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The Egyptian fruit bat *Rousettus aegyptiacus* (Chiroptera: Pteropodidae) in the Palaearctic: Geographical variation and taxonomic status

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Abstract: Two metrically defined subspecies have traditionally been recognised within the Palaearctic distribution range of the Egyptian fruit bat *Rousettus aegyptiacus*; the larger *R. a. aegyptiacus* in Egypt and the northern part of the Middle East and the smaller *R. a. arabicus* in the southern Middle East. An extensive material of *R. aegyptiacus* from all parts of this area, i.e. the Levant (incl. Turkey and Cyprus), Egypt (incl. Sinai), northern Sudan, Yemen, Oman, Iran, and Pakistan, as well as comparative samples from its sub-Saharan range, were tested using both morphological and genetic approaches in order to revise the species' taxonomic status. The results indicated two possible processes, depending on the method used. Genetic analysis of the mitochondrial genome (nd1 and cyt*b*) indicated low variation (< 2.0% of genetic distance) and lack of geographical structure while morphometric analysis indicated significant metric differences. Two basic size morphotypes were found within the Palaearctic range, with a rather mosaic-like geographical distribution and a lack of clear size distinction between the two categories, though intermediate types were detected. Thus, we suggest that all Palaearctic populations of *R. aegyptiacus* represent one form, the nominotypical subspecies, which is uniform in genetic traits but plastic in metric traits.

Key words: *Rousettus*; morphometrics; mtDNA; taxonomy; distribution

Introduction

The Egyptian fruit bat, *Rousettus aegyptiacus* (Geoffroy, 1810), is the only member of the Pteropodidae family that has the large part of its distribution range in the Palaearctic region (Fig. 1), and the only one whose type locality lies in the Palaearctic. Considering the distribution range, *R. aegyptiacus* is mostly an Afro-tropical species, which reaches the southwestern part of the Palaearctic in the Middle East (Juste & Ibañez 1993; Bergmans 1994; Kwiecinski & Griffiths 1999). In sub-Saharan Africa, it occupies areas around the Gulf of Guinea from Senegal to western Angola (including some islands in the Gulf) and savannah regions of south and east Africa from the Cape to Eritrea. In the Palaearctic, *R. aegyptiacus* occurs in Egypt and northern Sudan as well as in broad areas along the sea coasts of the Middle East, from south-western Turkey and Cyprus along the Levantine and Arabian shores up to southern Iran and Pakistan (Bergmans 1994; Benda et al. 2011). The Palaearctic range (here considered as Egypt, northern Sudan and the whole Middle East, as well as southern Pakistan) is reported to be isolated from the sub-Saharan African range by the Red Sea and desert areas of north-eastern Africa. The north-eastern Mediterranean represents the northern margin of the species' distribution range (Harrison & Bates 1991; Bergmans 1994; Benda et al. 2006, 2011).

The species' presence in the Middle East has traditionally been seen as something of an enigma as it is the only offshoot of the family found beyond the tropics. The species' occurrence depends upon continuous all-year-round availability of fruit, which is strictly related to anthropogenic plant cultivation over the vast majority of the Middle East and has undergone numerous dramatic spatial and temporal fluctuations. Despite this, the species is reported as exhibiting clear patterns of geographic phenotype variation that correspond with the variation pattern displayed over the rest of its range (Bergmans 1994; Kwiecinski & Griffiths 1999).

R. aegyptiacus is regarded as a polytypic species, with four subspecies recognised within its continental Afro-Asian range, i.e., *R. a. aegyptiacus* (Geoffroy, 1810), *R. a. leachii* (Smith, 1829), *R. a. unicolor* (Gray, 1870), and *R. a. arabicus* Anderson, 1902 (Eisentraut 1959; Hayman & Hill 1971; Bergmans 1994; Koopman 1994; Kwiecinski & Griffiths 1999; Simmons 2005). In addition, Juste & Ibañez (1993) described two subspecies from two islands in the African Gulf of Guinea;

R. a. princeps from Principe and *R. a. tomensis* from São Tomé.

Traditionally, two subspecies of the Egyptian fruit bat have been considered as meeting in the Middle East (Eisentraut 1959; Bergmans 1994), based on evidence of two distinct size categories occurring in two distinct ranges: the larger form, *R. a. aegyptiacus*, living in Egypt and the eastern Mediterranean (type locality: Great Pyramid of Giza, Egypt; restricted by Anderson 1902), and a smaller form, *R. a. arabicus*, living in southern Arabia and southern parts of Iran and Pakistan (type locality: Lahej near Aden, Yemen) (Eisentraut 1959; Harrison 1964; Corbet 1978; Harrison & Bates 1991; Bergmans 1994; Koopman 1994; Kwiecinski & Griffiths 1999; Horáček et al. 2000). Benda et al. (2008), however, in describing the first evidence of *R. aegyptiacus* populations in desert oases of Sinai, found that Sinaitic fruit bats conformed in body size almost completely with comparative samples from Yemen and Iran (*R. a. arabicus*), and only partly overlapped with those from the Levant and Lower Egypt (*R. a. aegyptiacus*). Benda et al. (2010) reported that the dimensions of south-Jordanian *R. aegyptiacus* samples were intermediate between the two Mediterranean (Levantine) and desert (Sinaitic) size-morphotypes and, therefore, they suggested that a continuous cline shift in size existed between the respective regions of the eastern Mediterranean. On the other hand, preliminary comparisons of mitochondrial genes, though based on a few samples only (Benda et al. 2007), showed very small genetic differences between *R. aegyptiacus* populations over the whole Middle East (from Cyprus to Iran). This tends to indicate a discontinuous geographical arrangement of variation categories, rather than two metrically and geographically distinct subspecies *sensu* Bergmans' (1994) revision. Hence, Benda et al. (2008) speculated that rearrangements in body size could have occurred over the course of just a few generations and represent adaptive changes rather than evidence of phylogenetic differences between geographically separated populations.

Over the last two decades, numerous new records of *R. aegyptiacus* have been obtained from the Middle East and north-east Africa (Benda et al. 2011), which provides an opportunity to re-examine patterns of geographic variation and interrelationships between local populations in the Palaearctic. Using both morphometric and genetic approaches, the present paper reports on the first results of this process, with particular focus on the taxonomic arrangement of the species within this part of its distribution range.

Material and methods

Morphological analysis

We compared morphological (morphometric) skull and forearm data from museum specimens covering all parts of the Palaearctic distribution range of *R. aegyptiacus* (Fig. 1), including most of the type material and several comparative specimens/taxa from the species' sub-Saharan range and from its Oriental promontory (Pakistan). See Appendix 1 for a detailed list of the material examined (see below for abbreviations used).

Specimens were measured with a mechanical calliper using standard methods according to e.g. Benda et al. (2004). Statistical analysis of the morphometric data was performed using Statistica 6.0 software. Principal component analysis (PCA) was used as a test of the importance of skull size and, in particular, skull dimensions for intraspecific variation. Sexual dimorphism was tested using the t-test and one-way analysis of variance (ANOVA). Cluster analysis (unweighted pair-group average, Euclidean distances) was used to evaluate size similarities among representatives of particular populations within the distribution range.

Molecular genetic analysis

Molecular genetic analysis was based on 79 *R. aegyptiacus* samples from the northern and southern parts of the Palaearctic range (one to four samples per site), with 44 samples from 27 sites in the northern regions (Egypt, northern Sudan and the Levant, including Cyprus, Turkey and Sinai) and 35 samples from 21 sites in the southern regions (Yemen, Oman and Iran). Several samples from African sub-Saharan populations were also added (17 samples from specimens originating in Senegal, Ghana, Gabon, Ethiopia, Kenya and Malawi). See Appendix 2 for detailed data and details of origin for the material examined.

Total genomic DNA was extracted from the tissue samples using the Nucleospin 96-well tissue kit (Macherey-Nagel, USA) or the JetQuick DNA extraction kit (Genomed, Germany). Nicotine-amid dehydrogenase 1 (nd1) and cytochrome *b* (cyt*b*), two mitochondrial (mt) genes, were amplified via Polymerase Chain Reaction (PCR) using ER65 and ER66 primers (Petit et al. 1999) and L14724 and H15915 primers (Irwin et al. 1991), respectively. Each PCR volume contained 12.5 µl Combi PPP mix (TopBio, Czech Republic), 2 µl of a 10 µM solution of each primer, and 2.5 µl of isolated DNA solution. The PCR began with initial denaturation at 94 °C for 3 min, followed by 35 cycles of denaturation for 40 s at 94 °C, annealing for 40 s at 58 °C (nd1) or 50 °C (cyt*b*), extension for 90 s at 65 °C, with a final extension at 65 °C for 5 min. Amplified products were purified using either the JetQuick PCR purification kit (Genomed, Germany) or commercially by Macrogen Inc. (Seoul, Korea). The latter company also sequenced all PCR products with the respective forward primer on an ABI 3137 XL sequencer using the BigDye sequencing kit. Sequences were checked for ambiguities and edited in Sequencher (Gene Codes, USA), then aligned manually in BioEdit (Hall 1999). For reconstruction of phylogenetic trees, sequences were narrowed to unique haplotypes. Sequences of *Rousettus lanosus* Thomas, 1906, and *Lissonycteris angolensis* (Bocage, 1898) were used as an outgroup (Appendix 2). Phylogenetic trees were computed in PAUP* 4.10b (Sinauer Associates, USA) using maximum parsimony (MP) and neighbour-joining (NJ). Characters were equally weighted and unordered during MP analysis, while distance in NJ analysis was based on the Kimura two-parameter (K2p) evolutionary model (Kimura 1980). K2p distances were also used for expressing genetic difference among haplotypes. Support for the branching pattern was assessed through 1000× nonparametric bootstrapping during MP analysis. Relationships among *R. aegyptiacus* sequences were also explored using the network approach in Network (Fluxus, USA) in order to recover structure among closely related haplotypes that may

