13 May 2014 on the premises of the Breeding Centre for Endangered Arabian Wildlife, Sharjah, United Arab Emirates (25.2836° N; and 55.6977° E). The climate of the United Arab Emirates (UAE) is characterised by a bi-seasonal type climate with high temperatures and low rainfall (Hellyer & Aspinall 2005). Rainfall generally occurs in the cooler winter months from November to April. Although rainfall in the mountainous regions of the United Arab Emirates is relatively higher, rainfall can be extremely region specific and highly localised (Hellyer & Aspinall 2005). Average annual rainfall is 106.9 mm (Feulner 2006).

Sand sheets and dunes of different types cover by far the largest proportion of the UAE, ranging from vast, flat expanses to the impressive mega-dunes which are a prominent feature, especially in the south of the country (Hellyer & Aspinall 2005). The habitat surrounding the Breeding Centre for Endangered Arabian Wildlife consists mainly of sandy desert with a mixture of low dunes and high dunes with intervening sand flats, classified as “Eastern sand sheet and dunes” (Jongbloed 2003). Camel farming, goat farming, date plantations and occasional alfalfa and hay farms are found in the area (personal observations by CJ).

The bat was collected and deposited in the museum of the Breeding Centre for Endangered Arabian Wildlife, Sharjah (Sharjah_01_BCEAW). Standard museum measurements of external features were taken by CJ. Blood was stored on

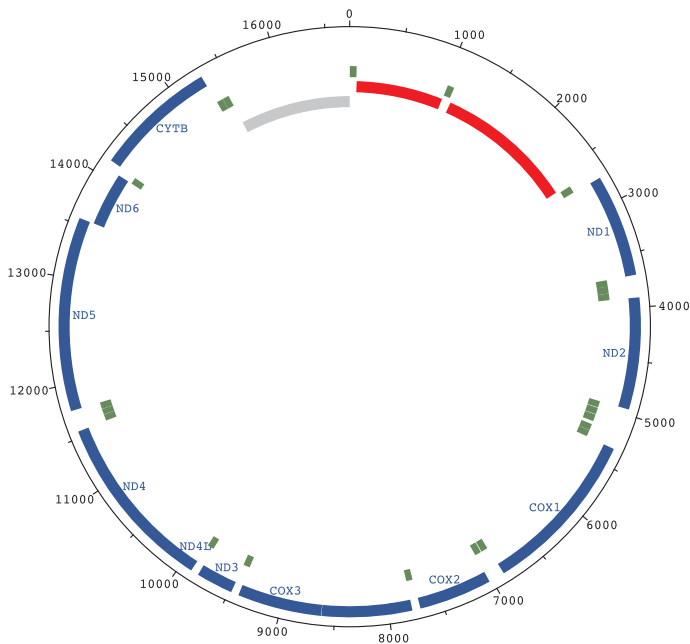


Fig. 1. Overview of the Sharjah bat specimen's mitochondrial genome. Genomic coordinates are shown on the outer circle. The location of protein-coding genes (blue), ribosomal genes (red), tRNAs (green), and the d-loop (grey) are indicated by bars.

Obr. 1. Přehled mitochondriálního genomu netopýra z Šardže. Genomické koordináty jsou umístěny při vnějším kruhu. Proužky je vyznačeno umístění genů kódujících bílkoviny (modře), transferové ribonukleové kyseliny (zeleně) a kontrolní oblast (šedě).

a Whatman FTA card, which was sent to the Natural History Museum of Denmark for DNA analyses. The specimen was loaned to the Durban Natural Science Museum where the skull was extracted and standard cranial and dental measurements taken by AM.

