ORIGINAL ARTICLE

Recently Discovered Iranian Population of *Rousettus leschenaultii* (Chiroptera; Petropodidae), Highlighting the Essential Need for Taxonomic Expertise in Applied Medical Sciences

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Abstract

Background: Bats are potential reservoir hosts for vector-borne diseases that can transmit many bacterial and viral agents to humans. Fruit bats are considered reservoirs of many viruses and they are of major importance in the medical sciences for controlling and preventing vector-borne diseases. This study aimed to compare the morphological and genetic differences of two fruit bat species, *Rousettus aegyptiacus* and *R. leschenaultii* in Iran, implying anessential need for taxonomic expertise in applied medical sciences.

Methods: In this experimental study, we collected and identified 26 specimens of bats belonging to two fruit bat species from the southeast of Iran. The morphological and genetic differences were characterized between recently recorded migratory fruit bat *R. leschenaultii* and the morphologically similar and resident *R. aegyptiacus*. The haplotype network was generated using the program POPART. The nucleotide variation and population differentiation were calculated in DNAsp. Furthermore, we considered additional records of 15 specimens from three widespread bat species in the north of Iran.

Results: Morphological characterization of two fruit bat species showed *R. aegyptiacus* is larger than *R. leschenaultii* in morphometric variables except tail length (TL) for both sexes. The demographic analyses revealed that all haplotypes of *R. leschenaulti* formed a single haplogroup in the species' distributional range. In contrast, haplotypes of *R. aegyptiacus* were divided into five haplogroups respecting taxa/localities. The results represent a strong signature of population expansion for these two species of bats.

Conclusion: Population of the fruit bats throughout their ranges in the Middle East may indicate an increased risk of the spread of viral agents over time. Therefore, precise identification of hosts and vectors in applied medical sciences is essential to conduct standard procedures.

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Introduction

Taxonomic studies are sometimes an inevitable field of study in medical investigations (e.g., identification of vectors, reservoirs, and hosts) although identifying species in many cases is cumbersome and requires proficiency. Bats (order Chiroptera) are the second most species of mammals and have been considered vectors and reservoirs for many zoonotic diseases. *Rousettus aegyptiacus* and *R. leschenaultii* are two morphologically similar megabats in the family Petropodidae, which are considered the primary hosts for henipaviruses, and filoviruses, which cause fatal diseases in humans.[1, 2]

Egyptian fruit bat *R. aegyptiacus* is one of the most widespread bats in the southern part of Iran.[3, 4] The very recent discovery of *R. leschenaultii* in Chabahar, southeast Iran, by Khajeh et al.[5] accentuates the importance of correct taxonomic assessments for accurate medical appraisals. This species appears to have been overlooked due to its similarity to *R. aegyptiacus*. Furthermore, unlike *R. aegyptiacus,* which is essentially non-migrant, *R. leschenaultii* is a migratory bat; therefore, it may increase concerns about the spread of infectious agents from the Indian sub-continent to the southeastern Iran.[4, 6] *Rousettus leschenaultii* is also a reservoir of different viruses such as *Betacoronavirus* (including MERS-CoV, SARS-CoV1, and SARS-CoV-2) and parainfluenza virus.[7-10] Detection of Chikungunya virus and *Ebolavirus* in *R. leschenaultii*[11, 12] and spread of Chikungunya virus in 2016 to 2017 throughout Pakistan[13] and poor coverage of public health services in some parts of Pakistan and India, from where seropositive and suspected cases of infection to *Ebolavirus* were detected,[14] increase the essential need for monitoring of *R. leschenaultii* and *R. aegyptiacus* populations in the southeast Iran by the health research services. Consequently, taxonomic studies are an integral component in medical investigations of the identification of vectors, reservoirs and hosts, although identifying wild species in many cases is a complicated process and needs proficiency.

The discovery of the migratory *R. leschenaultii* in Iran highlights the problem that satisfactory identification keys are not available for many taxa. Furthermore, in some cases, only certain forms of the species (males or females), or only certain age groups (larvae, juvenile, or adult), may be described in dichotomous identification keys. If such identification keys are created to cater for local demands, they may be based only on species that are already known to occur in the particular localities and would thus lack detection power for new species. Similarly, they would be unsatisfactory if used in other regions. Another potential problem is the character states described in dichotomous identification keys.

In many cases, these characters may have been selected by comparison with museum specimens and lack features that are only applicable to live specimens. Moreover, intra-species frequency in character states causes overlap between species in certain characters, making identifications more problematic in many cases. Additionally, taxonomy is a dynamic science and applying different species concepts, especially the phylogenetic approach for understanding the identity has caused many species to be revised in recent years. All these potential complications may result in species' existence in a specific region remaining undetected. This event is an important caveat, as if hosts or vectors are misidentified or taxonomy is changed, so downstream research may be misled. For example, *Trypanosoma grosi*[15] and the flea *Leptopsylla taschenbergi taschenbergi*[16] were reported to be hosted by *Apodemus sylvaticus* sampled in Iran.[17-19] However, due to taxonomic reassessment, *A. sylvaticus* is no longer considered part of the fauna of Iran, but instead *A. witherbyi* was mentioned in the checklist of mammlas from Iran.[20-22] Therefore, either the range of the parasites or the identity of their host species might be misunderstood, demonstrating that updated taxonomic knowledge is essential for a better understanding of the biotic factors affecting human public health.