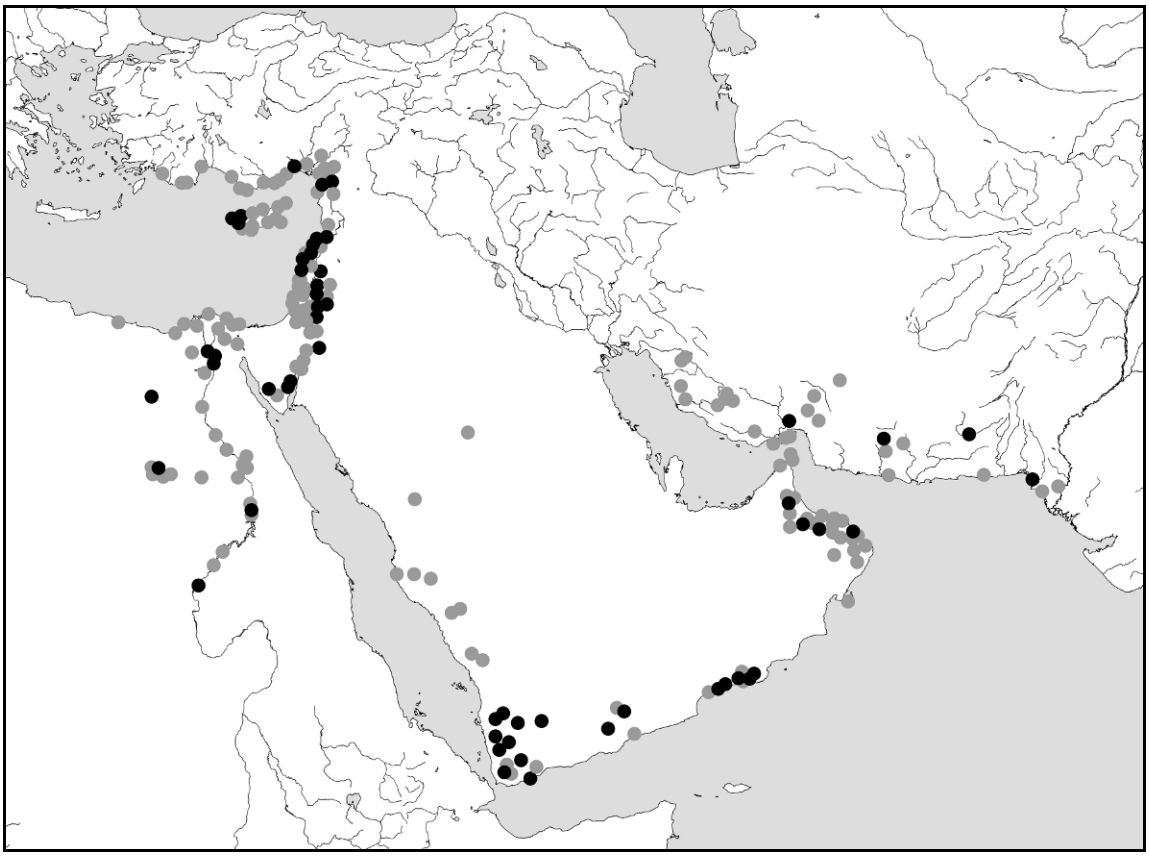

Fig. 1. Map of *Rousettus aegyptiacus* records in the Palaearctic, *sensu* Benda et al. (2011). Black dots – sites of origin of the material examined, grey dots – other records.

be in ancestor-descendant relationships and whose position in phylogenetic trees remained unresolved (Posada & Crandall 2001).

Abbreviations

Dimensions: LAt = forearm length (including wrist); LCr = greatest length of skull; LCb = condylobasal length; LaZ = zygomatic width; LaI = width of interorbital constriction; LaP = width of postorbital constriction; LaN = neurocranium width; AN = neurocranium height; CC = rostral width between upper canines (incl.); M^2M^2 = rostral width between 2nd upper molars (incl.); CM^2 = length of upper tooth-row between canine and 2nd molar (incl.); LMd = condylar length of mandible; ACo = height of coronoid process; CM_3 = length of lower tooth-row between canine and 3rd molar (incl.).

Collections: BMNH = Natural History Museum, London, United Kingdom; IVB = Institute of Vertebrate Biology, Academy of Sciences of the Czech Republic, Brno, Czech Republic; JOC = Ján Obuch Private Collection, Blatnica, Slovakia; MSNG = Civil Museum of Natural History Giacomo Doria, Genoa, Italy; NMP = National Museum (Natural History), Prague, Czech Republic; ZFMK = Alexander Koenig Zoological Institute and Museum, Bonn, Germany.

Other abbreviations: A = alcoholic preparation; B = skin (balg); df = degrees of freedom; f = female; F = F-value of ANOVA; m = male; M = mean; max, min = maximum and minimum range margins; P = probability (significance); PC = principal component; S = skull; SD = standard deviation; Sk = skeleton; T = t-value of t-test.

Results

Morphometric analysis

Morphometric comparisons revealed two basic size morphotypes among the population samples examined (Table 1). The dimension ranges of these groups overlapped only partially, with the largest skull measurements overlapping by less than 50% (Fig. 2). Larger representatives (LAt 86.6–98.1 mm; LCr 40.7–46.6 mm) were found in two separate areas: the Levant (Turkey, Cyprus, Syria and Lebanon) and continental Egypt (Cairo region, the Nile valley and Western Oases). The largest of all (LCr > 46.0 mm) were found among samples from the Western Oasis of Dakhla in Egypt. Smaller representatives (LAt 81.5–95.4 mm; LCr 38.0–43.7 mm) were also found in two largely separated geographical areas: the southern Middle East (Yemen, Oman, Iran and Pakistan) and Sinai. Smallest specimens (LCr < 38.5 mm) were documented from south-central Iran (Isin). The dimensions of samples from Jordan were intermediate between these two groups (LAt 86.4–96.5 mm; LCr 40.2–44.8 mm). Comparative sample sets from sub-Saharan Africa (Ethiopia and Senegal) were positioned similarly to the intermediate samples from Jordan, although slightly larger in their external dimensions (LAt 88.3–100.7 mm; LCr 39.7–44.2 mm; n=12).

Variation in sexual dimorphism was noted within

Table 1. Forearm and skull dimensions (in mm) and PC1 values of the examined sample sets of *Rousettus aegyptiacus* from the Palaearctic and results of sexual dimorphism analysis within the sets.

	Egypt									Levant							
	n	**M**	min	max	SD	df	*F*	*T*	*P*	*n*	**M**	min	max	SD	df	*F*	*T*	*P*
LAt	25	**92.36**	86.6	96.6	2.986	23	1.69	1.301	–	16	**94.53**	90.0	98.1	2.321	14	0.03	–0.173	–
LCr	45	**44.34**	41.47	46.57	1.290	28	3.88	1.970	–	19	**43.51**	40.68	45.19	1.130	16	0.43	0.654	–
LCb	44	**42.79**	39.64	44.86	1.253	27	3.67	1.917	–	18	**41.87**	39.24	43.57	1.129	15	0.46	0.677	–
LaZ	44	**27.02**	24.23	29.27	1.270	28	2.24	1.498	–	19	**26.88**	24.63	29.26	1.050	16	2.02	1.420	–
LaI	24	**8.63**	7.73	9.51	0.514	14	0.08	0.292	–	18	**8.37**	7.93	9.08	0.301	15	**4.96**	**2.227**	*
LaP	25	**7.87**	6.87	8.87	0.555	15	2.57	1.603	–	16	**7.78**	7.22	8.76	0.424	13	**5.86**	**2.420**	*
LaN	44	**17.45**	16.82	18.34	0.400	28	2.25	1.501	–	19	**17.31**	16.83	17.93	0.323	16	0.09	0.300	–
AN	45	**13.26**	12.31	14.28	0.532	28	1.39	1.177	–	19	**13.17**	11.64	13.93	0.546	16	0.01	0.078	–
CC	44	**8.83**	8.21	9.71	0.433	28	**8.76**	**2.959**	*	18	**8.89**	8.42	9.68	0.401	16	**5.43**	**2.329**	*
M^2M^2	40	**13.30**	12.35	14.08	0.426	27	0.93	0.963	–	17	**12.90**	12.29	13.50	0.380	15	0.12	0.353	–
CM^2	44	**16.87**	15.61	18.04	0.535	27	**5.25**	**2.290**	*	19	**16.54**	15.15	17.62	0.642	16	**5.76**	**2.400**	*
LMd	45	**34.47**	31.97	36.32	0.997	28	2.50	1.581	–	19	**33.87**	31.98	35.32	0.955	16	1.03	1.012	–
ACo	45	**16.03**	14.54	17.67	0.767	28	**6.39**	**2.529**	*	18	**15.13**	13.27	16.63	0.937	15	0.04	0.202	–
CM_3	44	**18.38**	17.08	19.85	0.622	28	0.40	0.632	–	18	**18.20**	17.56	19.20	0.464	15	1.49	1.222	–
PC1	45	**–0.980**	–2.039	0.262	0.591	28	**4.48**	**–2.117**	*	19	**–0.542**	–1.511	0.458	0.593	16	4.41	2.099	–
	Sinai									Jordan							
	n	**M**	min	max	SD	df	*F*	*T*	*P*	*n*	**M**	min	max	SD	df	*F*	*T*	*P*
LAt	14	**91.56**	87.0	95.4	2.358	12	3.26	1.805	–	11	**91.73**	86.4	96.5	3.290	9	3.04	1.742	–
LCr	13	**41.27**	38.91	43.66	1.329	11	**4.87**	**2.208**	*	10	**42.44**	40.24	44.82	1.481	8	**14.00**	**3.742**	*
LCb	13	**39.55**	37.53	41.47	1.173	11	4.74	2.176	–	10	**40.80**	38.67	42.84	1.427	8	**17.45**	**4.177**	**
LaZ	13	**24.95**	23.48	27.33	0.920	11	4.05	2.014	–	10	**25.85**	24.89	26.87	0.719	8	**21.27**	**4.612**	**
LaI	13	**7.87**	7.19	8.59	0.403	11	2.54	1.594	–	10	**8.30**	7.83	9.17	0.379	8	0.00	0.045	–
LaP	13	**7.39**	6.61	7.94	0.360	11	1.22	1.106	–	9	**7.87**	7.44	8.71	0.358	7	0.80	–0.896	–
LaN	13	**16.47**	16.02	17.91	0.530	11	2.39	1.546	–	10	**15.45**	6.54	17.71	3.476	8	0.03	0.162	–
AN	13	**12.53**	11.56	13.24	0.453	11	3.71	1.926	–	10	**13.18**	12.45	14.57	0.652	8	0.16	0.397	–
CC	13	**8.25**	7.82	8.84	0.296	11	**6.40**	**2.530**	*	10	**8.46**	7.72	9.02	0.460	8	**9.72**	**3.118**	*
M^2M^2	13	**12.38**	11.84	13.30	0.425	11	**5.15**	**2.269**	*	10	**12.62**	12.11	13.12	0.380	8	**11.32**	**3.365**	*
CM^2	13	**16.02**	14.91	16.99	0.583	11	**6.36**	**2.522**	*	10	**16.09**	15.31	17.07	0.587	8	**25.30**	**5.030**	**
LMd	13	**32.12**	30.08	33.82	1.048	11	**7.37**	**2.714**	*	10	**32.70**	30.75	34.37	1.237	8	**11.11**	**3.333**	*
ACo	13	**14.89**	14.18	16.17	0.614	11	**11.10**	**3.332**	*	10	**15.38**	14.08	16.56	0.969	8	5.16	2.272	–
CM_3	13	**17.29**	16.08	18.44	0.692	11	**6.24**	**2.498**	*	10	**17.35**	14.98	18.85	1.077	8	**8.16**	**2.856**	*
PC1	13	**0.532**	–0.936	1.418	0.626	11	2.02	–1.421	–	10	**0.124**	–0.892	1.161	0.641	8	0.01	–0.101	–
	South Arabia									Iran and Pakistan							
	n	**M**	min	max	SD	df	*F*	*T*	*P*	*n*	**M**	min	max	SD	df	*F*	*T*	*P*
LAt	35	**90.37**	85.2	94.8	2.416	33	**9.05**	**3.008**	**	19	**88.42**	81.5	92.9	2.973	17	**4.61**	**2.146**	*
LCr	29	**40.80**	38.48	42.94	1.061	27	**27.89**	**5.281**	***	18	**39.95**	38.00	42.07	1.200	16	**67.10**	**8.191**	***
LCb	29	**39.15**	36.64	41.47	1.104	27	**23.06**	**4.803**	***	18	**38.33**	36.14	40.49	1.271	16	**43.25**	**6.576**	***
LaZ	29	**24.57**	23.13	26.57	0.824	27	**24.06**	**4.905**	***	19	**24.49**	23.42	26.42	0.826	17	**22.87**	**4.782**	***
LaI	29	**7.90**	7.06	8.65	0.379	27	0.50	0.707	–	19	**7.73**	7.25	8.73	0.363	17	1.77	1.332	–
LaP	29	**7.52**	6.63	8.63	0.511	27	0.21	–0.459	–	19	**7.75**	6.97	8.98	0.490	17	1.41	–1.188	–
LaN	29	**16.41**	15.58	17.04	0.445	27	**14.07**	**3.751**	**	18	**16.49**	15.74	17.19	0.436	16	**28.51**	**5.340**	***
AN	29	**12.46**	11.90	13.29	0.370	27	**7.00**	**2.646**	*	18	**12.25**	11.61	13.23	0.406	16	**6.74**	**2.595**	*
CC	29	**8.14**	7.58	8.82	0.290	27	3.65	1.912	–	19	**8.64**	7.68	17.96	2.279	17	0.41	–0.644	–
M^2M^2	28	**12.13**	11.65	12.74	0.275	26	0.18	–0.419	–	16	**12.08**	11.34	12.83	0.437	14	**12.49**	**3.534**	**
CM^2	29	**15.81**	14.82	16.70	0.466	27	**19.14**	**4.375**	***	19	**15.56**	14.30	16.54	0.669	17	**47.73**	**6.909**	***
LMd	29	**31.70**	29.63	33.37	0.843	27	**22.52**	**4.745**	***	19	**31.30**	29.68	32.89	1.016	17	**81.27**	**9.015**	***
ACo	29	**14.42**	12.88	15.58	0.750	27	**6.87**	**2.621**	*	19	**13.49**	12.38	14.36	0.602	17	4.25	2.061	–
CM_3	29	**17.22**	16.38	18.23	0.490	27	**24.24**	**4.923**	***	19	**16.93**	15.75	18.32	0.681	17	**13.36**	**3.655**	**
PC1	28	**0.720**	–0.096	1.612	0.454	26	0.08	0.291	–	19	**1.038**	–0.083	1.951	0.790	17	0.06	0.241	–