Bat DNA was extracted from the Whatman FTA card using the Qiagen DNA Investigator Kit (Valencia, CA, USA) following the protocol (Handbook April 2010) for isolation of total DNA from FTA and Guthrie cards although omitting opening the lid in step 16. The DNA extract was enriched for genomic mitochondrial DNA (Hansen et al. 2015). In brief, the DNA was incubated with 15 U of Plasmid-Safe™ ATP-dependent DNase (Epicentre) in the presence of 112.5 nmol ATP (3 hours at 37°C and 30 minutes at 70°C) in order to digest linear dsDNA. The remaining DNA was amplified using the REPLI-g® Midi kit (Qiagen) for 16 hours at 30°C and 3 min at 65°C according to the manufacturers' instructions. A Bioruptor® NGS (Diagenode) was used to fragment 2 µg of DNA to an average length of 350 bp, as verified by running the samples on a High Sensitivity chip on the Agilent 2100 Bioanalyzer instrument. The NEBnext® DNA Library Prep Master Mix Set for 454 kit (New England Biolabs) was used to create an Illumina sequencing library with a modified protocol with blunt end Illumina adapters (Meyer & Kircher 2010).

The library was sequenced on an Illumina HiSeq 2000 platform, yielding 19,703,126 2×100 paired-end sequence reads. Removal of adapters, quality trimming and merging of paired reads were carried out using AdapterRemoval (Lindgreen 2012). Reads were assembled using The ORGanelle ASSEMBler (<http://pythonhosted.org/ORG.asm/index.html>), SPAdes (Bankevich et al. 2012), SGA (Simpson and Durbin 2010), and Ray Meta (Boisvert et al. 2012). Assembly was attempted using all sequence reads as well as subsets thereof. None of these assemblers produced contigs or scaffolds exceeding 10 kbp. Guided by similarity to the mitochondria of the Japanese house bat, *Pipistrellus abramus* (accession NC_005436), a selection of long scaffolds from the different assemblers could be manually assembled to a 16,721 bp long mitochondrial genome. Coverage across the mitochondrial genome was determined by mapping all sequence reads back onto the consensus sequence using BWA (Li and Durbin 2010). The sequence was annotated using the DOGMA web service (<http://dogma.cccb.utexas.edu/>) (Wyman et al. 2004) and visualised by DNAPlotter (Carver et al. 2009). The assembled mitochondrial genome, which we refer to as “Sharjah” hereafter, was uploaded to Genbank Accession No. KU161588. Sequence reads have been deposited at the European Nucleotide Archive, accession no. PRJEB11821.

Neighbor-joining phylogenetic tree was constructed using ClustalW (Thompson et al. 1994, Larkin et al. 2007) with bat cytochrome *b* sequences obtained from the Genbank (<http://www.ncbi.nlm.nih.gov/>). Species and accession numbers of bats used in the phylogenetic analysis: *Barbastella leucomelas*, EF534766; *Rhyneptesicus nasutus*, EU786840; *Eptesicus serotinus*, KF111725; *Hypsugo savii*, AJ504450; *Myotis myotis*, AF376860; *Myotis blythii*, AF376840; *Myotis nattereri*, AB106606; *Myotis emarginatus*, AF376849; *Myotis aurascens*, DQ182698; *Myotis capaccinii*, AF376845; *Nyctalus noctula*, JX570902; *Nycticeinops schlieffenii*, JX276308; *Neoromicia guineensis*, KF019055; *Otonycteris hemprichii*, HM030844; *Pipistrellus pipistrellus*, AJ504443; *Pipistrellus kuhlii*, AJ504445; *Pipistrellus rueppellii*, JX276316; *Plecotus christii*, EU743799; *Plecotus balensis*, AF513799; *Plecotus macrobullaris*, NC_027977; *Scotophilus leucogaster*, JX281748; *Vespertilio murinus*, AB287358; *Vespertilio sinensis*, KJ081440; *Vespertilio superans* [= *sinensis*], AB085738.

Results

Sequencing and the following assembly yielded a 16,721 bp mitochondrial genome containing the expected repertoire of genes (Fig. 1). Mapping individual reads onto the mitochondrial genome revealed an average coverage of 811×. Coverage was continuous across the entire sequence and did not drop below 100× coverage at any position (not shown). Initial analysis of the mitochondrial genome revealed that the sequence displayed high levels of similarity to the mitochondria of *Vespertilio* species. We therefore constructed a phylogeny based on the cytochrome *b* gene from available sequences from the Arabian Vespertilionidae family and other *Vespertilio* specimens. With strong support, the novel sequence clustered with the *Vespertilio* sequences, showing high similarity to *Vespertilio murinus* (Fig. 2). The size of the d-loop varies greatly in bats (Meganathan et al. 2012), and the d-loop of our assembled genome is highly repetitive. This is particularly evident when aligning part of the d-loop against a haplotype previously described in Swiss populations of *Vespertilio murinus* (Safi et al. 2007) (Fig. 3). The 331 bp long Sharjah sequence differs from the published haplotype at 18 positions, but these differences are very unevenly distributed and all reside in the first repeated structure (Fig. 3). This may indicate that our assembly of the repetitive bat d-loop is incorrect and that elucidating the correct sequence is not feasible using next-generation sequencing reads with short insert sizes.