Phylogenetic studies have rendered an effective diagnostic method for medical investigations and a reliable tool to cross-examine the relationship between hosts and infection agents. For example, Hassanin et al.[23] investigated circulating hosts of ebolavirus applying comparative phylogeny and phylogeography of fruit bats in West Africa. The similarity between severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) to Bat/Yunnan/RaTG13 CoV has also been detected by phylogenetic studies.[24] Nesi et al.[25] carried out a resolved phylogeny of the threatened Old World fruit bats applying coalescent-based methods.

Considering that *R. leschenaultii* migrates annually back and forth between Iran and India and also this species is a potential reservoir of several viruses, such as ebolavirus, coronaviruses, and flaviviruses, we assessed morphological and population genetic differences between this species and the morphologically similar *R. aegyptiacus* to provide diagnostic tools for future medical research. Furthermore, the hypothesis of the range expansion of these two species of fruit bats mentioned by Chen et al.[26] and Stribna et al.[27] was also examined to predict the long-term dynamic of bat-borne pathogens in response to climate change based on epidemiological triad. We also refer to genetic and morphological differences between these two species of megabats from Iran as they are a potential reservoir of the coronavirus. Moreover, we reported additional records of three species of bats, *Pipistrellus kuhlii*, *Myotis blythii*,and *Rhinolophus hipposideros* from the north of Iran, considering their potential role in transmission of different viral agents to provide morphological information about some bat species for future medical research.

Methods

Sampling and Morphometric Measurements

41 individuals of the genera *Rousettus* (26 specimens), *Pipistrellus* (4 specimens), *Myotis* (5 specimens), and *Rhinolophus* (6 specimens) were collected using mist-nets during field trips from seven localities in Iran (Table 1 and Figure 1). The sex of the specimens was registered, and six external and two cranial measurements of the specimens were measured using a vernier caliper, accurate to the nearest 0.05 mm. The abbreviations of morphometric variables are as follows: Head and body length (HBL to tail), Head and body length (BL to anus), Ear length (EL), Ear width (EW), Foot length (FL), Tail length (TL), Forearm length (FAL), the greatest length of the skull (GL), and Condylobasal length of the skull (CBL). A descriptive analysis was performed in R 3.5.2 (R Core Team[28]). The bat species were identified using morphological identification keys.[29-31] Laboratory procedures were accomplished following the ethical protocols in the Ethics Committee on Animal Research, Golestan University of Medical Sciences (code: IR.GOUMS.REC.1399.072).

Demographic Analyses

To evaluate the potential risk of expansion of megabat populations and the zoonoses that serve as reservoirs, we used the dataset from our previous paper in Khajeh et al.[5] consisting of 10 cytochrome *b* (cytb) sequences (Accession numbers: MW250906 to MW250915) of the genus *Rousettus* together with the cytb dataset from GenBank (74 sequences). The 1098 base pairs (bp) of the cytb dataset and 84 sequences were used for population genetic analyses. The unrooted median-joining haplotype network was generated using the program POPART ver. 1.7[32] using equal weights for all mutations. The descriptive statistics of nucleotide variation and population differentiation, including numbers of haplotypes (H), haplotype diversity (h), and nucleotide diversity (p) were calculated in DNAsp 5.0[33] for each lineage. Fu's Fs,[34] Tajima's D,[35] and R2[36] were calculated in DNAsp using 1000 simulated replicates for each lineage to test significance and identify some of the

Table 1: Number of sampled individuals from Iran according to species and localities.

Locality	NO. on map	Species				
		R. aegyptiacus	***R. leschenaultii***	***P. kuhlii***	***M. blythii***	***R. hipposideros***
Ghasr Ghand, Sistan & Baluchestan	6	16				
Anbarabad, Kerman	5	6				
Chabahar, Sistan & Baluchestan	7		4			
Gorgan, Golestan	3			2		
Sari, Mazandaran	2			2		
Galikesh, Golestan	4				5	
Masal, Guilan	1					6
		n=22	n=4	n=4	n=5	n=6

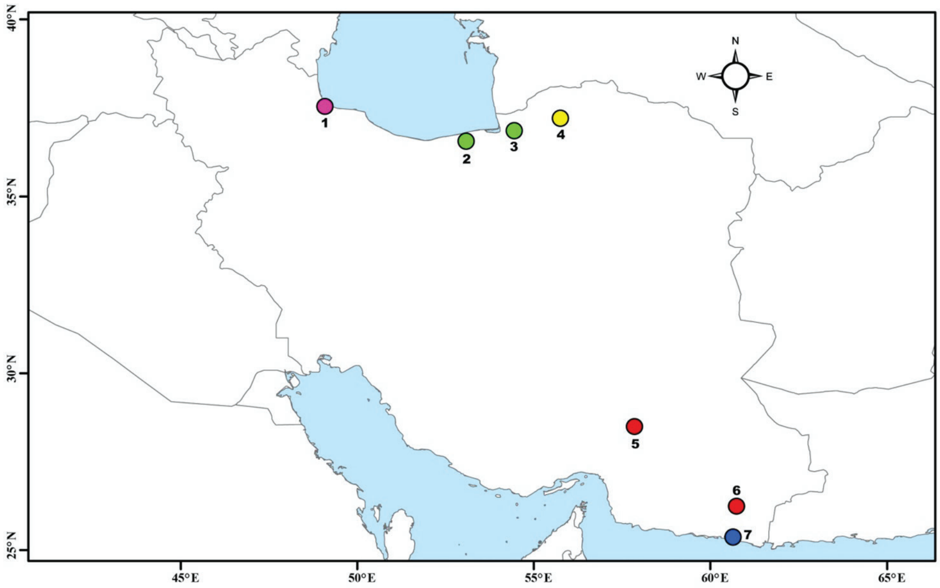

Figure 1: The map shows the sampling points of the five bat species (*Roussetus aegyptiacus* (NO: 5 & 6 on map), *R. leschenaultii* (NO: 7), *Myotis blythii* (NO: 4), *Rhinolophus hipposideros* (NO: 1), and *Pipistrellus kuhlii* (NO: 2 & 3)). For details of the localities and numbers, see Table 1.

demographic evolutionary processes associated with genetic diversity.