Explanations: *F* – *F* value of ANOVA; *T* – *t*-value of *t*-test; *P* – significance). See the Abbreviations section for an explanation of the dimension abbreviations used.

the Palaearctic *R. aegyptiacus* sample sets (Table 1). While larger bats of the Levant and Egypt had less pronounced dimorphism, observed mainly in skull width (LaI, LaP, CC) and upper tooth-row length (CM^2), highly significant sexual dimorphism was detected in smaller bats from the southern Middle East and Sinai, mainly in skull length dimensions (LCr, LCb, CM^2, LMd, CM_3) and a number of skull width measurements (LaZ, LaN, CC, M^2M^2). Dimorphism in the majority of sample sets, however, was not highly significant (see the test results for PC1 in Table 1 and compare factor loadings of particular dimensions for both sexes in Table 2). Sexual dimorphism, therefore, does not appear to be a significant factor affecting the separation of samples into the geographically limited size morphogroups observed.

The mutual positions of the geographical sets were indicated through PCA of the 13 cranial dimensions (Fig. 3). Samples along the 1st PC (affected mainly by size characters) were clustered into three groups,

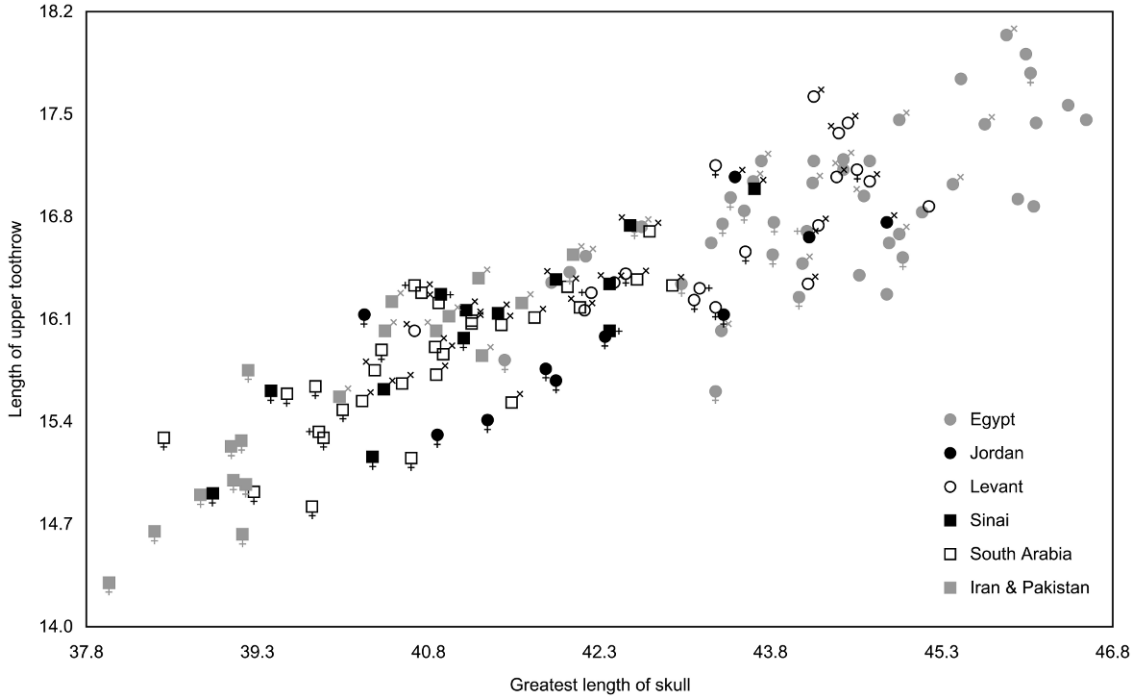

Fig. 2. Bivariate plot of *Rousettus aegyptiacus* samples from the Palaearctic: greatest length of skull against the length of upper tooth-row. Symbols associated with × represent males, symbols with + represent females.

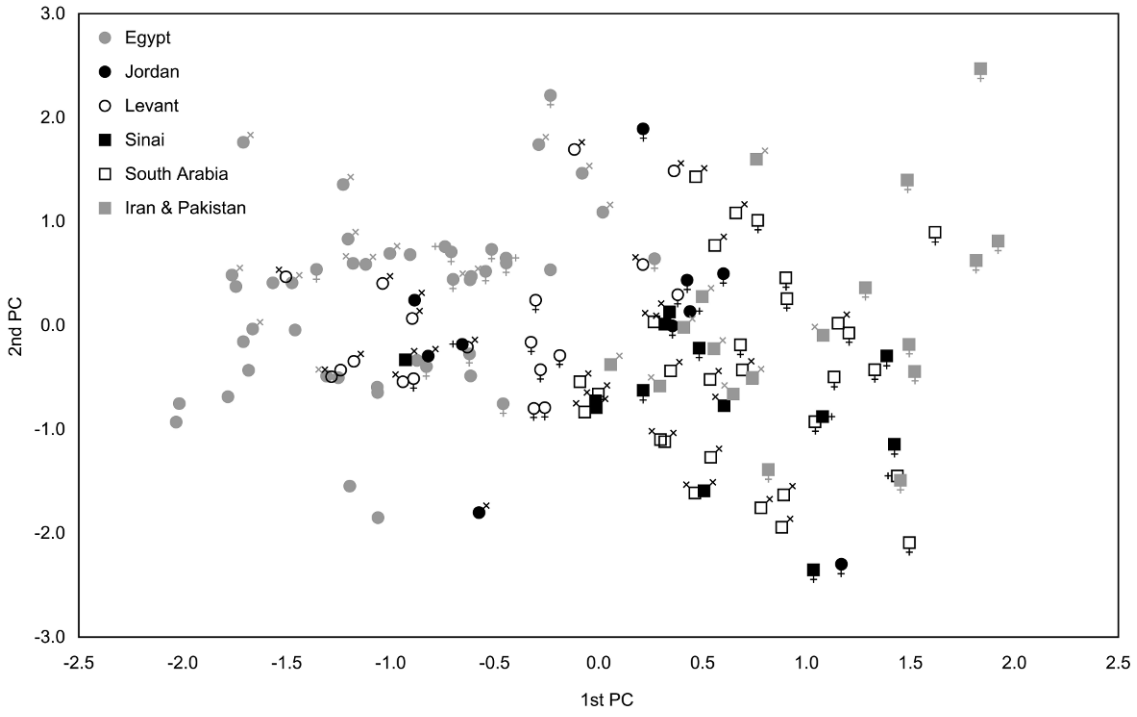

Fig. 3. Bivariate plot of *Rousettus aegyptiacus* samples of from the Palaearctic: results of principal component analysis for all cranial dimensions (first and second factors, principle component). Symbols associated with × represent males, symbols with + represent females.

with larger bats from Egypt and the Levant (PC1 –2.0–0.5), smaller bats from the southern Middle East and Sinai (PC1 –0.9–2.0), and intermediate bats from Jordan (PC1 –0.9–1.2). Along the 2nd PC axis (presumably, less affected by size characters), however, there appeared to be no geographical variation (see Table 2). The percentages of variance, as well as the factor loadings for each sex, were very similar, suggesting similar levels of sex-biased influence affecting the separation of samples into geographical morphogroups.