Table 1. Measurements of the *Vespertilio murinus* specimen collected in the United Arab Emirates (UAE), with comparative measurements (mean, range and sample size) from Iran (taken from Benda et al. 2012)
 Tab. 1. Rozměry jedince *Vespertilio murinus* dokladovaného ve Spojených arabských emirátech (UAE) v porovnání s rozměry (průměr, rozmezí hodnot a počet jedinců) jedinců z Iranu (podle Bendy et al. 2012)

measurement / rozměr [mm]	<i>Vespertilio murinus</i> (UAE)	<i>Vespertilio murinus</i> (Iran)
<i>external / vnější</i>		
head-body length / délka těla	61	62.7 (61–64, n=3)
tail length / délka ocasu	40	45.3 (42–47, n=3)
forearm length / délka předloktí	42.5	43.6 (42.2–44.5, n=4)
ear length / délka ušního boltce	13.8	18.1 (17.4–18.8, n=3)
3rd digit length / délka třetího prstu křídla	75.8	
5th digit length / délka pátého prstu křídla	52.9	
<i>skull / lebeční</i>		
greatest skull length / délka lebky	16.1	14.9 (14.7–15.2, n=4)
condylo-canine length / kondylokaninová délka	15.3	
zygomatic width / zygomatická šíře	10.3	9.6 (9.4–9.7, n=4)
width of interorbital constriction / meziočnicová šíře	4.2	4.2 (4.2–4.3, n=4)
mastoid width / mastoidální šíře	9.3	8.8 (8.5–9.2, n=4)
width of braincase / šíře mozkovny	8.6	7.7 (7.4–8.1, n=4)
height of neurocranium / výška mozkovny	5.8	5.1 (5.1–5.2, n=4)
length of mandible / délka spodní čelisti	11.6	11.0 (10.7–11.1, n=4)
<i>dental / zubní</i>		
length of upper toothrow / délka horní zubní řady	5.3	5.3 (5.2–5.5, n=4)
width across upper canines / šíře přes horní špičáky	5.0	5.2 (5.1–5.3, n=4)
width across upper molars / šíře přes horní stoličky	6.5	6.4 (6.2–6.5, n=4)
length of lower toothrow / délka dolní zubní řady	5.8	5.6 (5.6–5.8, n=4)

The results of the molecular and haplogroup analysis were supported by morphological observations and measurements. The shape of the ears, tragus and characteristically frosted pelage with silver-whitish tips on the head and dorsum as well as the contrasting black-and-white coloration of the dorsum and belly (Fig. 4) are consistent with this species (Dietz et al. 2009). The skull is robust with a broad rostrum and a relatively flattened braincase in lateral profile (Fig. 5). In dorsal view, there is a deep V-shaped notch in the nasal region. The two upper incisors are visible in the ventral view (Fig. 5), with the outer incisor smaller than the inner one. The anterior upper premolar is absent and the posterior upper premolar is in contact with the canine (Fig. 5). The external, dental and cranial measurements of the specimen are presented in Table 1, and fall within the limits of *Vespertilio murinus*.

