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Phylogeny and systematics of Old World serotine bats (genus *Eptesicus*, Vespertilionidae, Chiroptera): an integrative approach

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Integrative taxonomy aims to document biodiversity by incorporating all useful characters to increase confidence in hypotheses about phylogenetic relationships. In this study, we combine data obtained independently from morphology, two maternally inherited mtDNA genes and two biparentally inherited nuDNA genes to make phylogenetic and taxonomic hypotheses about the Palaearctic members of the bat genus *Eptesicus* (Vespertilionidae). This genus is distributed worldwide (except for Antarctica) and is highly diversified, presenting one of the most entangled taxonomic puzzles among all mammals. Our results support restoring the genus *Rhyneptesicus* and separating *E. isabellinus* and *E. pachyomus* from *E. serotinus* and *E. ognevi* and *E. anatolicus* from *E. bottae*. Differences in the phylogenetic hypotheses from mtDNA and nuDNA data suggest the occurrence within *E. serotinus* of evolutionary processes such as mtDNA capture and secondary contacts between partially differentiated ecomorphs. These two evolutionary processes deserve more in-depth studies within the group.

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Introduction

DNA-based approaches provide an extraordinary powerful tool for studying evolutionary relationships among organisms. They are particularly helpful in disentangling relationships and clarifying taxonomy within groups, such as bats in which morphology has been tightly constrained by functional or ecological pressures and therefore may be of limited value in species recognition. In fact, molecular techniques have helped clarify misleading morphological arrangements resulting from convergent evolution (Ruedi & Mayer 2001) or from morphological conservatism and its related cryptic diversity. Cryptic diversity has been overlooked by traditional taxonomy (Bickford *et al.* 2007) and appears to be particularly important in many groups of bats such as vespertilionids (e.g. Mayer & von Helversen 2001; Ibáñez *et al.* 2006; Mayer *et al.* 2007; Moratelli *et al.* 2011).

Unfortunately for taxonomists, genes often differ in their evolutionary pathways and as a result they often disagree in their species definition hypotheses (e.g. Edwards 2008; Degnan & Rosenberg 2009). This disagreement among data sets brings about a big potential for confusion in their derived taxonomic inferences. Among other proposed solutions, the integrative approach (Padial *et al.* 2010) aims to incorporate all the data types available in an increasing confidence-building process to document biodiversity. The rationale is that congruence among data sets is strong evidence that the underlying historical pattern is being

recovered, and that the taxonomic conclusions derived from them are robust and stable.

The bat genus *Eptesicus* Rafinesque, 1820 (Vespertilionidae) poses one of the most entangled taxonomic puzzles among mammals. It consists in an evolutionary successful group of open-flyers bats that lived in a wide variety of environments (from forests to xeric shrubs) and that was once considered distributed in temperate and tropical areas across all continents except Antarctica (Hill & Smith 1984). *Eptesicus* was later restricted geographically to the Palaearctic, Africa and the Americas, based on skull and baculum structure and on banded karyotyping (Hill & Harrison 1987; Volleth & Tideman 1989; Volleth & Tidemann 1991; Volleth *et al.* 2001; Kearney *et al.* 2002). Recently, molecular studies of the phylogenetic relationships within the family Vespertilionidae have shown that the American *Eptesicus* are paraphyletic with regard to the Palaearctic forms, and the definition of the genus Eptesicus was extended to include also the American genus *Histiotus* (Hoofer & Van Den Bussche 2003; Hoofer *et al.* 2006; Rohers *et al.* 2010). Even before this change, the number of recognized species within the genus varied significantly according to the authors and all arranged by Simmons (2005) in three main groups (*nasutus*, *nilssonii* and *serotinus*). The highest diversity within the Palaearctic *serotinus* is found in the Middle East, where different forms distinguished by coloration, dental and skull features and habitat preferences have been known for over a century. However, their taxonomic relationships have long been disputed. They have been included in or split from the two main species *E. serotinus* and *E. bottae*. Moreover, recent molecular approaches have suggested, based only on mitochondrial DNA, species rank for the taxa *E. isabellinus* and *E. anatolicus* (Ibáñez *et al.* 2006; Mayer *et al.* 2007; Artyushin *et al.* 2009); this arrangement is also supported by morphological differences (Benda *et al.* 2004, 2006).

From the analyses of mitochondrial (mtDNA) and nuclear DNA (nuDNA)markers, and using an extensive sampling throughout its vast putative distribution area (from the Atlantic to the Pacific Ocean), we have studied the taxonomy and evolutionary relationships of *E. serotinus* together with its close *E. bottae* and examined the validity of most of the taxa described within the subgenus *Eptesicus* as defined by Hill & Harrison (1987) and recognized by Simmons (2005), paying special attention to the forms described from and around the Mediterranean Basin. Benda *et al.* (2006, 2007, 2010, 2011) used morphological characters to revise the taxonomy of most of the specimens examined in this molecular study. This information permits us to evaluate the taxonomic position of all these taxa using an integrative approach, comparing the conclusions obtained independently from morphology, two maternally inherited mtDNA genes and two biparentally inherited nuDNA genes and accepting with confidence only those taxonomic conclusions in which a higher corroboration is obtained by the different approaches (Padial & De la Riva 2010).

Material and methods

Sampling

A total of 128 bats from 26 countries in Europe, Africa, America and Asia were included in the study (Appendix 1). The in-group comprises 102 individuals belonging to the genus *Eptesicus*. The morphological assignment of the vouchers in the studies by Benda *et al.* (2006, 2007, 2010, 2011) is used as a starting taxonomic consideration in this study. Sampling includes the extremes of the distribution area of *E. serotinus*, (from England to Laos) as well as several European populations of the species. We include also samples of putative Palaearctic sister species to *E. serotinus* (Fig. 1) and *E. bottae* (Fig. 2) from the Middle East to China and Laos, plus *E. furinalis*, *E. diminutus* and *E. fuscus* from America and *E. hottentotus* from South Africa, all included within the '*serotinus*' group (as defined by Hill & Harrison 1987). Additionally, we included samples from the related species *E. nasutus* and *E. nilssonii*. To study the evolutionary relationships of the genus *Eptesicus* within the family, we included in the analyses of 26 specimens belonging to 14 species representing most of the groups defined within the subfamily Vespertilioninae. A detailed list of the specimens analysed is provided in Appendix 1.