Results

41 bats from seven localities in Iran were morphologically characterized, including Pteropodidae; *R. aegyptiacus* (22 specimens), and *R. leschenaultii* (4 specimens), Rhinolophidae: *Rhinolophus hipposideros* (6 specimens), and Vespertilionidae; *Pipistrellus kuhlii* (4 specimens) *Myotis blythii* (5 specimens). Besides, *R. aegyptiacus* and *R. leschenaultii* were genetically studied due to the cryptic diversity reported in southeast Iran.

Morphological Identification

The most prominent characteristics of the family members Pteropodidae are dog-like head; absence of nose leaf, and simple ear pinna without tragus and anti-tragus. The genus *Rousettus* tail is longer than 10 mm and separates wing membranes. This difference is the most obvious one between the genus *Rousettus* and other megabats distributed in the Middle East. Forearm longer than 75 mm (Table 2). Ears without any white margins. *Rousettus leschenaultii* is the new record from Iran as a reservoir of viruses from Chabahar, south of Sistan and Baluchestan province, where is sympatric with the other reservoir of the virus *R. aegyptiacus*. The forearm is shorter in *R. leschenaultii* compared to *R. aegyptiacus* (Table 2). The tips of the ears are conical and not attenuated, pollex and wings are shorter than those in *R. aegyptiacus* collected in Sistan and Baluchestan. The snout is well-haired, slender, and shorter than that in *R. aegyptiacus*. In general, *R. aegyptiacus* is larger than *R. leschenaultii* in morphometric variables except for both sexes' tail length (TL) (Table 2). External measurements in *R. aegyptiacus*are FAL: 83-90 mm; TL: 14-20 mm; CBL: 36.9-40.4 mm whereas in *R. leschenaultii* are FAL: 75-86 mm; TL: 8-21 mm, and CBL: 33.5-37.7 mm (Table 2).

Table 2: The descriptive statistics including mean and standard deviation for six external and two cranial characters in five bat species from Iran. Parameters include Head and body length (HBL), Snout to anus length (BL), Ear length (EL), Foot length (FL), Tail length (TL), Forearm length (FAL), the greatest length of the skull (GL), Condylobasal length (CBL), and Number (n).

Species	*R. aegyptiacus* **(n=22)**		*R. leschenaultii* **(n=4)**		*P. kuhlii***(n=4)**		*M. blythii* **(n=5)**	*R. hipposideros* **(n=6)**	
Sex	**Female (n=16)**	**Male (n=6)**	**Female (n=2)**	**Male (n=2)**	**Female (n=1)**	**Male (n=3)**	**Male (n=5)**	**Female (n=5)**	**Male (n=1)**
Character	**Mean±SD**	**Mean±SD**	**Mean±SD**	**Mean±SD**	**Mean**	**Mean±SD**	**Mean±SD**	**Mean±SD**	**Mean±SD**
HBL	130.4±10.6	139.3±8.7	121.0±2.8	129.0±5.7	77.2	76.6±1.5	120±5.1	65.5±2.1	67.0
BL	118.9±8.4	131.3±7.9	113.5±6.4	116.5±2.1	45.0	44.7±3.0	69.2±2.3	41.5±2.1	42
EL	22.1±0.9	22.5±1.2	20.0±0.0	20.0±0.0	10	9.7±0.5	22.8±0.5	15±0	13
FL	39.8±2.8	40.7±3.6	37.0±1.4	38.0±1.4	5.5	6.2±0.8	10.6±0.9	7±0.0	7
TL	13.8±1.8	13.3±2.7	16.0±0.0	16.5±0.7	32	33.0±1	53.2±6.7	24±2.3	25
FAL	87.8±3.1	90.5±2.3	76.5±0.7	80.5±3.5	35	36.7±2.1	57.8±0.5	37.5±0.7	36
GL	40.3±0.9	40.9±0.5	36.1±0.6	37.6±0.4	14.12	-	22.74±	-	-
CBL	38.8±0.6	39.5±0.7	35.2±0.6	35.4±0.4	13.62	-	21.4±	-	-