UPGMA cluster analysis of cranial dimensions from 45 localities of origin for Palaearctic *R. aegyptiacus* samples, and for the eight comparative samples from sub-Saharan Africa and Pakistan (calculated from

Table 2. Factor loadings and percentage of variance resulting from principal component analysis of 13 cranial dimensions for all samples examined (see also Fig. 3) and of particular sex groups.

	All samples		Males		Females	
Dimension \ Factor	PC1	PC2	PC1	PC2	PC1	PC2
LCr	–0.965	–0.037	–0.961	–0.028	–0.964	0.038
LCb	–0.860	–0.053	–0.956	–0.041	–0.723	–0.043
LaZ	–0.908	–0.034	–0.867	–0.004	–0.915	0.048
LaI	–0.776	0.101	–0.734	0.117	–0.718	0.271
LaP	–0.057	0.964	–0.229	0.887	–0.098	0.751
LaN	–0.549	0.270	–0.533	0.448	–0.442	0.064
AN	–0.785	0.012	–0.751	–0.014	–0.784	0.105
CC	–0.410	–0.122	–0.854	0.003	–0.144	0.698
M^2M^2	–0.842	0.163	–0.868	0.128	–0.839	0.051
CM^2	–0.886	–0.016	–0.886	–0.123	–0.825	–0.146
LMd	–0.959	–0.021	–0.947	–0.047	–0.953	0.001
ACo	–0.826	–0.205	–0.777	–0.270	–0.770	0.236
CM_3	–0.846	–0.074	–0.749	–0.264	–0.832	–0.118
% var	61.522	8.521	64.288	9.075	55.570	9.532

mean values of all dimensions from all specimens from the respective site), revealed similar pattern of geographical variation (Fig. 4). As with the above comparison, the two basic size groups and one intermediate group were indicated; however, cluster analysis better illustrated the relationships observed (see Figs 4A, B). Six locality samples from Egypt, the only sample from Turkey, two (of three) samples from Cyprus, three (of five) samples from Lebanon, one (of two) sample from Syria, and one (of three) sample from Jordan were all placed within the cluster of larger bats (LCr 43.5–45.3 mm, LaZ 26.2–28.7 mm). In addition, the type specimen of *Eleutherura unicolor* (= *Rousettus aegyptiacus unicolor*) from Gabon (LCr 46.2 mm, LaZ 27.3 mm) was also clustered within this group. All three locality samples from Sinai, ten (of twelve) samples from Yemen [including the type specimen of *Rousettus arabicus* (YEM7)], five (of six) samples from Oman, both samples from Iran, and both samples from Pakistan were clustered amongst the smaller bats (LCr 39.4–41.8 mm, LaZ 23.7–26.6 mm). In addition, a cluster (LCr 41.4–42.5 mm, LaZ 23.2–24.7 mm) containing the type specimen of *Cynopterus angolensis* (= *Lissonycteris angolensis*) from Angola, the type specimen of *Rousettus lanosus* from Uganda, and a sample of *R. lanosus* from Ethiopia were all classed as a sister group to the cluster of smaller Egyptian fruit bats. Finally, one sample (of three) from Cyprus, one (of two) samples from Syria, two (of three) samples from Jordan, two (of five) samples from Lebanon, two (of twelve) samples from Yemen, and one (of six) sample from Oman formed a cluster of intermediate bats (LCr 41.9–43.4 mm, LaZ 24.6–27.6 mm). Also clustered within this group were the comparative samples of *R. aegyptiacus* from localities in sub-Saharan Africa, one sample of *R. a. leachii* from Ethiopia (LCr 41.4–42.9 mm, LaZ 24.2–26.9 mm) and one sample of *R. a. unicolor* from Senegal (LCr 39.7–44.2 mm, LaZ 23.2–28.5 mm). A separate cluster (LCr 37.2–38.8 mm, LaZ 20.1–23.4 mm) containing reference samples of *R. leschenaulti* from Pakistan and *Lissonycteris angolensis* from Sierra Leone (type specimen of *Rousettus smithii*) and Uganda (including the type specimen of *Rousettus angolensis ruwenzorii*) was ranked as a sister group to the cluster containing all locality samples of *R. aegyptiacus*.

Molecular genetic analysis

We obtained 83 nd1 sequences and 68 cyt*b* sequences for *R. aegyptiacus* from the species' distribution range. The sequences were assembled into a 520 bp alignment of nd1 and an 867 bp alignment of cyt*b*. Nd1 sequences corresponded to 17 unique haplotypes and cyt*b* sequences to 30 unique haplotypes (Appendix 2). These haplotypes were submitted to GenBank under accession numbers JX274443–JX274462 for nd1 (N1–N20) and JX274463–JX274497 for cyt*b* (C1–C35), respectively.

In the nd1 alignment, 25 positions were variable with nine sites parsimony informative. MP search analysis yielded one shortest tree (160 steps) with very short branches within the ingroup arranged in a comb-like pattern, indicating shallow phylogenetic structure. Aside from 100% bootstrap support for the whole *R. aegyptiacus* ingroup, only two internal groups (which mostly contained the Middle Eastern haplotypes) were significantly supported, i.e., >70% support (Fig. 5). In the cyt*b* alignment, 80 positions were variable with 36 sites parsimony informative. MP search analysis yielded nine shortest trees (279 steps) with very short branches within the ingroup. Several haplogroups were consistently present in each recovered MP tree and significantly supported by bootstrap (Fig. 6). *R. aegyptiacus* from Africa were represented by two haplogroups and a solitary haplotype, the remaining haplogroup containing samples from the Middle East. The topology of NJ trees for both markers basically agreed with the respective MP trees except for minor rearrangements for terminal taxa; the former, however, were used for presentation purposes due to clearer structure (Fig. 5).

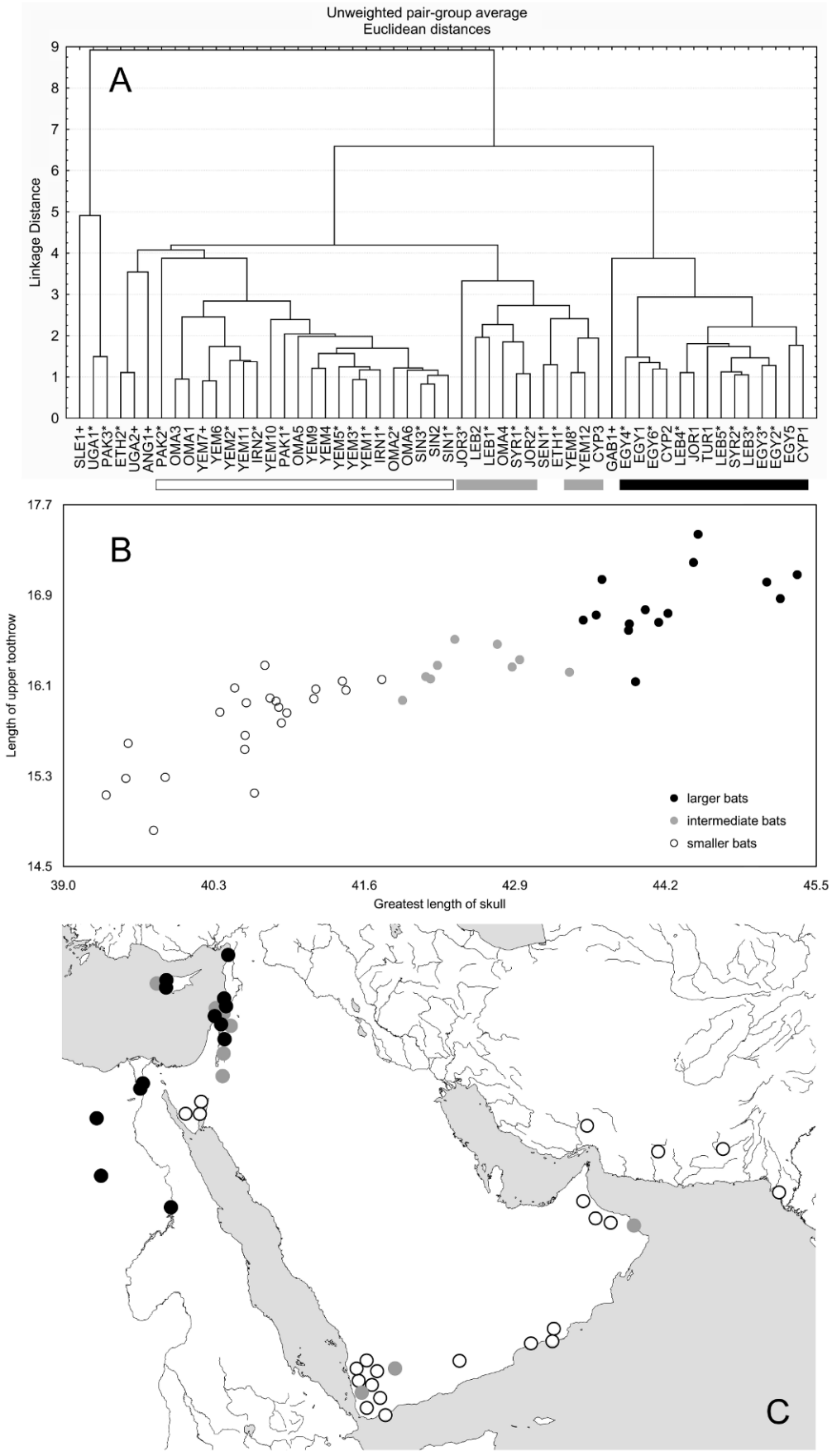

Fig. 4. Cluster analysis of localities for *Rousettus aegyptiacus* and comparative taxa samples; A – results of cluster analysis (unweighted pair-group average, Euclidean distances, calculated from mean values of all cranial dimensions from all specimens from the respective site; * average values for multiple samples from one site; + type specimens; see Appendix 1 for explanations of acronyms); B – metric distribution of Palaearctic samples separated by cluster analysis (colours of symbols according to divisions in A); C – geographical distribution of Palaearctic samples separated by cluster analysis (colours of symbols according to divisions in A).