Sequencing

Genomic DNA was extracted from tissue samples preserved in alcohol by proteinase K digestion and standard phenol–chloroform protocol (Higuchi *et al.* 1988; Maniatis *et al.* 1989). After trying different primer combinations, fragments of the two mtDNA genes, Cytochrome *b* (*Cytb*) and NADH dehydrogenase (*ND1*), were amplified from all samples with the primer pairs MOLCIT-F (Ibáñez *et al.* 2006) and MVZ-16 (Smith & Patton 1993), and ND1-F2 and ND1-R (Kawai *et al.* 2002), respectively. The amplifications for both fragments were carried out in a volume of 20 μL containing 0.1% BSA, 2.5 mM $MgCl_2$, 0.5 μL of each primers, 0.2 mM of each dNTP, 0.5 units of taq-polymerase with appropriate buffer and H_2O. *Cytb* thermocycling consisted of a four-minute initial denaturation at 94 °C followed by 35 cycles of 60 s at 94 °C, 30 s at 45–50 °C, 90 s at 72 °C and then a final extension of 10 min at 72 °C. For the *ND1* fragment, thermocycling was the same except that the annealing temperature was 60 °C. A fragment of the nuDNA gene recombination-activating gene (*RAG2*) was amplified using the primers RAG2-F1, RAG2-R2, RAG2-R1 and RAG2-F1int (Baker *et al.* 2000)

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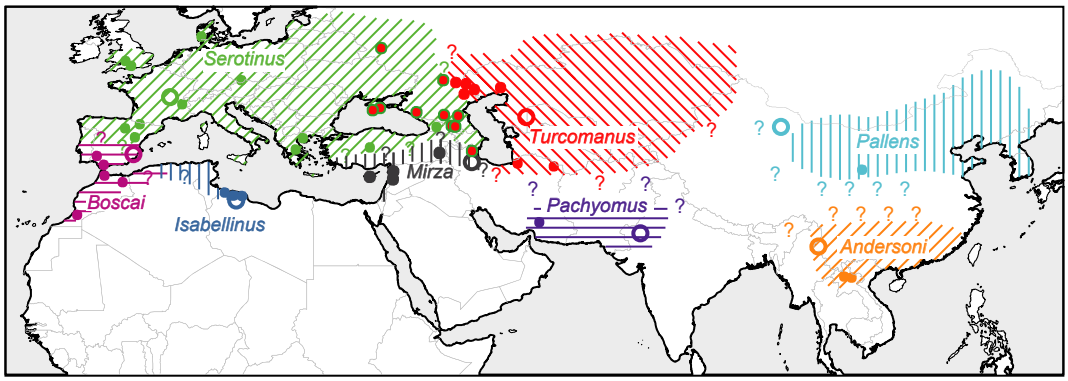

Fig. 1 Approximate distribution map of the taxa included within *Eptesicus serotinus* according to Simmons (2005) and morphologically identified by Benda *et al.* (2006, 2007, 2010, 2011) and used in this study plus the recently studied by Artyushin *et al.* (2009). Open circles indicate the type localities for each taxon and full-coloured circles indicate sampling localities for this paper. Two-coloured cycles indicate individuals with morphological and mitochondrial taxonomic disagreement.

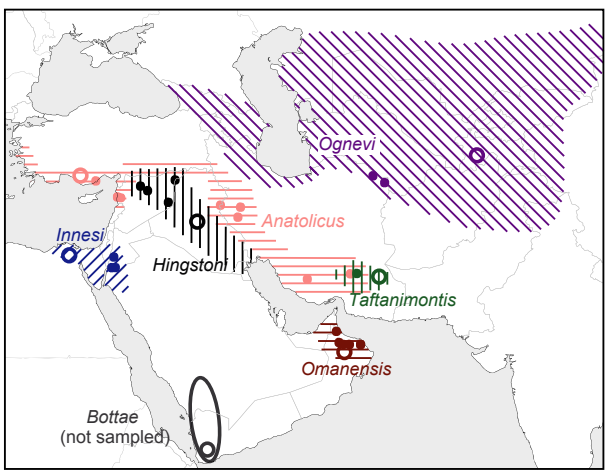

Fig. 2 Approximate distribution map of the taxa included within *Eptesicus bottae* according to Simmons (2005) and morphologically identified by Benda *et al.* (2006, 2007, 2010, 2011) and used in this study. Open circles indicate the type localities for each taxon and full-coloured circles indicate sampling localities for this paper.

as internal primers. In this case, the PCRs were conducted with 0.75 μL of each primer and 2 mM of $MgCl_2$. Thermocycler steps for this nuclear gene were as follows: a 2-min initial denaturation at 94 °C followed by 35 cycles of 60 s at 94 °C, 30 s at 45 °C, 90 s at 72 °C and then a final extension of four minutes at 72 °C. As a second nuDNA marker, we sequenced the intron 4 of the X-linked gene *BGN* using the primers BGN-F and BGN-R (Lyons *et al.* 1997). Genes linked to sex chromosomes are known to evolve faster than autosomes due to their smaller effective population size. PCRs were carried out for this gene in 20-μL simplex reactions consisting of 2 μL DNA (10 ng/μL), 2.0 μL 10X PCR buffer without $MgCl_2$, 0.8 μL $MgCl_2$ (1.5 mM), 0.16 μL dNTPs (25 mM), 1 μL of each primer (10 μM), 0.12 μL (1 U) Taq DNA polymerase and ddH_2O. Thermocycling consisted of 10-min initial denaturation at 95 °C followed by 30 cycles of 15 s at 95 °C, 30 s at 55 °C, 60 s at 72 °C and then a final extension of 5 min at 72 °C. All PCR products were purified and most of them sequenced in both directions using an ABI 3100 automated sequencer (PE Biosystems, Warrington, UK), following the manufacturer's protocols. Sequences from a few samples were obtained after repeating the sequencing with only the forward primer and several times until the ambiguities could be solved. The molecular sequences generated by this study have been deposited in GenBank under the accession numbers listed in Appendix 1.

Sequence analyses

The evolutionary relationships within the genus *Eptesicus* were reconstructed independently from the mtDNA and the nuDNA data set. The *Cytb* and *ND1* fragments and the *RAG2* and the *BGN* genes were concatenated into single sequences respectively following Wiens (1998), and because no highly supported incongruence was found comparing reconstructions from each single marker (Figs S1 and S2). In fact, both mtDNA markers produced the same clusters with almost identical internal relationships, whereas the differences in the two nuDNA markers were clearly associated with differences in resolution between the genes, being *RAG2* more conserved and leaving unsolved many terminal groupings (Figs S1 and S2). All reconstructions were rooted with the species *Myotis myotis* and/or *M. schaubi* from the closer and recently recognized by subfamily Myotinae (Hoofer & Van Den Bussche 2003).