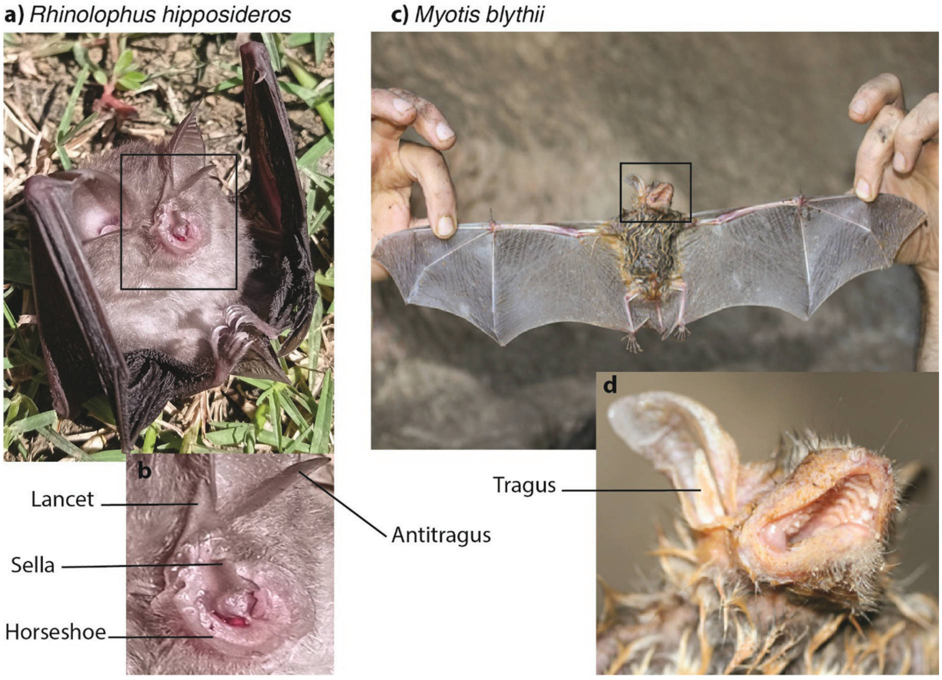

Figure 2: Morphological characters of *Rhinolophus hipposideros* (a,b) and *Myotis blythii*(c,d).

The Family Rhinolophidae is represented by a complex horseshoe-shaped nose leaf, and tragus is missing; however, the antitragal lobe is distinct (Figure 2a). The tail is enclosed within the interfemoral membrane and the triangular nose leaf consists of sella between the nostrils. Rhinolophid bats are characterized by pointed lancet raising near or above the forehead. The genus *Rhinolophus* nose leaf is complex and sella located above nostrils with a single triangular process (Figure 2a, b). The Lesser Horseshoe Bat *Rh. hipposideros* is the smallest horseshoe bat in Iran (mean value of forearm (FAL) is less than 43 mm, and condylobasal length (CBL) is less than 16 mm (Table 2). Sella lacks lateral basal lappets, and the tip of the connecting process is rounded and pointed more or less downwards (FAL: 35.9-40 mm).

The family Vespertilionidae in Iran is characterized by the absence of a nose leaf (Figure 2c). The muzzle has no dermal ridge. The tragus is present. Phalanges are reduced on the second digit of the wing and lack claws. The second phalanx of the third finger is only slightly longer than the first one. Vespertilionid bats have a well-developed interfemoral membrane by which the tail is completely enclosed (Figure 2c). In the genus *Myotis,* the mean value of the forearm is 30–62.1 mm. It is 50.5–62.1 mm in *M. blythii* (Table 2). The diagnostic character of the species is a long narrow tragus reaching half of the ear length (Figure 2d). In *Pipistrellus kuhlii*, the last tail vertebrae projected 1-2 mm beyond the tail membrane, and the forearm is 30.3-37.1 mm (Table 2).

Genetic Comparison and Demographic Structure

Rousettus leschenaultii and *R. aegyptiacus* are sibling species and may be morphologically difficult to differentiate. The unrooted median-joining network based on cytb including 84 samples (10 samples this study+74 samples from GenBank) supported the identification of five haplogroups (A1 to A5) in the species range of *R. aegyptiacus* and one haplogroup in *R. leschenaultii* (Figure 3). Additionally, we detected higher levels of nucleotide diversity for *R. aegyptiacus* (0.024) compared to *R. leschenaultii* (0.008; Table 3). It is noteworthy that demographic statistics revealed a strong signature of population expansion of *R. leschenaultii* throughout its range, which can be

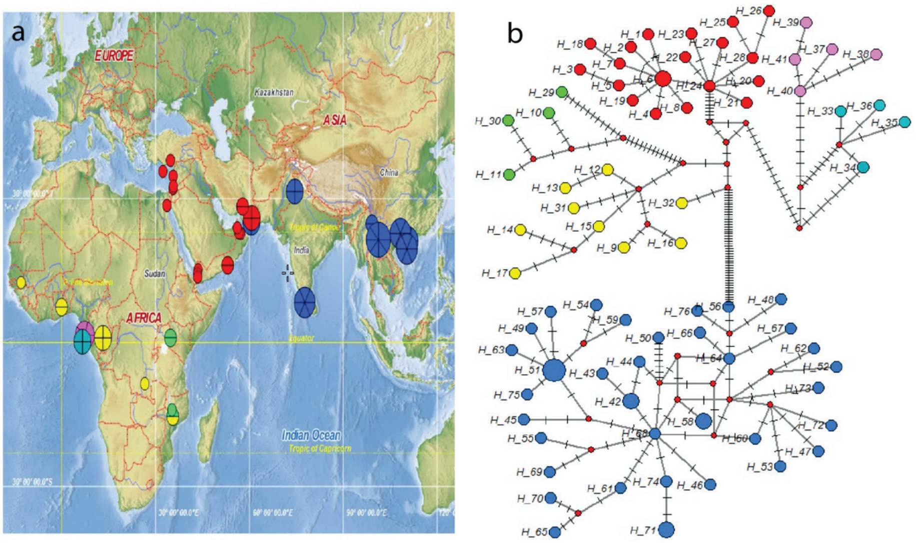

Figure 3: (a) The mtDNA diversity (cyt *b*) for 84 individuals of the genus *Rousettus* (42 *R. aegyptiacus* and 42 *R. leschenaultii*) placed on an elevation map. The pie chart's color and subdivision correspond to the lineages and number of haplotypes. (b) Median-joining haplotype network based on cyt *b* marker for *R. aegyptiacus* and *R. leschenaultii*. The size of the circle indicates the relative abundance of haplotype. Six lineages of the genus *Rousettus* are marked in different colors: *R. aegyptiacus*; Red: haplogroup A1, Yellow: haplogroup A2, Green: haplogroup A3, Purple:haplogroup A4, Turquoise: haplogroup A5, and Sky Blue: *R. leschenaultii*. See also Table 3 for related information.