Discussion

We generated the full mtDNA genome for the Sharjah individual. However, due to limited full mtDNA genomes from related bat species the phylogenetic analysis is based on the cytochrome *b* sequence of a selection of bat species. This phylogenetic analysis supports the finding that the individual is a member of the *Vespertilio* group, as it is embedded in a monophyletic *Vespertilio* group with all relevant branches having maximum bootstrap support (1000). Hence, from a genetic point of view, the individual is unambiguously identified as *Vespertilio murinus*. Further analysis of the d-loop haplotype shows a high similarity to haplogroup 15, but also reveals multiple varia-

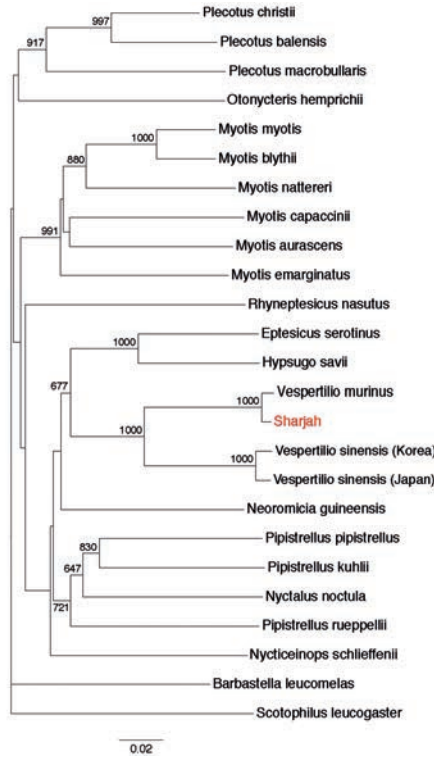


Fig. 2. Neighbor-joining tree of bat cytochrome *b* gene sequences. The Sharjah sequence is highlighted in red. Branches with bootstrap support values (1000 replicates) above 500 are indicated on the tree. Bar below tree denotes genetic distance.

Obr. 2. Dendrogram vytvořený metodou spojování sousedních objektů sekvencí mitochondriálního genu pro cytochrom *b*. Sekvence netopýra ze Šardže je zabarvena červeně. Větvě s hodnotami bootsrapu vyššími než 500 jsou v dendrogramu uvedeny (1000 replikací). Příčka pod dendrogramem označuje genetickou vzdálenost.

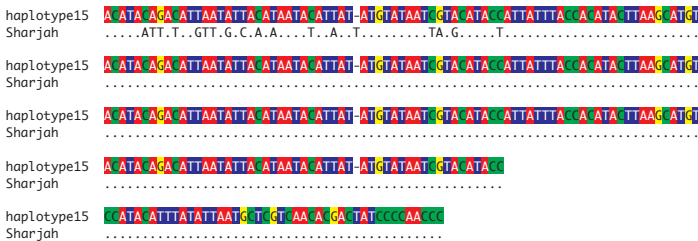


Fig. 3. Alignment of partial d-loop sequences from the Sharjah genome and haplotype 15 from Safi et al. (2007), GenBank accession: DQ162752. Residues are colored and adjusted vertically to highlight the repetitive nature of the sequence.

Obr. 3. Porovnání části kontrolní oblasti genu netopýra z Šardže a haplotypu 15 podle Safiho et al. (2015), přístupové číslo GenBank DQ162752. Jednotlivé base jsou značeny barevně a svisle uspořádány pro ukázení opakujících se částí genu. Base genu netopýra z Šardže, shodné s haplotypem 15 jsou označeny body.



Fig. 4. Photograph of the *Vespertilio murinus* specimen collected in the United Arab Emirates, showing: the characteristic silver-whitish tips to hairs on the head and dorsum giving a frosted effect (left); and the face, ears and broad short tragus (right).

Obr. 4. Fotografie jedince *Vespertilio murinus* dokladovaného ve Spojených arabských emirátech; typické stříbrobílé zbarvení vrcholků chlupů na hlavě a hřbetě dávající dojem ožinění (vlevo); tmavý obličej a ušní boltce, jakož i krátký tragus (vpravo).

tions in the repeated part of the assembled d-loop sequence of the Sharjah mtDNA genome. These variations are probably caused by incorrect assembly, due to the repeated nature of the d-loop sequence, which also is observed in other bat species (Meganathan et al. 2012).