For both mtDNA and nuDNA data set phylogenetic, hypotheses were obtained using three optimality criteria: maximum parsimony (MP), maximum-likelihood (ML) and

Bayesian posterior probabilities (BPP). MP phylogenetic analyses were conducted using PAUP* 4.0b10 (Swofford 2001), ML analyses were implemented in PhyML (Guindon & Gascuel 2003) and BPP were performed in MrBAYES v. 3.1.2. (Huelsenbeck & Ronquist 2001). Under MP, trees were obtained after heuristic search with an initial tree obtained by stepwise addition (random input order) of the taxa, followed by a complete tree-bisection–reconnection (TBR) branch swapping. This process was repeated 25 times. Topologies were obtained both by unweighting changes and differentially weighting transversions according to likelihood estimates of ts/tv ratios for each data set to take into account the heterogeneity of the sequences. The robustness for each topology was then assessed through bootstrapping (Felsenstein 1985) after 2000 iterations. In both (mtDNA and nuDNA) data set, complex models were selected using the Akaike information criterium implemented in JMODELTEST 0.1 (Posada 2008). Accordingly, for the following analyses (MLs and BPPs), substitution models were used with all parameters allowed to vary and empirically estimated. Under ML, trees were obtained using PhyML fast algorithm (Guindon & Gascuel 2003) implemented on line (http://www.atgc-montpellier.fr/phyml) to perform Nearest Neighbour Interchanges (NNIs) and using a BIONJ distance-based tree as starting trees. Bootstrap values were obtained after 1000 replicates. The BPPs were obtained with random starting trees without constraints and the data set partitioned: (i) by character position (six partitions) allowing specific rates to vary across sites and (ii) by gene (two partitions), being in this case, model parameters estimated independently for each fragment. For both designs, the Bayesian topologies that were obtained after five simultaneous Markov chains were run for 3 million generations; trees were sampled every 300 generations. The resulting burn-in values were determined empirically after likelihood scores reached stationary values. Analyses were repeated in two separate runs to ensure that trees converged on the same topology and similar parameters. The best-fitting partitioning model was chosen estimating the Bayes factor (BF) between the two alternatives and for each data set. We calculated the BFs using the differences between the harmonic mean (HM) of the likelihood scores from each posterior distribution as an approximation to the differences between marginal likelihoods. According to Pagel & Meade (2006), a BF value >10 was considered as strong support of the alternative model.

The genetic differentiations within and between groups were estimated according to a Kimura 2-parameter (K2P) model and for the *Cytb* fragment using MEGA v. 5 (Tamura *et al.* 2011) to produce a measure of a 'standardized genetic distance' between taxa.

Results

For each fragment, alignments were obtained with SEQUENCHER v. 4.1 (GeneCodes, Corp., Ann Arbor, MI, USA) and inspected visually for ambiguities. Due to differences in the quality of the DNA related to the variety of conservation conditions and origins, amplification success varied greatly among samples. Therefore, mtDNA sequences were trimmed for each marker to a fragment in which peaks could unequivocally be assigned for all individuals. Neither incongruent sequences or stop-codon/indels (indicating possible nuclear copies) nor double peaks (evidencing heteroplasmy) were found in the selected fragment for any sequence. In non-coding sequences, indels were corrected manually to minimize alignment gaps. A unique 234-bp insertion present only in the *BGN* fragment of *E. anatolicus* was excluded from the alignment to avoid possible homology uncertainty (Dool *et al.* in press).

In the final mtDNA alignment, and to avoid losing significant lineages, the sequences of the *Cytb* and *ND1* genes were trimmed to a length of 755 bp and 665 bp respectively for a total of 120 sequences (see Appendix 1). The combined alignment consisted of 1420 positions of which 742 characters were constant, 65 parsimony uninformative and 613 parsimony informative. The equally weighting MP heuristic search retained seven equally most-parsimonious trees of 3402 steps (first tree: CI = 0.31; HI = 0.69; RI = 0.83). Down-weighting transitions (1:9), the search produced 12 best trees of 2900 steps (first tree CI = 0.57; HI = 0.42; RI = 0.91). Both MP designs recovered a similar consensus topology with also similar bootstrap values, although slightly higher when weighting transversions. Consequently, the results of down-weighting transitions are the only presented (Fig. 3). ML topology (not shown) was very similar to the BPP approach and produced the lowest node supports (as expected). With respect to the BPP approach, both partition designs reached stationarity after 400 000 generations. Consensus trees showed almost identical topology, but according to the BF ratio, partition by character (HM = −15821.65) was selected against partition by gen (HM = −15887.71). The mtDNA topologies inferred by the MP, ML and BPP criteria were basically identical. The only main disagreement was regarding the relative positions of *E. isabellinus* and the American group, which switched positions according to the analysis at the base of a single large *Eptesicus* group (Fig. 3).

In the final nuDNA alignment, the complete *RAG2* gene (1054 bp) and the *BGN* intron (550 bp) were assembled for a total of 80 individuals (see Appendix 1). Heterozygote positions were treated as ambiguities and gaps as a fifth state; stop-codons were not found in any sequence. The concatenated alignment consisted of 1604 positions, of