Table 3: Genetic diversity of *R. aegyptiacus* and *R. leschenaultii* based on cyt *b* (84 individuals). Statistical parameters indicate the number of samples (N), number of haplotypes (nH), haplotype diversity (Hd±SD), nucleotide diversity (π±SD), singleton variable sites (S), number of variable sites (V), parsimony informative sites (P), Tajima's D, Fu's Fs, and R2 (Ramos-Onsin and Rozas' statistic). Pvalues higher than 0.05 are in bold.

Taxa/Group	N	nH	Hd	π	V	S	P	Tajima's D	Fu's Fs	R2
Haplogroup A1	20	19	0.99±0.018	0.003±0.003	19	15	4	-1.90*	-23.12***	0.04***
Haplogroup A2	9	9	1.00±0.052	0.008±0.001	26	21	5	-1.48	-3.91*	0.08**
Haplogroup A3	4	4	1.00±0.17	0.012±0.004	21	20	1	-0.71	0.41	0.22
Haplogroup A4	5	5	1.00±0.12	0.004±0.001	8	7	1	-0.80	-1.51*	0.09**
Haplogroup A5	4	4	1.00± 0.17	0.003± 0.0005	5	5	0	-0.81	-2.00*	0.23
R. leschenaultii	42	36	0.99± 0.009	0.008± 0.0005	63	33	30	-1.78*	-30.452 ***	0.05**

*P<0.05; **P<0.01; ***P<0.001

inferred from highly significant Tajima's D and Fu's Fs (Table 3). The median-joining network within the Middle East samples of *R. aegyptiacus* implies colonization from a few refugia following extensive population bottleneck during severe conditions.

Discussion

Fruit bats' phylogeny has been an interesting subject of investigation in recent years due to the role of megabats in the Ebola hemorrhagic fever (EHF) outbreak.[23, 25, 27, 37] *Rousettus aegyptiacus* and *R. leschenaultii* are two morphologically similar megabats included in the family Petropodidae. They were considered the primary hosts for some viruses which cause fatal diseases in humans. Morphological and molecular data showed unexpected diversity within fruit bats in south Iran. In this study, two species of megabats (the larger species *R. aegyptiacus* and the smaller one *R. leschenaultii*) were identified in Iran. Population genetic analyses revealed five lineages of *R. aegyptiacus* through its range, and the Iranian population of *R. aegyptiacus* clustered with the Middle East population. Our results also indicated that the population of *R. leschenaultii* shows no differentiation in the Indo-Himalayan and Indo-Chinese distributional range; however, there is a sampling gap regarding islands. Demographic analyses also revealed a strong signature of population expansion for Middle East population of *R. aegyptiacus* and *R. leschenaultii* from few refugia following extensive population bottleneck. The molecular signals of population expansion for *R. aegyptiacus* and *R. leschenaultii* throughout its range can be the consequence of climate change and raise more attention to these megabats in Iran as reservoir of different viral agents. In the recent decade, tropical fruits such as Jujube, guava, and papaya are part of the petropodid bats' diet[38-40] in Sistan and Baluchestan. They provided suitable habitat for fruit bats in southeast Iran. Migratory behavior of *R. leschenaultii,* which may imply the potential risk for viral agents spead with the establishment of new colonies of vectors into new invaded habitats should grab greater attention to the species.

Additionally, the other sympatric bat species and also potentially reservoir of coronavirus and other viral agents is *R. aegyptiacus*. This species has been shown to carry European bat lyssavirus 1, Orthonairoviruses (including Yogue virus (YOGV), Crimean-Congo Hemorrhagic Fever Virus (CCHF), and Kasokero orthonairovirus), Chikungunya virus, Paramyxovirus, Polyomavirus, Ebolavirus, and Poxvirus in different regions of the world.[1, 2, 41-43] So, screening of these pathogens in different populations of megabats in southeast Iran is proposed.

Moreover, the results of this study, which is consistent with different recent studies, indicated that climate change and consequent global warming resulted in northward range shifts for many species. These climate-induced range shifts has also been predicted to be faster for volant species such as bats.[44-47] These population expansion may also increase concerns about the spread of infectious agents from the Indian sub-continent to the southeast of Iran. Unlike *R. aegyptiacus*, which is essentially non-migrant, *R. leschenaultii* is a migratory bat.[4, 6] Detection of *Ebolavirus* in *R. leschenaultii*[17] and poor coverage of public health services in some parts of Pakistan and India from where cases of Ebola fever were reported will increase the essential need for monitoring of *R. leschenaultii* and *R. aegyptiacus* populations in southeast Iran by the health research services. As a result, it can be dedicated that based on the epidemiological triad, which is relationships between pathogen, environment, and host.[48] The range of related bat-borne pathogens can also be shifted in the future.