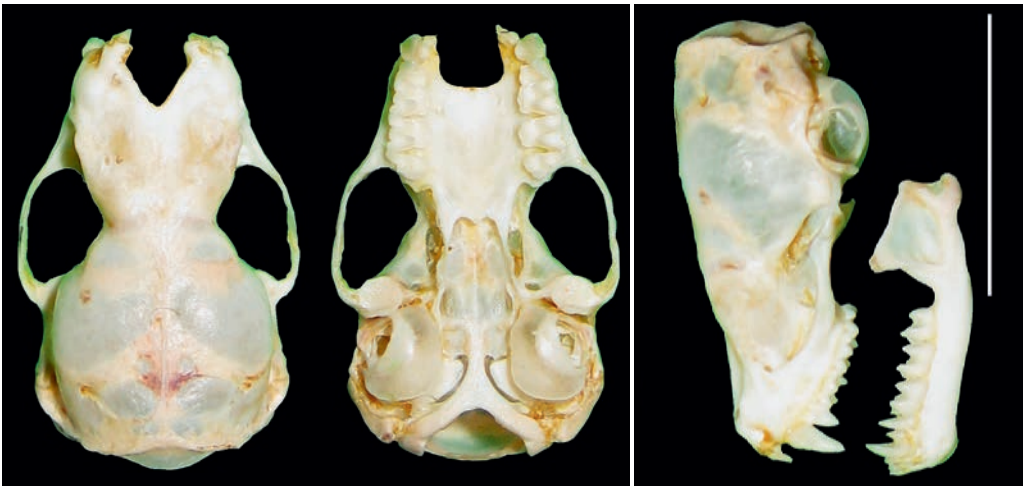


Fig. 5. Images of the skull of the *Vespertilio murinus* specimen collected in the United Arab Emirates in dorsal view (left), ventral view (middle) and lateral view (right). The bar on the right is 10 mm in length.

Obr. 5. Vyobrazení lebky jedince *Vespertilio murinus* dokladovaného ve Spojených arabských emirátech; hřbetní pohled (vlevo), břišní pohled (vprostřed) a stranový pohled (vpravo). Přímka vpravo značí délku 10 mm.

Table 2. Updated checklist of Vespertilionidae bat species recorded from the Arabian Peninsula
 Tab. 2. Aktualisovaný soupis druhů čeledi Vespertilionidae zaznamenaných z Arabského poloostrova

genus / rod	species / druh	authority / autor
<i>Barbastella</i>	<i>leucomelas</i>	Harrison 1964
<i>Eptesicus</i>	<i>bottae</i>	Harrison 1964
<i>Eptesicus</i>	<i>anatolicus</i>	Benda et al. 2006
<i>Eptesicus</i>	<i>serotinus</i>	Harrison 1964
<i>Hypsugo</i>	<i>arabicus</i>	Harrison 1979
<i>Hypsugo</i>	<i>ariel</i>	Harrison 1964
<i>Hypsugo</i>	<i>lanzai</i>	Benda et al. 2011a
<i>Hypsugo</i>	<i>savii</i>	Harrison 1964
<i>Myotis</i>	<i>myotis</i>	Harrison 1964
<i>Myotis</i>	<i>blythii</i>	Harrison 1964
<i>Myotis</i>	<i>nattereri</i>	Harrison 1964
<i>Myotis</i>	<i>emarginatus</i>	Harrison 1964
<i>Myotis</i>	<i>aurascens</i>	Benda et al. 2006
<i>Myotis</i>	<i>capaccinii</i>	Harrison 1964
<i>Nyctalus</i>	<i>noctula</i>	Harrison 1964
<i>Nycticeinops</i>	<i>schlieffenii</i>	Harrison 1964
<i>Neoromicia</i>	<i>guineensis</i>	Benda et al. 2011a
<i>Otonycteris</i>	<i>hemprichii</i>	Harrison 1964
<i>Pipistrellus</i>	<i>pipistrellus</i>	Harrison 1964
<i>Pipistrellus</i>	<i>kuhlii</i>	Harrison 1964
<i>Pipistrellus</i>	<i>rueppellii</i>	Harrison 1964
<i>Plecotus</i>	<i>christii</i>	Harrison 1964
<i>Plecotus</i>	cf. <i>balensis</i>	Benda et al. 2011a
<i>Plecous</i>	<i>macrobullaris</i>	Benda et al. 2006
<i>Rhynptesicus</i>	<i>nasutus</i>	Harrison 1964
<i>Scotophilus</i>	<i>colias</i>	Vallo et al. 2011
<i>Scotophilus</i>	<i>leucogaster</i>	Harrison 1964
<i>Vespertilio</i>	<i>murinus</i>	this paper