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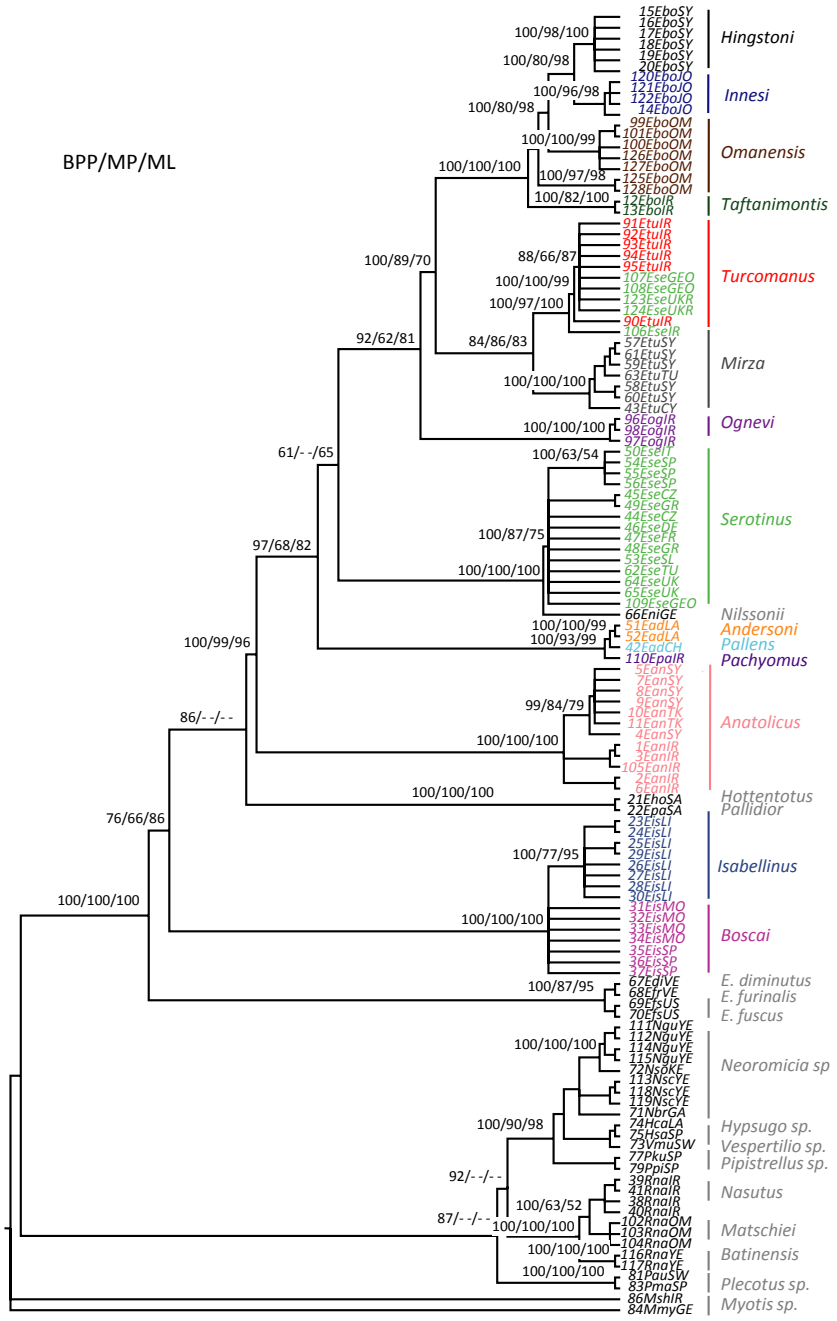

Fig. 3 Phylogenetic relationships of the Old World studied taxa of the genus *Eptesicus* based on concatenated mtDNA *Cytb* and *ND1*. The reconstruction presented is a Bayesian consensus tree with proportionally transformed branches allowing specific model rates to vary across characters. Above-selected nodes and from left to right: Bayesian posterior probabilities (BPP) partitioning the data set by character, bootstrap values from the Maximum Parsimony (MP) analysis weighting 9:1 transversions over transitions and bootstrap values from a Maximum-Likelihood (ML) analysis after 1000 iteractions.

which 1050 characters were constant and 366 were variable and parsimony informative. The equally weighting MP heuristic search retained 1257 equally most-parsimonious trees of 965 steps (first tree: CI = 0.72; HI = 0.28; RI = 0.64). Down-weighting transitions (1:2), the search produced 145 best trees of 737 steps (first tree CI = 0.85; HI = 0.14; RI = 0.94). Again, when down-weighting transitions, MP hypotheses were more robust and these results

are the only ones presented (Fig. 4). ML and BPP criteria produced similar topologies with lower nodes' support in the ML, particularly at the internal structure. Regarding the BPP approach, both designs reached stationarity after 300 000 generations. Consensus trees showed almost identical topology, and again according to the BF ratio, partition by character (HM = −7133.80) was selected against partition by gen (HM = −7364.92). All nuDNA topologies showed a deep split of a large 'serotinus group' differentiated from a 'bottae group' (Fig. 4). In fact, the topologies originated from both mtDNA and nuDNA data set disagree notably also at the tip groups and their internal relationships. For instance, the definition and relationships of the *'turcomanus'* samples group, the resolution was higher at the basal portion of the trees in the nuDNA-based reconstructions, particularly under the MP criterion.