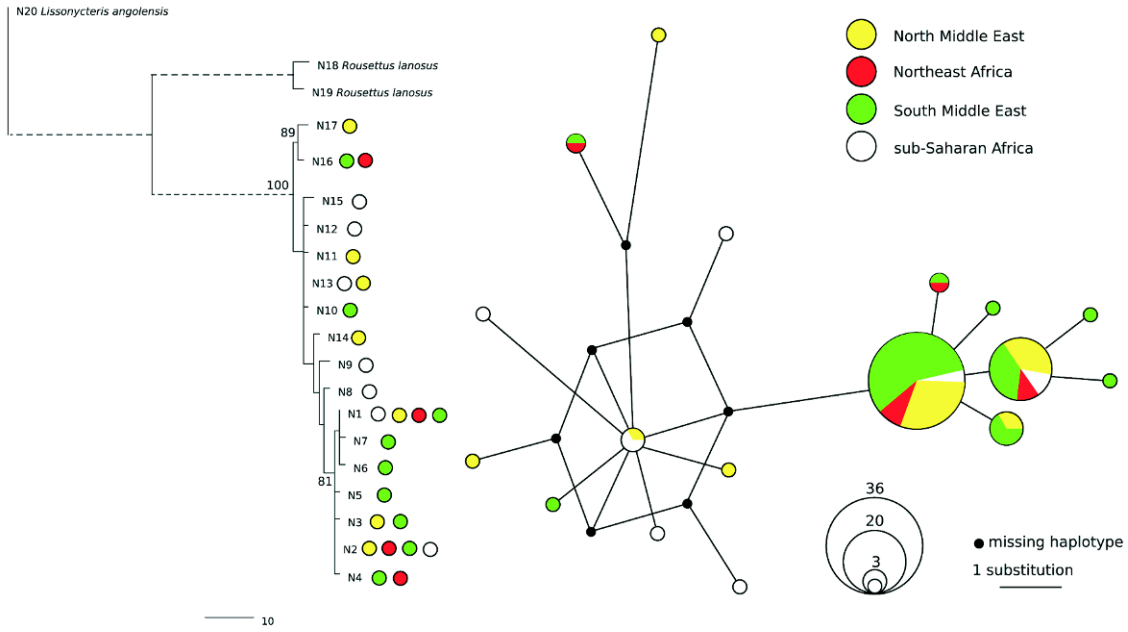

Fig. 5. Neighbour-joining tree and median-joining network depicting phylogenetic relationships among the *Rousettus aegyptiacus* samples based on nd1 sequences. Explanations: North Middle East – Cyprus, Turkey, Syria, Lebanon, Jordan, and Sinai; North-east Africa – continental Egypt and northern Sudan; South Middle East – Yemen, Oman and Iran; the dashed lines are not proportional to the number of substitutions (noted at the line); numbers at the tree nodes indicate the bootstrap support from maximum parsimony analysis.

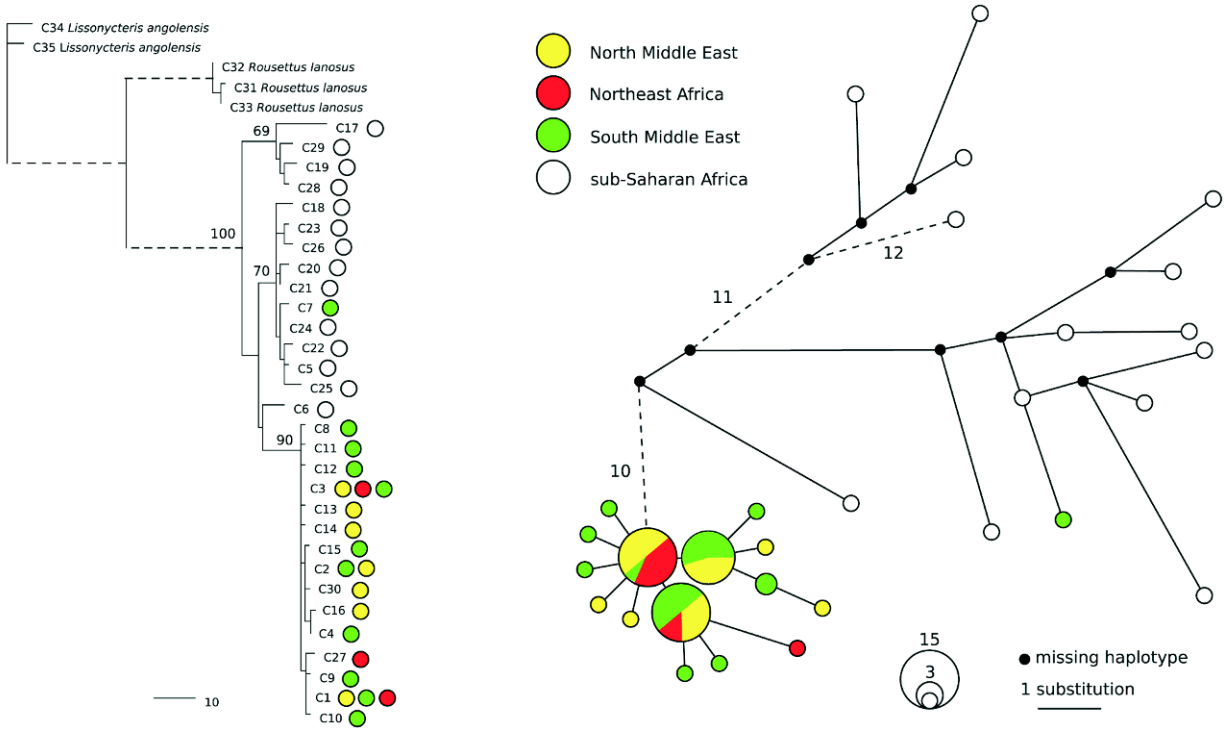

Fig. 6. Neighbour-joining tree and median-joining network depicting phylogenetic relationships among the *Rousettus aegyptiacus* samples based on cyt*b* sequences. For explanations see Fig. 5.

Genetic divergence between *R. aegyptiacus* and the outgroup taxa ranged from 15.4–16.9% for both markers. Within *R. aegyptiacus*, nd1 divergences ranged up to 2.0% and did not provide much distinction to the shallow structure of the phylogenetic tree. On the other hand, cyt*b* divergences reached values twice those for nd1, ranging up to 4.2%. Among the cyt*b* haplogroups (see Fig. 6), that containing four haplotypes, from Malawi and Kenya, differed by 2.1–3.3% from the other haplogroup and the solitary haplotype from sub-Saharan Africa, and by 2.8–4.2% from the Middle Eastern haplogroup. The Middle Eastern haplogroup further differed by 1.8–2.7% and 1.3–1.6% from the former two sub-Saharan units. Variability within the Mid-

dle Eastern haplogroup ranged from 0.1–0.6% and up to 1.2% and 2.0%, within the two sub-Saharan haplogroups.

The reconstructed nd1 haplotype network displayed a star-like pattern for the most frequent haplotypes (N1 and N2 extracted from more than 85% of specimens, plus five additional haplotypes – N3–N7) covering all four main geographic divisions, i.e., the northern (Turkey, Cyprus, the Levant and Sinai) and southern parts (Yemen, Oman and Iran) of the Middle East, and Egypt and sub-Saharan Africa (Senegal, Ghana, Gabon, Ethiopia and Malawi) (Fig. 5). Most of the less frequent haplotypes formed a second group with a more complicated structure, including closed loops and missing haplotypes.

The cyt*b* network displayed a similar star-like pattern for haplotypes from the Middle East and north-east Africa (Egypt and northern Sudan) (Fig. 6). Contrary to the nd1 network, however, cyt*b* haplotypes from sub-Saharan Africa (Senegal, Ghana, Gabon, Ethiopia, Kenya and Malawi) were placed separately, creating two distinct clusters and a solitary haplotype, i.e., the clustering corresponded with the recovered phylogenetic tree. As seen from both the phylogenetic tree and the network, substantial diversity is present in sub-Saharan Africa, while Middle Eastern and north-east African samples form a compact group with closely related haplotypes, three being present at high frequency throughout the area sampled (61.8% of the samples examined). Only two cyt*b* haplotypes disagree with the clear separation of Palaearctic and sub-Saharan samples, haplotype C6 from Ethiopia representing a lineage of its own clustered close to the Middle Eastern lineage, and the C7 haplotype from Yemen clustered within the sub-Saharan lineage (composed of samples from Malawi, Gabon, Ethiopia, Ghana and Senegal).

Discussion

Previous studies have concluded that variation in body size is the most pronounced aspect of geographic variation in *Rousettus aegyptiacus* in the Palaearctic and that, in the northern part of the species' range, this is characterized by the appearance of two distinct size groups representing separate subspecies: a larger-sized form (*R. a. aegyptiacus*) in the northern Middle East and Egypt and a smaller form (*R. a. arabicus*) in the southern Middle East (see Eisentraut 1959; Hayman & Hill 1971; Bergmans 1994; Koopman 1994; Kwiecinski & Griffiths 1999; Simmons 2005). Although, in general, the results of our study confirm this view, refining of geographic scale reveals patterns that tend to contradict it, with incongruent distribution of the respective size groups and/or a predominance of intermediate size characteristics over a considerable part of the species' Palaearctic range, stretching from Cyprus to Oman (see Fig. 4C). The situation in the southern Levant suggests a cline shift in size between the larger Mediterranean morphotype (Turkey, Cyprus and Lebanon) and the smaller desert morphotype [southern Jordan, Sinai and, presumably, southern Israel (Zelenova & Yosef 2003; Benda et al. 2008, 2010)]. In contrast, the small Sinaitic morphotype directly abuts a large morphotype (the largest of its representatives) in continental Egypt, where a very sharp step in size is evident. A further size shift might be expected along the Hijaz Range in western Arabia, from Jordan to Yemen, where individuals of intermediate body size (in the present sense) have previously been reported (see data by Nader 1975). However, very limited material has been examined from this extensive area (but see the discussion by Bergmans 1994: 103). Despite general agreement with difference in body size between the marginal populations within the species' Palearctic range (Egypt and northern Levant *vs.* southern Arabia, Iran and Pakistan), the prevailing pattern of geographic size variation tends to suggest a mosaic pattern rather than continuous distribution of two more or less categorically delimited and broadly distributed phenotypes. Aside from the populations in continental Egypt and the easternmost parts of the Middle East (which only fit the model well), the situation is obviously more complicated in other regions, appearing to include numerous local specificities such as the effects of local habitat conditions (cf. Benda et al. 2008). In conclusion, our results provide little support for the traditional subspecific arrangement of the species in the Palaearctic, a situation further supported by the results of genetic analysis.