Recent studies on the bats mostly focused on the western parts of Iran.[49-53] We presented here some new distribution records of bat species, especially fruit bats from eastern parts of Iran. However, the bats recorded in this study, including *Rhinolophus hipposideros*, *Pipistrellus kuhlii*, and *Myotis blythii* are the reservoir of some viral agents e.g., SARS-like coronavirus.[54,55] The Middle East Respiratory Syndrome Coronavirus (MERS CoV),[42, 56] *Rotavirus* and *Orthoreovirus*,[57] and *Astrovirus* (AstV) can harbor *Lyssavirus,* which causes rabies in humans.[58] Furthermore, alteration in landuse and deforestation enhanced the probability of human-bat interface in different places such as Hyrcanian forests in the north of Iran. It may increase the risk of spillover of zoonotic diseases. So, reporting these bat species close to humans may attract health authority's attention to control and screen their possible viral infections. Eventually, precise identification of hosts and vectors in applied medical sciences is essential to conduct standard procedures.

Conclusion

Haplotype network and demographic analyzes on the population of the genus *Rousettus* in Iran confirmed the presence of two separate evolutionary lineages, including *R. aegyptiacus* and *R. leschenaultii* in the country. Our results also indicated that the presence of populations of these two fruit bats in the region may occurred in response to climate change during Holocene. This climate-induced range shift can also result in northward alteration of the optimal range of pathogens hosted by these two species of bats. Moreover, the cultivation of tropical fruits in Sistan and Baluchestan provides suitable habitats for dense colonization of fruit bats and migratory behavior of *R. leschenaultii* will increase the essential need for monitoring and screening of related pathogens in *R. leschenaultii* and *R. aegyptiacus* populations in southeast Iran by the health research services.

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Conflicts of interest: None declared.

References

1 Amman BR, Bird BH, Bakarr IA, et al. Isolation of Angola-like Marburg virus from Egyptian rousette bats from West Africa. Nature Communications. 2020; 11:510.

2 Markotter W, Coertse J, De Vries L, Geldenhuys M, Mortlock M. Bat-borne viruses in Africa: a critical review. Journal of zoology (London, England: 1987). 2020; 10.1111/jzo.12769.

3 Benda P, Abi-Said M, Bartonička T, Bilgin R, Faizolahi K, Lučan RK, Nicolaou H, Reiter A, Shohdi WM, Uhrin M, Horáček I. *Rousettus aegyptiacus* (Pteropodidae) in the Palaearctic: List of records and revision of the distribution range.Vespertilio. 2011; 15: 3–36.

4 Benda P, Vallo P, Hulva P, Horáček I. The Egyptian fruit bat *Rousettus aegyptiacus* (Chiroptera: Pteropodidae) in the Palaearctic: geographical variation and taxonomic status. Biologia. 2012; 67: 1230–1244.

5 Khajeh A, Mohammadi Z, Ghorbani F, Jahantigh H. Molecular and morphometric characterization of fruit bats of the genus *Rousettus* Gray, 1821 (Chiroptera: Pteropodidae) in Iran. Zoology in the Middle East. 2021;67: 1-11.

6 De Blase AF. The bats of Iran: systematics, distribution, ecology. Fieldiana: Zoology, New Series; 1980; 424 pp.

7 Pavri K, Singh K, Hollinger F. Isolation of a new parainfluenza virus from a frugivorous bat, *Rousettus leschenaultii*, collected at Poona, India. American Journal of Tropical Medicine and Hygiene. 1971; 20: 125–130.

8 Li W, Shi Z, Yu M, et al. Bats are natural reservoirs of SARS-like coronaviruses. Science. 2005; 310: 676–79.

9 Lau SK, et al. Ecoepidemiology and complete genome comparison of different strains of severe acute respiratory syndrome-related *Rhinolophus* bat coronavirus in China reveal bats as a reservoir for acute, self-limiting infection that allows recombination events. Journal of Virology. 2010; 84: 2808–2819.

10 Zhou P, Yang XL, Wang XG, Hu B, Zhang L, Zhang W, Si HR, Zhu Y, Li B, Huang CL, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020; 579: 270–273.

11 Zhang YZ, Zhang HL, Mi ZQ. Investigation of mosquitoes and arboviruses in Hekou City Yunnan Province [in Chinese]. Yi Xue Dong Wu Fang Zhi. 1998; 14:37–38.

12 Olival KJ, Islam A, Yu M, Anthony SJ, Epstein JH, Khan SA, et al. *Ebola* virus antibodies in fruit bats, Bangladesh. Emerging Infectious Diseases. 2013; 19:270.

13 Badar N, Salman M, Ansari J, Aamir U, Alam MM, Arshad Y, Mushtaq N, Ikram A, Qaz J. Emergence of Chikungunya Virus, Pakistan, 2016–2017. Emerging Infectious Diseases. 2020; 26(2): 307–310.

14 Dovih P, Laing ED, Chen Y, Low DHW, Ansil BR, Yang X, et al. Filovirus-reactive antibodies in humans and bats in Northeast India imply zoonotic pillover. PLOS Neglected Tropical Diseases. 2019; 13(10): e0007733.

15 Yousefi A, Rahbari S, Karimi AS. The first report of *Trypanosoma* (*herpetosoma*) *grosi* in rodents of Razan plain, western Iran. Journal of Veterinary Microbiology. 2016; 33; 131-135.

16 Yousefi A, Nosrati MRC, Karimi A, Naisi S. *Leptopsylla taschenbergi taschenbergi* (Siphonaptera: Leptopsyllidae), new flea from Iran. Asian Pacific Journal of Tropical Disease. 2015; 5(8): 606-607.