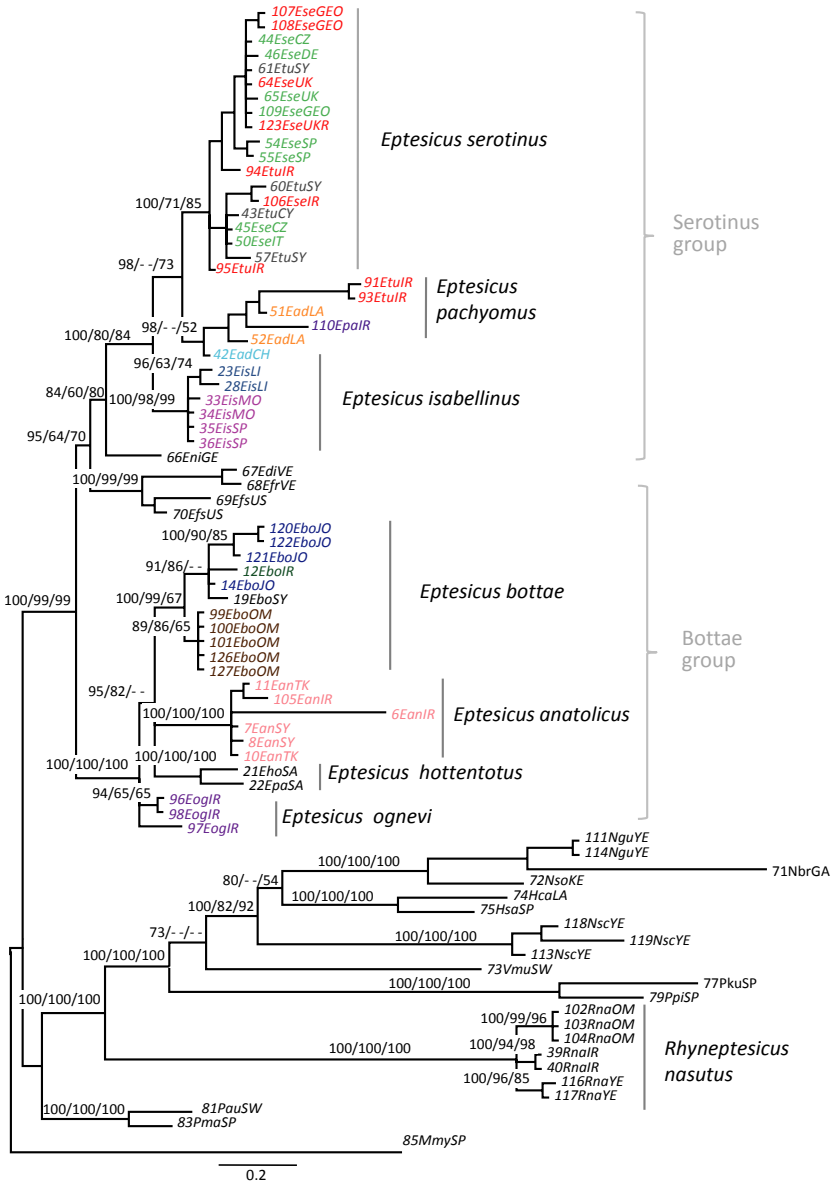

Fig. 4 Phylogenetic relationships of the studied Old World taxa of the genus *Eptesicus* based on concatenated fragments of the nuclear *RAG2* and *BGN* genes. The species taxonomic arrangement proposed within the genus is indicated at the right side. The reconstruction presented is a Bayesian consensus tree allowing specific model rates to vary across characters. Above-selected nodes and from left to right: BPP partitioning the data set by character, bootstrap values from the Maximum Parsimony (MP) analysis weighting 3:1 transversions over transitions, and bootstrap values from a Maximum-Likelihood (ML) analysis after 1000 iteractions.

Table 1 Summary of the support shown by each data set and final taxonomic proposal for each of the different Old World forms studied within the genus *Eptesicus*

Form	Morphology	mtDNA	nuDNA	Taxonomic proposal
hingstoni Thomas, 1919	X	X	X	*E. bottae hingstoni*
innesi (Lataste, 1887)	X	X	X	*E. bottae innesi*
omanensis Harrison, 1976	X	X	X	*E. bottae omanensis*
taftanimontis de Rouguin, 1988	X	X	X	*E. bottae taftanimontis*
ognevi Bobrinskii, 1918	X	X**	X	*E. ognevi*
anatolicus Felten, 1971	X	X**	X	*E. anatolicus*
serotinus (Schreber, 1774)	X	X	X	*E. serotinus*
turcomanus Eversmann, 1840	X	X	–	*E. serotinus (=turcomanus)*
mirza de Filippi, 1865	–	X	–	*E. serotinus mirza*
pachyomus Tomes, 1857	X	X	X	*E. pachyomus*
andersoni Donson, 1871	?	X	–	*E. pachyomus andersoni*
pallens Miller, 1911	?	X	X*	*E. pachyomus pallens?*
isabellinus Temminck, 1840	X	X	X	*E. isabellinus*
boscai Cabrera, 1904	?	X	X*	*E. isabellinus boscai*
hottentotus (A. Smith, 1833)	X	X	X	*E. hottentotus*
pallidior Shortridge, 1942	X	X	–	*E. hottentotus pallidior*
nasutus (Dobson, 1877)	X	X	X	*Rhyneptesicus nasutus*
matschiei Thomas, 1905	X	X	X	*Rhyneptesicus nasutus matschiei*
batinensis Harrison, 1968	X	X	X	*Rhyneptesicus nasutus batinensis*

*X No total agreement among the used reconstructions criteria.

**X Possible further cryptic diversity within the taxon.

Remarkably, all mtDNA- and nuDNA-based phylogenies showed that *Eptesicus* is not monophyletic. All '*nasutus*' samples branched off distantly from the rest of *Eptesicus* which otherwise made a monophyletic clade. The relationships of '*nasutus*' remained unresolved in all reconstructions although both mtDNA-based and nuDNA topologies supported a sister relation with a cluster including *Hypsugo*, *Neoromicia*, *Vespertilio* and *Pipistrellus* basally to the clade of Vespertilionini (Figs 3 and 4).

The mtDNA-based topologies (Fig. 3) indicated a deep grouping structure within the *Eptesicus* clade that is in general agreement with the recent picture based on morphology (Benda *et al.* 2006) and supported by most of the geographically based intraspecific subdivisions. Nevertheless, several of these groups were not sustained by the nuDNA dataset. For instance, whereas the morphologically defined *turcomanus* and *mirza* groups were clearly supported by the mtDNA data set (Fig. 3), nuDNA did not support this arrangement and allocated all *turcomanus* and *mirza* specimens sparse and mixed within a group morphologically identified as *serotinus* or located even farther along the trees (Fig. 4). The *turcomanus* + *mirza* clade joined in all the mtDNA topologies a cluster that morphologically corresponds to '*bottae*' and which showed a clear geographical structure distinguishing four groups corresponding to the samples from Syria, Jordan, Oman and Iran, respectively. The 'bottae group' and its subdivisions were clearly supported also by the nuDNA-based topologies and related to a group including all the specimens identified morphologically as *ognevi*. The mtDNA analysis placed the '*ognevi*' specimens as a sister group to *bottae* and '*turcomanus* + *mirza*'. In fact, the nuDNA hypotheses joined '*ognevi*' and '*bottae*' in the well-supported 'bottae group' which also included the group '*anatolicus*'. The latter is another monophyletic group well supported by both mtDNA and nuDNA data sets from Turkey, Syria and Iran.

All Western European '*serotinus*' bats clustered with some Eastern samples in a monophyletic group in the mtDNA-based topologies. These were closely attached to the much smaller *E. nilssonii*, but apart from other '*serotinus*' from Georgia, Iran and Ukraine. Instead, the nuDNA-based topologies grouped all western and eastern '*serotinus*' in a well-supported monophyletic group which included also the specimens morphologically identified as '*turcomanus*' and/or '*mirza*'. The nuDNA-based topologies placed *E. nilssonii* in a position distant from this cluster. Other groups identified in the mtDNA-based reconstructions included: (i) a clade constructed from serotine samples from China and Laos and including, interestingly enough, a sample corresponding morphologically to the taxon '*pachyomus*' from Iran, (ii) an Afrotropic '*hottentotus*' group from South Africa and (iii) an '*isabellinus*' cluster which showed a further differentiation of the specimens from Libya. The nuDNA also supported these last groupings but with a better defined topological structure. In fact, the

Far East clade (now including '*pachyomus*' and specimens from Iran) and '*isabellinus*' appeared as part of the 'serotinus group' which is linked to an American cluster. Interestingly, the other African species appeared, instead, as part of the other supra-specific 'bottae group'.