In general, we found very low levels of genetic variation in the mt markers studied and a lack of any robust phylogeographic structure throughout the distribution range of *R. aegyptiacus* (including the sub-Saharan regions). Identical haplotypes were found in very distant parts of the range, e.g. the nd1 haplotype N1 (found in 23 specimens or 27.7% of samples analysed) appeared in several populations over the Palaearctic range (Lebanon, Jordan, Sinai, Egypt and Yemen) as well as in Senegal. Similarly, haplotype N2 was found in extremely high numbers, appearing in 36 samples (52.9%) covering the whole Palaearctic range (all countries of origin except Syria) as well as sub-Saharan Gabon and Malawi. Other nd1 haplotypes, differing from each other by a single or few substitutions only (see Fig. 5), also showed quite extensive distribution, such as N3 (Lebanon, Jordan, Yemen and Oman) and N4 (Egypt and Yemen).

Considering the mutual positions of the Palaearctic haplotypes, the pattern of genetic variation demonstrated by analysis of cyt*b* haplotypes differed only slightly from that of nd1 (see Fig. 6). Three haplotypes were most widespread, while a number of adjacent variants differ by one or two substitutions only. The C1 haplotype was observed in 15 samples (22.1% of samples analysed) and found over almost the whole of the Middle East (Cyprus, Lebanon, Jordan, Egypt, Yemen and Oman), while C2 was found in 12 samples (17.6%) from Jordan, Sinai and Iran. The most widely distributed cyt*b* haplotype was C3, found in 15 samples collected from north-eastern Africa (N Sudan and Egypt), eastern Mediterranean (Turkey, Syria, Lebanon

and Jordan) and in Yemen. Although clear separation of the Palaearctic and sub-Saharan samples is obvious within the results of cyt*b* analysis (unlike the nd1 results), two haplotypes disagree with this arrangement: one Ethiopian sample that clusters close to those from the Middle East and one Yemeni sample that groups with the sub-Saharan samples.

The above findings suggest three possible explanations for the evolutionary history of *R. aegyptiacus*: (1) the genes examined (and especially nd1) are highly conservative and not informative for the history of the species; (2) there is an unstructured gene flow over the whole range, mediated by long-distance migrations (at least in females) interconnecting during historical time even the most distant parts of the range (e.g., Senegal to Lebanon, Malawi to Turkey, Cyprus to Oman, etc.); or (3) the contemporary Palaearctic range was colonised relatively recently from a geographically limited population, possibly of Afro-tropical origin.

The difference in phylogeographic signal for the two markers suggests putative validity of hypothesis (1), at least in the case of the nd1 gene. However, many other phylogenetic studies of bats have convincingly demonstrated a considerable degree of within- and between-species variation in the nd1 gene (see for example Mayer et al. 2007; Benda et al. 2004 and Benda & Gvoždík 2010), often even higher than that for the more commonly used cyt*b* gene. The relevance of this argument, and the low level of variation found in both markers, means that one cannot exclude the possibility that genotypic variation in pteropodid bats differs from that in microbats in general, as is the case for genome size (Smith & Gregory 2009). In connection with this, it is worth mentioning that Goodman et al. (2010), when studying genogeographic variation in *Rousettus madagascariensis* Grandidier, 1928, one of the sister taxa of *R. aegyptiacus*, also found no phylogeographic signal in cyt*b* sequences, and very shallow between-population distances (not exceeding 1.1%) throughout the 1,600 km distance between Madagascar and its neighbouring islands. Similarly, Chen et al. (2010) found unexpectedly low variation in mtDNA in fruit bats of the genus *Pteropus* Brisson, 1762 colonising islands in the Indian Ocean. In both cases, the extensive population mixing and continuous gene flow hypothesis, which could explain low genotypic variation, was refuted for lack of any direct support, i.e., no long-distance migrations were observed and the geographic distances between particular sample sites exceeded by a large degree the assumed dispersal capacity of the taxa concerned. Campbell et al. (2004: 774), who found considerable incongruence between patterns of molecular and morphological variation in the *Cynopterus brachyotis* (Müller, 1838) species complex, commented on the unexpectedly low geographic variation in mtDNA in this clade using the following words: "we cannot determine whether the absence of genetic structure in the Sunda lineage is solely a consequence of the effect expected when source populations undergo rapid demographic expansion, or if variation has been further reduced by fixation of a positively selected mitochondrial haplotype via a selective sweep [...]. Thus, without comparison of nuclear markers, we cannot rule out the possibility that this lineage may have a significantly longer and more complex evolutionary history than that inferred from mitochondrial haplotype data." The surprisingly low genetic variation found in Palaearctic *R. aegyptiacus* could be commented on using identical words. An alternative explanation for the lack of phylogeographic signal in the Mediterranean could be based on the effects of recent expansion promoted by post-Neolithic spread of cultural fruit trees. In the case of the sub-Saharan range, however, such an explanation would necessarily have to rely on other mechanisms driving extensive range expansion and/or enormous vagility, i.e., qualities not known for the species and, with regard to its cave-dwelling specialization, quite improbable.

In contrast to some other fruit bat species, such as *Eidolon helvum* (Kerr, 1792) from the Afro-tropics, therefore, there is no direct support for hypothesis (2) with regard to *Rousettus aegyptiacus* as regular mass long-distance migrations have never been observed for the species, despite its considerable capacity for movement (Tsoar et al. 2011). Such a sedentary life history for *R. aegyptiacus* is also supported by the morphological record (see above).

Consequently, hypothesis (3) appears to be the most plausible explanation. The cyt*b* phylogeographic signal appears to conform to a single invasion from Africa and subsequent multiple radiations in the Middle East, followed by episodic changes among populations of north-eastern Africa and south-western Arabia (as the findings of the Yemeni cyt*b* haplotype in Ethiopia and African haplotype in the Middle East suggest; see Fig. 6). The latter two regions can be looked upon as Afro-Arabian transition areas that could serve as gateways for the original invasion as they currently represent the most closely situated parts of the sub-Saharan and Palaearctic parts of the species' range (Bergmans 1994; Kwiecinski & Griffiths 1999).

As demonstrated elsewhere (see the review by Benda et al. 2007), the occurrence of *R. aegyptiacus* in the Mediterranean arboreal zone, as well as in oases of the Saharo-Sindian eremic zone, is strictly controlled by fruit supply in winter. In addition to the autochthonous carob (*Ceratonia siliqua* L., 1758) and date palm (*Phoenix dactylifera* L., 1753), fruit availability includes alien plants such as chinaberry (*Melia azedarach* L., 1753) (Korine et al. 1999). The winter survival of *R. aegyptiacus* in this region, therefore, is closely dependent upon anthropogenic agricultural production; colonisation of the present Mediterranean part of the range being largely contingent on anthropic crop production in the post-Neolithic Holocene period (cf. Bergmans 1994). Indeed, it appears highly probable that most of the species' present Mediterranean range was colonised during the Holocene period. Questions regarding the actual dynamics of the Holocene expansion, including whether *R. aegyptiacus* represents the apo-

choric element of the Holocene in all regions within its Palearctic range or whether some areas were colonised by resident local populations prior to the Holocene radiation, cannot be answered at the present time. Up till now, no fossil record for *R. aegyptiacus* older than the Holocene is available in the Mediterranean Levant (Tchernov 1988) and further studies using more sensitive genetic markers are urgently needed.

This study suggests that the current taxonomic arrangement of *Rousettus aegyptiacus* in the Palaearctic should be revised and the traditional subspecific division refused. Following from this, all Palaearctic *R. aegyptiacus* populations should now be considered as representing one form, the nominotypical subspecies, which is uniform in its genetic traits but plastic in its metric traits. Unfortunately, the exciting questions regarding the origin, history and evolutionary dynamics of this unique biogeographic phenomenon, i.e., the Palaearctic offshoot of the Afro-tropical range of *R. aegyptiacus*, cannot be answered definitively at the present time. These issues need to be re-investigated using an extended line of further evidence and we hope that the present survey of geographic variation within the species has provided a reliable platform for such studies.

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Appendix 1. List of material examined during morphological analysis. Acronyms for the respective sites used in the cluster analysis appear in brackets (Fig. 4).

***Rousettus aegyptiacus* (Geoffroy, 1810)**

Cyprus: 1 m (NMP 90435 [S+A]), Androlikou Gorge, 2 km SW Prodromi [CYP1], 20 April 2005, leg. P. Benda & V. Hanák; – 1 ind. (NMP 90399 [S]), Akamas, Baths of Aphrodite [CYP2], 10 April 2005, leg. P. Benda & V. Hanák; – 1 m (NMP 91274 [S+A]), Akamas, Smigies Trail, Magnesia Mine [CYP3], 27 March 2005, leg. I. Horáček, P. Hulva & R. Lučan.

Egypt: 1 m (NMP 92582 [S+A]), Aswan [EGY1], 24 January 2010, leg. P. Benda, R. Lučan & I. Horáček; – 8 m, 6 f, 1 ind. (IVB 1–3, 5–10, 13–16, 18 [S+B], MUB 1.1.106 [S]), Cairo, Sultan Hamid Mosque [EGY2], 23 April 1969, June 1971, leg. J. Gaisler & J. Groschaft; – 3 m, 7 f (ZFMK 63.267–63.272, 63.274 [S+B], 63.275–63.277 [S]), Cairo, Sultan Hassan Mosque [EGY3], 11 May 1951, leg. H. Hoogstraal; – 1 m (NMP 92570 [S+A]), Bawiti, Bahariya Oasis [EGY5], 18 January 2010, leg. P. Benda, R. Lučan & I. Horáček; – 2 m, 1 f, 2 inds. (ZFMK 62.199, 62.200 [S], 94.499, 94.501 [S+B], 94.502 [S+Sk]), [Lower] Egypt (undef.) [EGY4], coll. Möhres, 17 August 1994, ded. Flughafen Düsseldorf; – 13 inds. (NMP 91817–91820 [S], 92101 [S+A]), Qasr, Dakhla Oasis [EGY6], 17 April 2002, leg. P. Munzlinger & P. Nová, 21 January 2010, leg. P. Benda, R. Lučan & I. Horáček; – 1 m, 1 f (NMP 90527, 90528 [S+A]), Sinai, Ain El Furtaga [SIN1], 16 September 2005, leg. M. Andreas, P. Benda, J. Hotový & R. Lučan; – 1 m (NMP 90520 [S+A]), Sinai, Ain Hudra [SIN2], 14 September 2005, leg. M. Andreas, P. Benda, J. Hotový & R. Lučan; – 5 m, 6 f (NMP 90501, 90510 [Sk], 90502–90509, 90511 [S+A]), Sinai, Feiran [SIN3], 10 September 2005, leg. M. Andreas, P. Benda, J. Hotový & R. Lučan.

Ethiopia: 3 m, 5 f (NMP 92170–92176 [S+A], JOC unnumbered [S+Sk]), Gilo River bridge, 5 km S of Tepi [ETH1], 8 May 2003, leg. P. Benda & J. Obuch.