This is the first record of *Vespertilio murinus* on the Arabian Peninsula and is 900 km from the nearest record in Sar Dasht near Lordegan, Iran (Fig. 6) (Benda et al. 2012). The Sharjah record is separated from the Iranian locality by the Persian Gulf, which presumably does not form a barrier for this migratory species. *Vespertilio murinus* has been infrequently recorded on offshore platforms in the North Sea more than 80 km from land (Boshamer & Bekker 2008), suggesting that this species is capable of some movement across open seas. If, for the sake of comparison, we assume that *Vespertilio murinus* followed an overland route, then the nearest distance between these two points is almost 1,700 km. Furthermore, the possibility of a ship-assisted crossing cannot be ruled out. This is not the first record of a Palearctic bat species in the Arabian Peninsula. The record of *Nyctalus noctula*, also a migratory species, from the Masira island, Oman (Harrison & Jennings 1980, Harrison & Bates 1991) suggests that such isolated records may in fact be more common than currently assumed.

This record of *V. murinus* on the Arabian Peninsula raises the question as to whether the species has been overlooked in other parts of Arabia. *Vespertilio murinus* is a migratory species and all the records from Turkey are suspected to represent bats on migration (Benda & Horáček 1998). It is not known if the species breeds in Iran, but presumably not since they fall on the same line of latitude as those in Turkey. This suggests that the Sharjah record represents a vagrant bat,



Fig. 6. Map showing (top) the global distribution of *Vespertilio murinus* (IUCN 2015) (shaded grey) with the Sharjah record (black dot); and (below) the localisation of the Sharjah record in relation to those from Iran (based on Benda et al. 2012) (white dots), which represent the closest records to Sharjah.

Obr. 6. Mapka ukazující (nahoře) celosvětový areál *Vespertilio murinus* (IUCN 2015) (šedá plocha) s vyznačeným nálezem ze Šardže (plný kroužek) a (dole) umístění nálezu z Šardže ve vztahu k nálezům v Iranu (podle Benda et al. 2012) (prázdné kroužky), jež jsou nejbližšími nálezmi lokality v Šardže.

perhaps “overshooting” its normal migratory stopping point; or having been carried further by strong winds, as happens frequently in migratory birds (Burger et al. 1980).

This record brings the total number of Vespertilionidae genera known for the Arabian Peninsula up to 13. A revised checklist of Vespertilionidae species from the Arabian Peninsula is presented in Table 2, which includes 28 species. Although the general resolution of phylogeny (Fig. 2) is low and only a few branches exhibit high bootstrap support, it nevertheless raises two interesting additional observations: (1) the paraphyly of the genus *Eptesicus* (as *E. serotinus* forms a highly supported clade with *Hypsugo savii*); and (2) the position of *Nyctalus* embedded within *Pipistrellus*, albeit with very moderate support. The paraphyly of *Eptesicus* was recently shown by Juste et al. (2013), with the species *nasutus* being assigned to a separate genus *Rhyneptesicus*. Our results are consistent with this conclusion. The position of *Nyctalus* within *Pipistrellus* has also been reported before (Lack & van den Bussche 2010, Roehrs et al. 2010).

Souhrn

První nález netopýra pestrého (*Vespertilio murinus*) z Arabského poloostrova. Na pozemku Chovného centra ohožených zvířat Arabie v Šardže ve Spojených arabských emirátech byl 13. května 2014 odchycen jedinec netopýra pestrého (*Vespertilio murinus*). Druh byl nepochybně určen za pomoci molekulárně genetické analýsy (genu pro cytochrom *b*) a studia morfologických znaků. Nález představuje první záznam tohoto druhu z Arabského poloostrova. Předložen je také revidovaný soupis netopýrů čeledi Vespertilionidae z Arabského poloostrova, který nyní zahrnuje 28 druhů náležejících 13 rodům. Fylogenetická analýza arabských druhů této čeledi znovu potvrdila parafylii rodu *Eptesicus* a posici rodu *Nyctalus* uvnitř rodu *Pipistrellus*.

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