Discussion

The joint analyses of morphology, mtDNA- and nuDNA-based phylogenetic reconstructions suggest a number of important changes in the traditional view of the genus *Eptesicus* (Table 1) and its phylogenetic relationships:

The taxonomic status of Eptesicus nasutus

Our analyses confirm the close relationships between the tribes Vespertilionini and Pipistrellini as defined by Hoofer & Van Den Bussche (2003) within the subfamily Vespertilioninae. These results also indicate a close phylogenetic relationship between the *nasutus* samples and these tribes, and a distant relationship from the other *Eptesicus*, which, according to Hoofer & Van Den Bussche (2003), belong to the tribe Nycticeini. In fact, *nasutus* appears in the topologies associated with other genera that were once related to *Eptesicus*, but that are currently separated (e.g. the Australian *Vespadelus* or the Afrotropic *Neoromicia*). These topologies indicate that the '*nasutus*' group cannot be included in *Eptesicus*, but belongs instead to a different genus whose close evolutionary relationships are still unclear (Figs 3 and 4). An available name for this taxon is *Rhyneptesicus*, a name that Bianchi (1917) proposed to distinguish *nasutus* on the basis of a lack of an epiblema as a diagnostic character. Although all *nasutus* used for this study have epiblema, (thereby indicating that this diagnostic character is not valid), the formal description and name are still applicable. In fact, *Rhyneptesicus* was recovered as a genus by Horáček & Hanák (1986) and as a subgenus by Horáček *et al.* (2000). The valid morphological characters which differentiate this genus are the relatively narrow pointed ears, long tragus and relative short fur. There are also dental characters that support this distinction such as the unicuspidal first upper incisor and the complete molar including protocrista. *Rhyneptesicus* has the typical baculum morphology of the *Eptesicus* (Hill & Harrison 1987), but the structure of its karyotype is still unknown. Both mtDNA and nuDNA markers indicate a strong and geographically sound genetic structure within its discontinuous distribution. Taxonomically, the reconstructions validate subspecific recognition for the nominal *nasutus* from the samples of Iran, close to the terra typica in Pakistan plus the forms *matschiei* (for Yemeni specimens) and *batinensis* from Oman. This arrangement is also supported by values of K2P-corrected distances of 3.36 and 6.55 % between them (Table S1).

The genus Eptesicus and the *American clade*

Apart from the *nasutus* samples, all *Eptesicus* from the different continents cluster in the analyses in a well-supported basal group (Figs 3 and 4) sustaining the monophyly of the clade and its taxonomic validity. The genus *Eptesicus* Rafinesque, 1820 is well defined on the basis of a series of morphological characters such as absence of the pm^2, myotodont lower molars, well-defined basisphaenoidal pits, a triangular-shaped baculum and a $2n = 50$ / NF = 48 karyotypic formula (Heller & Volleth 1984; Horáček & Hanák 1986; Hill & Harrison 1987; Morales *et al.* 1991). Recent molecular studies have placed the genus *Eptesicus* in the tribe Nycticeini and separated it from the pipistrelles (Hoofer & Van Den Bussche 2003; Hoofer *et al.* 2006). In their comprehensive study of the family Vespertilionidae, these authors also found that the American *Eptesicus* and the genus *Histiotus* form a unique American clade, which makes the American *Eptesicus* paraphyletic with respect to the Old World members of the genus. To resolve this situation they suggest relegating *Histiotus* to subgeneric rank and propose restoring the name *Cnephaeus* to include the Old World forms, as a subgenus. We support this option for the sake of taxonomic stability because it will bring less turmoil to the taxonomy of the Palaearctic forms.

All American species of *Eptesicus* included in our analyses cluster in a monophyletic group that corresponds to the American clade suggested by Hoofer & Van Den Bussche (2003); Hoofer *et al.* (2006) and Roehrs *et al.* (2010). This would indicate that a single penetration event of *Eptesicus* has occurred from one continent to another. The oldest fossil records of *Eptesicus* in North America correspond to Early Upper Miocene (Czaplewski & Morgan 2003). This date coincides also with the estimated dating of the American split of the bats of the genus *Myotis* whose diversification has been related to the global cooling and the development of temperate conditions during this period (Stadelmann *et al.* 2004, 2007). Finally, our results also show a small degree of differentiation (both at nuDNA and mtDNA) found between the small American *Eptesicus* species *E. furinalis* and *E. diminutus*. A molecular review of these taxa seems particularly needed.

Taxonomic inferences of Palaearctic forms

The classical taxonomic arrangements (Gaisler 1970; Harrison 1975; Nader & Kock 1990; Horáček *et al.* 2000) have considered that the systematics of the Palaearctic *Eptesicus* revolved around two main species: the smaller *E. bottae* and the larger *E. serotinus*, to which most of the described forms have been ascribed either as subspecies (e.g.'*turcomanus*') or synonymized (e.g. '*intermedius*'). This basal division in two main groups is supported by all our nuDNA-based topologies (Fig. 4).

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The 'bottae group'

Within the small bats grouped in the 'bottae group', our analyses support *E. bottae* as a valid monophyletic entity at specific level although showing some differences in composition and structure from previously suggested groupings (e.g. Harrison 1975; Nader & Kock 1990). The deep molecular structure found in mtDNA and nuDNA trees coincides with its consideration of the species as formed by discontinuous populations morphologically differentiated on the basis of pelage colour and size. The patchy distribution may be related to the fact that inhabits oases and relatively humid areas in a variety of extreme arid habitats along the edge of the southern Palaearctic (Nader & Kock 1990). The nuDNA analyses support the distinction of the following taxa: (i) ' *innesi*' from Sinai, the outskirts of Cairo, southern parts of Israel and Jordan and related to, (ii) ' *hingstoni*' found from Syria all the way to south-eastern Iraq, (iii) ' *taftanimontis*' from Kerman and Baluchestan provinces of south-eastern Iran and (iv) ' *omanensis*', apparently linked to mountains and high altitudes of north-eastern Oman (Harrison 1975) and this being the most distinct morphologically. All these forms are tentatively maintained as subspecies within *bottae*, as recognized by Nader & Kock (1990), but need confirmation in relation to the rare nominotypical form of *E. bottae* which could not be included in our analysis.