Gabon: 1 f (BMNH 62.8.26.1. [S+B], type of *Eleutherura unicolor* Gray, 1870), Afrique Occidentale (Gabon) [GAB1], purch. Verreaux.

Iran: 5 m, 5 f (NMP 48377–48386 [S+A]), Espakeh [IRN1], 10 April 2000, leg. P. Benda & A. Reiter; – 3 m, 6 f (NMP 40467 [S+B]), Isin [IRN2], 29 April & 2 May 1977, leg. B. Pražan.

Jordan: 1 m (NMP 92558 [S+A]), Kufranja, Iraq al Wahaj Cave [JOR1], 26 May 2009, leg. P. Benda, A. Reiter & J. Obuch; – 1 m, 1 f (NMP 92362, 92411 [S+A]), Wadi as Sir, Iraq al Amir Cave [JOR2], 10 October 2008, 10 May 2009, leg. P. Benda, A. Reiter & J. Obuch; – 1 m, 7 f (NMP 92430, 92431, 92433, 92434, 92436, 92438, 92440, 92442 [S+A]), Wadi Dhana, 4 km ENE of Feinan Ecolodge [JOR3], 14 May 2009, leg. P. Benda, A. Reiter & J. Obuch.

Lebanon: 1 m (AUB M021 [S]), Antelias [LEB1], 19 March 1960, leg. J. E. Stencel; – 1 m, 1 f (NMP 91799, 91910 [S+A]), Antelias [LEB1], Kassarat Cave, 25 January 2007, 25 January 2008, leg. P. Benda, R. Černý, I. Horáček, R. Lučan & M. Uhrin; – 1 f (AUB M006 [S]), cave 4 km SE of Beit Meri [LEB2], 4 October 1959, leg. R. E. Lewis; – 1 m, 1 f (NMP 91904, 91905 [S+A]), Dahr el Mghara, Mgharet el Aaonamie cave [LEB3], 19 January 2008, leg. P. Benda, I. Horáček, R. Lučan & M. Uhrin; – 2 f (NMP 93697, 93699 [S+A]), Jeita Cave [LEB4], 20 March 2009, leg. T. Bartonička, P. Benda, I. Horáček & R. Lučan; – 2 m, 1 f (NMP 91765, 91766, 91899 [S+A]), Tarabulus, Mtal al Azraq Cave [LEB5], 21 January 2007, 18 January 2008, leg. P. Benda, R. Černý, I. Horáček, R. Lučan & M. Uhrin.

Oman: 1 f (NMP 92733 [S+A]), Ain Jarziz [OMA1], 27 October 2009, leg. P. Benda, A. Reiter & M. Uhrin; – 1 m, 1 f (NMP 92679, 92680 [S+A]), Al Nakhar [OMA2], 22 October 2009, leg. P. Benda, A. Reiter & M. Uhrin; – 1 f (NMP 92654 [S+A]), Dhahir Al Fawaris [OMA3], 21 October 2009, leg. P. Benda, A. Reiter & M. Uhrin; – 1 m (NMP 92778 [S+A]), wadi 7 km W of Dibab [OMA4], 2 November 2009, leg. P. Benda, A. Reiter & M. Uhrin; – 1 m (NMP 92651 [S+A]), Khutwa [OMA5], 20 October 2009, leg. P. Benda, A. Reiter & M. Uhrin; – 1 m (NMP 92756 [S+A]), Taiq [OMA6], 30 October 2009, leg. P. Benda, A. Reiter & M. Uhrin.

Pakistan: 2 m (BMNH 20.1.17.1., 20.1.17.2. [S]), Karachi, Sind [PAK1], leg. C. B. Tycehurst; – 2 m, 1 f (BMNH 19.11.7.1., 19.11.7.2., 19.11.7.4. [S]), Panjgur, Baluchistan [PAK2], leg. J. E. Hotson.
Senegal [SEN1]: 1 m, 2 f (IVB S172–174 [S+B]), Dakar, 22 October 2004, leg. J. Červený & P. Koubek; – 1 m (IVB S1267 [S+A]), Niokolo, 19 August 2006, leg. J. Červený & P. Koubek.
Syria: 1 m, 1 f (NMP 48865, 48866 [S+A]), Talsh'hab [SYR1], 25 May 2001, leg. M. Andreas, P. Benda, A. Reiter & D. Weinfurtová; – 1 m, 1 f (NMP 48264, 48265 [S+A]), Ya'ar Oden forest (Golan Heights) [SYR2], 18 July 1999, leg. P. Benda.
Turkey: 1 m (ZFMK 65.205 [S+B]), Dermustlu Köy, Höhle bei Antakya [TUR1], 2 January 1952, leg. H. Kumerloeve.
Yemen: 3 m, 2 f (NMP pb3112–pb3116 [S+A]), 5 km W of Hammam Ali [YEM1], 27 October 2005, leg. P. Benda; – 2 f (NMP pb3056, pb3057 [S+A]), Al Khuraybah, Wadi Daw'an [YEM2], 19 October 2005, leg. P. Benda; – 4 m, 2 f (NMP pb3628–3630, 3632, 3633 [S+A], pb3631 [A]), Assala at Mashgab, S of Taiz [YEM3], 26 October 2007, leg. P. Benda & A. Reiter; – 1 m (NMP pb3758 [S+A]), Halhal, 10 km NE Hajja [YEM4], 2 November 2007, leg. P. Benda & A. Reiter; – 2 m, 1 f (NMP pb2959–pb2961 [S+B]), Hawf [YEM5], 12 October 2005, leg. P. Benda; – 2 f (NMP pb3118, pb3119 [S+B]), Jebel Bura, W of Riqab [YEM6], 30 October 2005, leg. P. Benda; – 1 m (BMNH 95.6.1.47. [S+B], holotype of *Rousettus arabicus* Anderson, 1902), Lahej, Aden; shot near Sultans garden [YEM7], 21 March 1895, leg. G. W. Yerbury; – 2 m (NMP pb2943, pb2944 [S+A]), Ma'arib [YEM8], 9 October 2005, leg. P. Benda; – 1 m (NMP pb2956 [S+A]), Sah, Wadi Hadramawt [YEM9], 11 October 2005, leg. P. Benda; – 1 f (NMP pb3159 [A]), Wadi Al Lahm, W of Al Mahwit, 1 October 2005, leg. P. Benda; – 1 m (NMP pb2917 [S+A]), Wadi Dhahr, 15 km N of Sana'a [YEM10], 6 October 2005, leg. P. Benda; – 2 f (NMP pb3089, pb3090 [S+A]), Wadi Maytam, 12 km SE of Ibb [YEM11], 26 October 2005, leg. P. Benda; – 1 m (NMP pb3728 [S+A]), Wadi Zabid, SE of Al Mawkir [YEM12], 30 October 2007, leg. P. Benda & A. Reiter.

***Rousettus leschenaultii* (Desmarest, 1820)**
Pakistan: 1 ind. (BMNH 69.484 [S]), Lahore, Old Tomb, W Pakistan [PAK3], 2 February 1968, leg. T. J. Roberts; – 2 m, 1 f (BMNH 67.1105, 67.1106 [S]), Lahore, Tomb of Ali Mordan Khan [PAK3], W Pakistan, 16 November 1966, leg. T. J. Roberts.

***Rousettus lanosus* Thomas, 1906**
Ethiopia: 5 m, 2 f (NMP 92181–92187 [S+A]), Baro River bridge, 15 km N of Masha [ETH2], 9 May 2003, leg. P. Benda & J. Obuch.
Uganda: 1 m (BMNH 6.4.1.2. [S+A], holotype of *Rousettus lanosus* Thomas, 1906), Ruwenzori [UGA2].

***Lissonycteris angolensis* (Bocage, 1898)**
Angola: 1 f (BMNH 97.8.6.1. [S+A], type of *Cynopterus angolensis* Bocage, 1898), Quihula, Benguella [ANG1].
Sierra Leone: 1 f (BMNH 8.9.11.1. [S+B], holotype of *Rousettus smithii* Thomas, 1908), Sierra Leone [SLE1], leg. Canon F. C. Smith.
Uganda: 4 f (MSNG 15303–d [S+B]), Arcip. Sesse: Maiba [UGA1], 1908, leg. E. Bayon; – 2 m (MSNG 13616a, b [S+B]), Bukasa (Iss. Sesse) [UGA1], 15 June 1908, leg. E. Bayon; – 2 m, 1 f (BMNH 6.12.4.1. [S+B], holotype of *Rousettus angolensis ruwenzorii* Eisentraut, 1965, MSNG 6414, 6415 [S+B]), Ruwenzori East [UGA1], 5500 ft., 8 & 12 March 1906, leg. R. R. Dent.

Appendix 2. List of material examined during molecular genetic analysis; nd1, cyt*b* = haplotypes of the respective genes.

nd1		cyt*b*		Voucher	Country	Site	Coordinates
haplotype	GenBank Acc. No.	haplotype	GenBank Acc. No.				
Rousettus aegyptiacus							
N2	JX274457	–	–	NMP 93850	Turkey	Sayköy, W of Tarsus	36°57′N, 34°47′E
N2	–	C3	JX274479	NMP 93851	Turkey	Sayköy, W of Tarsus	36°57′N, 34°47′E
N2	–	C3	–	NMP 93852	Turkey	Harbiye	36°09′N, 36°08′E
N2	–	C1	JX274474	NMP 90435	Cyprus	Prodromi, Androlikou Gorge	35°00′N, 32°23′E
N2	–	–	–	NMP 91274	Cyprus	Neo Horio, Magnesia Mine	35°03′N, 32°20′E
N17	JX274446	–	–	NMP 48865	Syria	Talsh'hab	32°42′N, 35°58′E
N11	JX274452	–	–	NMP 48867	Syria	Talsh'hab	32°42′N, 35°58′E
N13	JX274450	C3	–	NMP 48264	Syria	Ya'ar Oden, Golan	33°12′N, 35°46′E
N16	JX274447	C13	JX274482	NMP 91909	Lebanon	Aamchite, Saleh Cave	34°09′N, 35°40′E
N2	–	–	–	NMP 91799	Lebanon	Antelias, Kassarat cave	33°55′N, 35°36′E
N3	JX274462	C1	–	NMP 91910	Lebanon	Antelias, Kassarat cave	33°55′N, 35°36′E
N2	–	C1	–	NMP 93697	Lebanon	Jeita Cave	33°57′N, 35°39′E
N2	–	C3	–	NMP 93699	Lebanon	Jeita Cave	33°57′N, 35°39′E
N2	–	C3	–	NMP 91765	Lebanon	Tarabulus, Mtal al Azraq Cave	34°25′N, 35°50′E
N1	JX274456	C1	–	NMP 91766	Lebanon	Tarabulus, Mtal al Azraq Cave	34°25′N, 35°50′E
–	–	C3	–	NMP 91899	Lebanon	Tarabulus, Mtal al Azraq Cave	34°25′N, 35°50′E
N1	–	C1	–	NMP 93712	Lebanon	Wadi Jilo	33°14′N, 35°19′E
N1	–	–	–	*biopsy*	Israel	Beit Oren	32°43′N, 35°01′E
N2	–	C2	JX274468	*biopsy*	Jordan	Jufat al-Qafrayn	31°53′N, 35°37′E
N7	JX274458	C2	–	*biopsy*	Jordan	Jufat al-Qafrayn	31°53′N, 35°37′E
N3	–	C14	JX274483	NMP 92558	Jordan	Kufranja, Iraq al Wahaj Cave	32°19′N, 35°43′E