17 Yousefi A, Eslami A, Mobedi I, Rahbari S, Ronaghi H. Helminth Infections of House Mouse (*Mus musulus*) and Wood Mouse (*Apodemus sylvaticus*) from the Suburban Areas of Hamadan City, Western Iran. Iranian Journal of Parasitology. 2014; 9(4): 511–8.

18 Esfandiari B, Nahrevanian H, Pourshafie MR, Gouya MM, Khaki P, Mostafavi E, Darvish J, Hanifi H. Epidemiological distribution of rodents as potent reservoirs for infectious diseases in the Provinces of Mazandaran, Gilan and Golestan, Northern Iran. Infectious disease reports. 2017; 9(2): 6900.

19 Ranjbar MJ, Sarkari B, Mowlavi GR, Seifollahi Z, Moshfe A. Khabisi SA, Mobedi I. Helminth infections of rodents and their zoonotic importance in Boyer-Ahmad District, southwestern Iran. Iranian Journal of Parasitology. 2017; 12(4): 572.

20 Macholán M, Filippucci MG, Benda P, Frynta D, Sadlova J. Allozyme variation and systematics of the genus *Apodemus* (Muridae: Rodentia) in Asia Minor and Iran. Journal of Mammalogy.2001; 82:799–813.

21 Darvish J, Mohammadi Z, Ghorbani F, Mahmoudi A, Dubey S. Phylogenetic relationships of *Apodemus* Kaup, 1829 (Rodentia: Muridae) species in the eastern Mediterranean inferred from mitochondrial DNA, with Emphasis on Iranian Species. Journal of Mammalian Evolution. 2015; 22(4): 583–595.

22 Arzamani K, Mohammadi Z, Shirzadi MR, Alavinia SM, Jafari B, Darvish J. Faunistic study of the rodents of north Khorasan province, north east of Iran, 2011-2013. Journal of Arthropod-Borne Diseases. 2018; 12(2): 127–134.

23 Hassanin A, Nesi N, Marin J, Kadjo B, Pourrut X, Leroy É, Gembu G-C, Akawa PM, Ngoagouni C, Nakoune´E, Ruedi M, Tshikung D, Shongo CP, Bonillo C. Comparative phylogeography of African fruit bats (Chiroptera, Pteropodidae) provide new insights into the outbreak of Ebola virus disease in West Africa, 2014–2016. Comptes Rendus Biologies. 2016: 339: 517–528.

24 Huang J, Jan SS, Wei X, Wan Y, Ouyang S. Evidence of the Recombinant Origin and Ongoing Mutations in Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). BioRxiv. 2020. DOI: 10.1101/2020.03.16.993816.

25 Nesi N, Tsagkogeorga G, Tsang S, Nicolas V, Lalis A, Scanlon A, Rossiter S. Interrogating Phylogenetic Discordance Resolves Deep Splits in the Rapid Radiation of Old World Fruit Bats (Chiroptera: Pteropodidae).Systematic Biology. 2021; 70(6), 1077–1089.

26 Chen J, Rossiter SJ, Flanders JR, Sun Y, Hua P, Miller Butterworth C, Zhang S. Contrasting genetic structure in two co distributed species of Old World fruit bat. PLoS ONE. 2010; 5, e13903.

27 Stribna T, Romportl D, Demjanovič J, Vogeler A, Tschapka M, Benda P, Horáček I, Juste J, Goodman SM, Hulva P. Pan African phylogeography and palaeodistribution of rousettine fruit bats: Ecogeographic correlation with Pleistocene climate vegetation cycles. Journal of Biogeography. 2019;00:1–14.

28 R Core Team. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. 2018. Retrieved from https://www.R-project.org/

29 Bergmans W. Taxonomy and biogeography of African fruit bats (Mammalia, Megachiroptera). 4. The genus *Rousettus* Gray, 1821. Beaufortia. 1994; 44(4): 79-126.

30 Kwiecinski GG, Griffiths TA. *Rousettus egyptiacus*. Mammalian Species. 1999; 611: 1–9.

31 Srinivasulu C, Racey PA, Mistry S. A key to the bats (Mammalia: Chiroptera) of South Asia. Journal of Threatened Taxa. 2010; 12:1001–1076.

32 Leigh JW, Bryant D. POPART: full-feature software for haplotype network construction. Methods in Ecology and Evolution. 2015; 6: 1110–1116.

33 Librado P, Rozas J. DnaSp v5: A software for comprehensive analysis of DNA polymorphism data. Bioinformatics. 2009; 25: 1451–1452.

34 Fu YX. Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. Genetics. 1997; 147: 915–925.

35 Tajima F. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics. 1989; 123: 585–595.

36 Ramos-Onsins SE, Rozas J. Statistical Properties of New Neutrality Tests Against Population Growth. Molecular Biology and Evolution. 2002; 19: 2092–2100.

37 Hassanin A, Bonillo C, Tshikung D, Shongo CP, Pourrut X, Kadjo B, Nakouné E, Tu VT, Prié V, Goodman SM. Phylogeny of African fruit bats (Chiroptera, Pteropodidae) based on complete mitochondrial genomes. Journal of Zoological Systematics and Evolutionary Research. 2020; 00: 1–16.

38 Win SS, Mya KM. The diet of the Indian Flying Fox *Pteropus giganteus* (Brünnich, 1782) (Chiroptera: Pteropodidae) in Myanmar—conflicts with local people? Journal of Threatened Taxa. 2015; 7: 7568-7572.