The species *E. ognevi* Bobrinskoj, 1918 also stands in the mtDNA-based analyses as a well-defined group also supported by the nuDNA analyses, which include '*ognevi*' clearly within the 'bottae group'. The large differentiation shown by one of the samples (97EogIR) suggests further cryptic differentiation within the clade. This pale little form from deserts and steppes of the northern part of the Middle East was described from Western Tajikistan and soon after its description was synonymized with *sodalis* and later included within *E. bottae* (Hanák & Gaisler 1971; Harrison 1975; Nader & Kock 1990; Artyushin *et al.* 2009). In contrast to other recent examples of newly recognized species, for example within *Otonycteris* (Benda & Gvoždík 2010), morphological differences between *bottae* and *ognevi* are very subtle.

Both our mtDNA- and nuDNA-based results validate also *E. anatolicus* Felten, 1971 as a fully distinct species. This taxon was originally described from south-western Anatolia based on external (e.g. pelage coloration) and skull characters (e.g. high braincase), and was later included within *E. bottae* (Harrison 1975). However, it was recognized as the most distinct form within *E. bottae* and vindicated as probably valid species by Hanák *et al.* (2001) and Benda *et al.* (2006), who also pointed new ecological differences with respect to *E. bottae*. Contrary to *E. bottae*, *E. anatolicus* seems to be a Mediterranean forest-related species with echolocation calls that are also quite different from those of *E. bottae*: peak frequency of 28 kHz in *E. anatolicus* (von Helversen 1998) against 32.5 kHz in *E. bottae* (Holderied *et al.* 2005). Our results suggest a close phylogenetic relationships between *E. anatolicus* and both *E. bottae* and *E. ognevi* within the group. *E. anatolicus* represents a rather common faunal element throughout the Mediterranean forests of the Levantine Sea from Rhodes (Greece) and Cyprus in the west to southern Anatolia and Lebanon in the south-east, north-western Syria and western Iran. It avoids open xeric areas and reaches southwards as far as Kerman (Spitzenberger 1994; von Helversen 1998; Benda *et al.* 2006, 2007). A recent mtDNA-based revision of Palaearctic bat species has also supported this specific consideration (Mayer *et al.* 2007), although the mtDNA internal structure and the large differentiation shown by one of the samples (6EanIR) suggest again further cryptic differentiation within the clade.

The two *E. hottentotus* samples stand as another very distinct group. The taxonomy of the different forms described within *E. hottentotus* is still confused and requires further research. The two forms included in our analysis, *hottentotus* and *pallidior*, were synonymized in the most recent morphological revision by Schlitter & Aggundey (1986). Our molecular analyses indicate that our *hottentotus* sample from Cape Province is highly differentiated (over 12% K2P distance in the *Cytb*, Table S1) from the sample *pallidior* from Goodhouse, near the border with Namibia and found in xeric '*karoo*' habitats. Probably related to these habitat differences, *pallidior* is much paler than *E. hottentotus* (M. Ruedi, pers. comm.). Therefore, we validate the form *pallidior* Shortridge, 1942 and recognize it at least as a valid subspecies that would extend through north-western Cape and Namibia. Its final taxonomic consideration needs more thorough studies that will probably raise this taxon to specific rank. This Ethiopian clade appears basal to the rest of the 'bottae group' and distant from the larger African '*isabellinus*' which belongs to the 'serotinus group'. Thus, the hypothesis that there is an African monophyletic lineage within *Eptesicus* is not supported.

The 'serotinus group'

Morphologically, there are three forms closely related to '*serotinus*': one large, normally pale, known as '*mirza*' (Turkey, South Iran, Levant, Cyprus); a second medium sized, dark, corresponding to the nominal '*serotinus*' (Europe, Anatolia and Caucasus); and finally, a small, with 'sandy' fur colour and pale-face form (Strelkov & Iljin 1992; Benda *et al.* 2006) known as '*turcomanus*' (central Asia and north-east Iran). Both mtDNA and nuDNA support clades identified morphologically as '*serotinus*' (Figs 3 and 4), but interestingly the composition and structure of this clade

vary significantly according to the markers. The mtDNA-based reconstructions show two paraphyletic groups of '*serotinus*' samples (Fig. 3), one made up by all Western European samples clustering together with *E. nilssonii* as distinct from another group made of '*serotinus*' from Ukraine, Georgia and Iran together with '*turcomanus*'. This clade is connected with the samples from Syria, Turkey and Cyprus that morphologically correspond to *'mirza'*. In summary, the mtDNA distinguishes three lineages within '*serotinus*' one linked to *E. nilssonii*, another that groups '*serotinus*' and '*turcomanus*' morphotypes and another that corresponds to *'mirza'*.

The nuDNA hypotheses invalidated the three groups because all its members appear now sparse in a well-supported unique clade corresponding to *E. serotinus* (Schreber, 1774), which is now located far apart from *E. nilssonii* (Fig. 4). According to these reconstructions, it seems appropriate to keep only *mirza* as s subspecies within *E. serotinus*. The form '*turcomanus*' would be included in and synonymized within *E. serotinus* despite its morphological differences which are not supported by any marker. Similar results are obtained by Artyushin *et al.* (2012) using other nuclear markers. Neither is the third mtDNA lineage validated because it seems linked to a mitochondrial capture by other species (see below). Within this definition, *E. serotinus* extends its distribution from England and Western Iberia to Central Asia (Benda *et al.* 2006).

The *Eptesicus* samples from the Far East (Laos and China) are distinguished from the 'serotinus group' in a well-supported clade in both mtDNA- and nuDNA-based analyses (Figs 3 and 4). Although the whole lineage clearly needs further research, the level of differentiation shown in all markers supports the species rank of this Oriental lineage that comprises Far Eastern as well as Indian forms. The samples from the Far East cluster in all topologies with two samples identified morphologically as well as by mtDNA as *turcomanus* from Iran plus another sample from Southern Iran (Dehbarez). Contrary to the former, this last sample was obtained from a bat with a pale face and brownish grey (not dark) dorsal pelage with whitish tips. All these characters indicate closer relationships with the Indian forms and accordingly it was identified as *pachyomus*. The topologies suggest thus an evolutionary connection between the two lineages. The levels of genetic differentiation within the clade validate taxonomically the forms '*andersoni*' (Dobson, 1871) described from Yunnan, southern China and *'pallens*' (Miller, 1911) from central China. According to our rank topologies, they are tentatively considered as subspecies of *E. pachyomus* (Tomes, 1857) because this last one has priority on the other two names. Genetic analyses have supported species rank for other extreme Oriental forms of Palaearctic bats such as *Barbastella* (Zhang *et al.* 2007) and *Nyctalus* (Salgueiro *et al.* 2007), which were once considered as part (or at best as subspecies) of extremely widespread morphologically uniform units.