Appendix 2. (continued)

nd1		cyt*b*		Voucher	Country	Site	Coordinates
haplotype	GenBank Acc. No.	haplotype	GenBank Acc. No.				
N2	–	C1	–	*biopsy*	Jordan	Nahla	32°17′N, 35°49′E
N1	–	–	–	NMP 47975	Jordan	Tabaqat Fahl	32°27′N, 35°37′E
N2	–	C2	–	*biopsy*	Jordan	Wadi al Walah	31°33′N, 35°44′E
N1	–	C3	–	NMP 92362	Jordan	Wadi as Sir, Iraq al Amir	32°13′N, 35°53′E
N2	–	C3	–	NMP 92411	Jordan	Wadi as Sir, Iraq al Amir	32°13′N, 35°53′E
N2	–	C2	–	NMP 92440	Jordan	Wadi Dhana, near Feinan	30°39′N, 35°32′E
N1	–	–	–	NMP 92430	Jordan	Wadi Dhana, near Feinan	30°39′N, 35°32′E
N1	–	–	–	NMP 90527	Egypt	Sinai, Ein El Furtaga	29°03′N, 34°33′E
N2	–	C2	–	NMP 90528	Egypt	Sinai, Ein El Furtaga	29°03′N, 34°33′E
N1	–	C2	–	NMP 90520	Egypt	Sinai, Ein Khudra	28°54′N, 34°25′E
N1	–	–	–	NMP 90503	Egypt	Sinai, Wadi El Feiran	28°42′N, 33°40′E
–	–	C30	JX274496	NMP 90504	Egypt	Sinai, Wadi El Feiran	28°42′N, 33°40′E
N1	–	C2	–	NMP 90508	Egypt	Sinai, Wadi El Feiran	28°42′N, 33°40′E
N1	–	C2	–	NMP 90509	Egypt	Sinai, Wadi El Feiran	28°42′N, 33°40′E
N1	–	C3	–	NMP 92582	Egypt	Aswan	24°07′N, 32°54′E
N2	–	C1	–	NMP 92570	Egypt	Bahariya, Bawiti	28°21′N, 28°52′E
N16	–	C3	–	*biopsy*	Egypt	Cairo, Zamalek, Fish Garden	30°03′N, 31°13′E
N4	JX274461	C3	–	*biopsy*	Egypt	Cairo, Zamalek, Fish Garden	30°03′N, 31°13′E
N2	–	C1	–	*biopsy*	Egypt	Cairo, Zamalek, Fish Garden	30°03′N, 31°13′E
N2	–	C3	–	NMP 92101	Egypt	Dakhla, Qasr	25°42′N, 28°53′E
N1	–	C3	–	NMP 92102	Egypt	Dakhla, Qasr	25°42′N, 28°53′E
–	–	C27	JX274486	NMP 93677	Sudan	Ferqa	20°54′N, 30°35′E
–	–	C3	–	NMP 93678	Sudan	Ferqa	20°54′N, 30°35′E
N3	–	–	–	NMP pb3056	Yemen	Al Khuraybah, Wadi Daw'an	15°09′N, 48°26′E
N2	–	–	–	NMP pb3057	Yemen	Al Khuraybah, Wadi Daw'an	15°09′N, 48°26′E
N1	–	C1	–	NMP pb3629	Yemen	Assala, Mashgab	13°21′N, 43°57′E
N1	–	–	–	NMP pb3630	Yemen	Assala, Mashgab	13°21′N, 43°57′E
N2	–	C1	–	NMP pb3631	Yemen	Assala, Mashgab	13°21′N, 43°57′E
N1	–	C12	JX274481	NMP pb3758	Yemen	Halhal, NE of Hajja	15°44′N, 43°37′E
N2	–	–	–	NMP pb3113	Yemen	Hammam Ali	14°41′N, 44°07′E
N5	JX274460	–	–	NMP pb3115	Yemen	Hammam Ali	14°41′N, 44°07′E
N3	–	C8	JX274476	NMP pb2959	Yemen	Hawf	16°40′N, 53°05′E
N1	–	C1	–	NMP pb2960	Yemen	Hawf	16°40′N, 53°05′E
N2	–	C9	JX274477	NMP pb2961	Yemen	Hawf	16°40′N, 53°05′E
N1	–	C10	JX274478	NMP pb3118	Yemen	Jebel Bura, W of Riqab	14°52′N, 43°25′E
N2	–	C1	–	NMP pb3119	Yemen	Jebel Bura, W of Riqab	14°52′N, 43°25′E
N1	–	–	–	NMP pb2943	Yemen	Ma'arib	15°24′N, 45°16′E
N2	–	C1	–	NMP pb2944	Yemen	Ma'arib	15°24′N, 45°16′E
N10	JX274453	–	–	NMP pb2956	Yemen	Sah, Wadi Haramawt	15°41′N, 48°52′E
N4	–	C3	–	NMP pb3159	Yemen	Wadi Al Lahm, W of Al Mahwit	15°26′N, 43°29′E
N6	JX274459	–	–	NMP pb2917	Yemen	Wadi Dhahr, N of Sana'a	15°27′N, 44°08′E
N1	–	C7	JX274475	NMP pb2918	Yemen	Wadi Dhahr, N of Sana'a	15°27′N, 44°08′E
N2	–	–	–	NMP pb3089	Yemen	Wadi Maytam, SE of Ibb	13°52′N, 44°18′E
N1	–	C11	JX274480	NMP pb3728	Yemen	Wadi Zabid, SE of Al Mawkir	14°10′N, 43°30′E
N3	–	–	–	NMP 92733	Oman	Ain Jarziz	17°06′N, 54°05′E
N2	–	–	–	NMP 92735	Oman	Ain Tabruq	17°06′N, 54°20′E
N2	–	C4	JX274497	NMP 92679	Oman	Al Nakhar	23°12′N, 57°13′E
N2	–	–	–	NMP 92680	Oman	Al Nakhar	23°12′N, 57°13′E
N2	–	C16	JX274485	NMP 92654	Oman	Dhahir Al Fawaris	23°39′N, 56°39′E
N3	–	C1	–	NMP 92714	Oman	Hagarir	16°42′N, 53°09′E
N16	–	C15	JX274484	NMP 92651	Oman	Khutwa	24°19′N, 56°08′E
N2	–	C1	–	NMP 92756	Oman	Taiq	17°09′N, 54°37′E
N2	–	C4	–	NMP 92778	Oman	Wadi Dibab	23°04′N, 58°59′E
N2	–	C2	–	NMP 48379	Iran	Espakeh	26°48′N, 60°10′E
N2	–	C2	–	NMP 48381	Iran	Espakeh	26°48′N, 60°10′E
N14	JX274449	–	–	NMP 48382	Iran	Espakeh	26°48′N, 60°10′E
N2	–	C2	–	NMP 48380	Iran	Espakeh	26°48′N, 60°10′E
–	–	C2	–	NMP 48383	Iran	Espakeh	26°48′N, 60°10′E
N2	–	–	–	IVB S1316	Senegal	Mako	12°51′N, 12°21′W
N1	–	C26	JX274495	IVB S1267	Senegal	Niokolo	13°04′N, 12°43′W
N1	–	–	–	IVB S1000	Senegal	Wassadou	12°53′N, 11°51′W
N12	JX274451	C22	JX274487	IVB pv113	Gabon	Belinga Mts	00°59′N, 13°12′E
–	–	C23	JX274488	IVB pv114	Gabon	Belinga Mts	00°59′N, 13°12′E
–	–	C24	JX274489	IVB pv115	Gabon	Belinga Mts	00°59′N, 13°12′E
N2	–	C25	JX274490	IVB pv116	Gabon	Belinga Mts	00°59′N, 13°12′E
–	–	C20	JX274491	IVB pv051	Ghana	Buoyem	07°43′N, 01°59′W
N15	JX274448	C21	JX274492	IVB pv052	Ghana	Buoyem	07°43′N, 01°59′W

Appendix 2. (continued)

nd1		cyt*b*		Voucher	Country	Site	Coordinates
haplotype	GenBank Acc. No.	haplotype	GenBank Acc. No.				
N9	JX274454	C5	JX274469	NMP 92170	Ethiopia	Gilo River, S of Tepi	07°07′N, 35°26′E
N13	–	C6	JX274470	NMP 92175	Ethiopia	Gilo River, S of Tepi	07°07′N, 35°26′E
N8	JX274455	–	–	NMP 92176	Ethiopia	Gilo River, S of Tepi	07°07′N, 35°26′E
–	–	C28	JX274493	NMP rs458	Kenya	Mt. Elgon	01°02′N, 34°47′E
–	–	C29	JX274494	NMP rs459	Kenya	Mt. Elgon	01°02′N, 34°47′E
N2	–	C17	JX274465	NMP mw128	Malawi	Mulanje-Chitakali	16°02′S, 35°31′E
–	–	C18	JX274466	NMP mw131	Malawi	Mulanje-Chitakali	16°02′S, 35°31′E
N13	–	C19	JX274467	NMP mw194	Malawi	Mpalanganga	15°27′S, 35°15′E
Rousettus lanosus							
N18	JX274444	C31	JX274471	NMP 92182	Ethiopia	Baro River, N of Masha	07°52′N, 35°29′E
N19	JX274445	C32	JX274472	NMP 92183	Ethiopia	Baro River, N of Masha	07°52′N, 35°29′E
–	–	C33	JX274473	NMP 92186	Ethiopia	Baro River, N of Masha	07°52′N, 35°29′E
Lissonycteris angolensis							
N20	JX274443	C34	JX274463	NMP 90938	Malawi	Zomba Plateau	15°21′S, 35°17′E
N20	–	C35	JX274464	NMP 90939	Malawi	Zomba Plateau	15°21′S, 35°17′E