39 Aziz SA, Olival KJ, Bumrungsri S, et al. The conflict between pteropodid bats and fruit growers: species, legislation and mitigation. In Bats in the Anthropocene: Conservation of Bats in a Changing World. Voight CC, Kingston T, Eds; Springer; 2016; 377–426.

40 Openshaw, JJ, Hegde S, Sazzad HMS, Khan SU, Hossain MJ, Epstein JH, Daszak P, Gurley ES, Luby SP. Bat Hunting and Bat–Human Interactions in Bangladeshi Villages: Implications for Zoonotic Disease Transmission and Bat Conservation. Transboundary and Emerging Diseases. 2017; 64: 1287-1293.

41 Kading RC, Kityo RM, Mossel EC, Borland EM, Nakayiki T, Nalikka B, Nyakarahuka L, Ledermann JP, Panella NA, Gilbert AT. Neutralizing antibodies against flaviviruses, Babanki virus, and Rift Valley fever virus in Ugandan bats. Infection Ecology & Epidemiology.2018; 8: 1439215.

42 Kuzmin IV, Niezgoda M, Franka R, Agwanda B, Markotter W, Beagley JC, Urazova OY, Breiman RF, Rupprecht CE. Lagos Bat Virus in Kenya. Journal of Clinical Microbiology. 2008; 46(4): 1451–1461.

43 Mishra AK, Moyer CL, Abelson DM, Deer DJ, El Omari K, Duman R, Lobel L, Lutwama JJ, Dye JM, Wagner A, Chandran K, Cross RW, Geisbert TW, Zeitlin L, Bornholdt ZA, McLellan JS. Structure and characterization of Crimean-Congo hemorrhagic fever virus GP38. Journal of Virology. 2020; 94(8): e02005-19.

44 Jones G, Jacobs DS, Kunz TH, Willig MR, Racey PA. Carpe noctem: the importance of bats as bioindicators. Endangered species research. 2009; 8(1-2): 93-115.

45 Rebelo H, Tarroso P, Jones G. Predicted impact of climate change on European bats in relation to their biogeographic patterns. Global Change Biology. 2010; 16(2): 561-576.

46 Sherwin HA, Montgomery WI, Lundy MG The impact and implications of climate change for bats. Mammal Review. 2013; 43(3): 171-182.

47 Wu J. Detection and attribution of the effects of climate change on bat distributions over the last 50 years. Climatic Change. 2016; 134(4): 681-696.

48 Oliveira ARS, Cohnstaedt LW, Cernicchiaro N, Japanese Encephalitis Virus: Placing Disease Vectors in the Epidemiologic Triad. Annals of the Entomological

Society of America. 2018; 111(6): 295–303.

49 Shahabi S, Akmali V, Sharifi M. Echolocation call frequency and mitochondrialcontrol regionvariation in the closely related bat species of the genus *Rhinolophus*(Chiroptera: Rhinolophidae) occurring in Iran: implications for taxonomy andintraspecific phylogeny. Mammal Research. 2019;64(4); 485-501.

50 Akmali V, Shahabi S, Zagmajster M. Bats of Tadovan Cave insouthern Iran: their occurrence in the cave, and conservation issues.Cave & KarstScience.2019; 46(2): 79-86.

51 Shahabi S, Akmali V, Sharifi M. Potential Geographic Distribution of the GreaterHorseshoe Bat, *Rhinolophus ferrumequinum* (Chiroptera: Rhinolophidae) in Iran. Journal of Wildlife and Biodiversity.2018; 3(2): 40-51(2019).

52 Eghbali H, Shahabi S, Najafi N, Mehdizadeh R, Shetav Y, Sharifi M.Postnatal growth, wing development and age estimations in the Mediterraneanhorseshoebat, *Rhinolophus euryale* (Chiroptera: Rhinolophidae) in Kerend Cave,Western Iran. Mammalia. 2018; 82(3): 276-287.

53 Shahabi S, Akmali V, Sharifi M. Taxonomic evaluation of the greaterhorseshoe bat *Rhinolophus ferrumequinum* (Chiroptera: Rhinolophidae) in Iran inferredfrom the Mitochondrial D-loop Gene. Zoological Science.2017; 34: 1–7.

54 Lelli D, Papetti A, Sabelli C, Rosti E, Moreno A, Boniotti MB. Detection of coronaviruses in bats of various species in Italy. Viruses. 2013; 5: 2679–2689.

55 Kemenesi G, Kurucz K, Dallos B, Zana B, Foldes F, Boldogh S, Gorfol T, Carroll MW, Jakab F. Re-emergence of Lloviu virus in *Miniopterus schreibersii* bats, Hungary, 2016. Emerging Microbes and Infections.2018; 7:66.

56 Memish ZA, Mishra N, Olival KJ, Fagbo SF, Kapoor V, Epstein JH, Alhakeem R, Durosinloun A, Asmari MAl, Islam A, et al. Middle East respiratory syndrome coronavirus in bats, Saudi Arabia. Emerging Infectious Diseases journal. 2013; 19: 1819-1823.

57 Lelli D, Moreno A, Lavazza A, Bresaola M, Canelli E, et al. Identification of Mammalian orthoreovirus type 3 in Italian bats. Zoonoses Public Health. 2013; 60: 84–92.

58 Leopardi S, et al. Active and passive surveillance for bat lyssaviruses in Italy revealed serological evidence for their circulation in three bat species. Epidemiology and Infection. 2018; 1–6.