E. isabellinus (Temminck, 1840) stands out as a clearly differentiated clade both in mtDNA- and nuDNA-based reconstructions (Figs 3 and 4). Originally described from Libya (type locality outskirts of Tripoli) and distributed across north-west Africa, it was traditionally included in *E. serotinus* as well as more recently (Simmons 2005), although it was vindicated as a species by other authors (e.g. Benda *et al.* 2004). Previous studies have supported its species rank and extended its distribution to the southern half of the Iberian Peninsula (Ibáñez *et al.* 2006; Mayer *et al.* 2007; García-Mudarra *et al.* 2009). The large mtDNA differentiation (over 13% K2P distances in *Cytb*, Table S1) with *E. serotinus* indicates a long independent evolutionary history despite the extraordinary morphological similarity between the two taxa. In all trees, all *E. isabellinus* from western Libya appears forming a supported clade, whereas samples from Morocco and Iberia in the West appear mixed in MP and ML mtDNA analyses forming another clade which is not fully supported in other analyses. The lack of differentiation between Iberian and Moroccan samples supports the finding (García-Mudarra *et al.* 2009; Juste *et al.* 2009) that the Straits of Gibraltar does not act as a geographical barrier for the species. The available name for the western form would be '*boscai*', Cabrera, 1904 from Muchamiel, Alicante (Spain) that will include the Moroccan and Iberian populations. The discontinuity in North Africa between the two clades needs a more comprehensive study, including samples from Algeria and/or Tunisia.

Evolutionary remarks from Morphology, mtDNA and nuDNA contrasting patterns

Sequence characteristics, such as the absence of stop-codons or indels and the high degree of congruence in the topologies of the two relatively distant mtDNA fragments (Fig. S1), allow assuming for this study that the discrepancies between mtDNA and nuDNA reconstructions result from actual different histories and are not resulting from molecular or analytical artefacts. The response of a molecular marker to an evolutionary process depends on intrinsic characteristics (e.g. mutation rate, effective population size, selection regime, etc.) and other stochastic processes acting on the whole genome such as genetic drift or bottlenecks; frequently, non-hierarchical processes like introgression further complicate the patterns (Edwards & Bensch 2009). The different responses imply different evolutionary pathways for each marker, and whether they represent or not the histories of the relevant species will depend on the interplay of these forces (Zhang & Hewitt 2003). By

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increasing the number of markers studied, we will also increase the chances of recovering evolutionary processes and of reconstructing the complete organismal history (Edwards *et al.* 2005), facilitating the inference of stable taxonomies. On the other hand, mtDNA-based historical reconstructions maybe partial (Ballard & Whitlock 2003) and by contrasting mtDNA- and nuDNA-patterns, we can get relevant information about the underlying evolutionary processes (e.g. Wiens *et al.* 2010; Toews & Brelsford 2012) due to their deep evolutionary differences, particularly the rapid attainment of reciprocal monophyly of mtDNA genes relative to nuDNA ones (Edwards *et al.* 2005). In our analyses, besides the differences clearly due to the higher resolution at the deep nodes of nuDNA in relation to mtDNA (e.g. the distinction of the 'serotinus and bottae groups'), the main disagreement between the mtDNA and the nuDNA topologies resides in the relative positions of *E. serotinus* and *E. nilssonii* and in the recognition of the *turcomanus* clade. In the first case, our mtDNA results are in agreement with Artyushin *et al.* (2009) who have already shown, with a larger geographical coverage, the presence of two clearly distinct mtDNA lineages within *E. serotinus*, one almost identical to *E. nilssonii* and the other clearly distinct. The contrasting distant relationship between the two species in our nuDNA-based topologies supports the hypothesis of a mitochondrial introgression and capture of *E. nilssonii*'s mtDNA by *E. serotinus*. This hypothesis was first suggested by Mayer & von Helversen (2001) and later supported by Artyushin *et al.* (2009, 2012). According to the model presented by Currat *et al.* (2008), hybridization would have occurred asymmetrically between front populations of *E. serotinus* and the resident (or earlier arrived) populations of *E. nilssonii*, a more cold-tolerant species, during the expansion of *E. serotinus* west and northwards to new opened suitable habitats. In the expansion along more mesic areas, the captured *E. nilssonii*'s mtDNA would have been transmitted to all present Western populations of *E. serotinus*, whereas the nuclear imprint of this hybridization event would have been diluted due to demographic factors (Currat *et al.* 2008). Asymmetrical hybridization with mtDNA capture has been convincingly demonstrated for other European bats of the genus *Myotis* Kaup, 1829 (Berthier *et al.* 2006) and more recently for Asian *Rhinolophus* (Mao *et al.* 2010) or the African *Scotophilus* (Vallo *et al.* 2012).

The second disagreement concerns the morphologically distinct lineages related to *E. serotinus*. This morphological variability is probably associated with the regional mosaic of habitats in the Middle East from diverse forests with open dry steppes and xeric habitats that point to a scenario of distinct populations isolated in different degrees during climatic cycles and possibly under different selective pressures. Secondary contacts during expansion episodes would have allowed for the homogenizing of the nuDNA of these not completely isolated ecomorphs and even the mtDNA in the case of the '*turcomanus*'. The differentiation of into desert/mesic ecomorphs has probably evolved several times under different cycles of environmental conditions, as suggested by the large mtDNA differentiation (circa 5% K2P distance, Table S1) between *serotinus* + *turcomanus* and *mirza*. Several examples of similar partial differentiation in ecomorphs are known, for instance, within the *Pipistrellus* complex, remarkably also around the Mediterranean basin (Hulva *et al.* 2010). The morphologically similar species *E. fuscus* in North America shows also strong concordance between morphological ecomorphs and mtDNA lineages in a complex that maintains high levels of nuDNA flow (Turmelle *et al.* 2011).

In summary, the net effects of past climate changes on the range of a species are largely determined by the consequences of these changes on its habitat requirements and its physiological tolerances (Hoskin *et al.* 2011). The evolution, in this case, of the ecologically plastic Palaearctic *Eptesicus* seems to be determined by the processes of fragmentation, contraction and range expansion that occurred in an area with highly variable geography, in which ecological conditions have changed dramatically in the recent cold/warm climatic cycles (Carrión *et al.* 2011). In fact, since at least Early to Middle Pleistocene, changes in vegetation during cold periods, leading to the fragmentation of forested landscapes and the development of open dry landscapes, were a general feature of the Mediterranean region and Western Asia (Leroy *et al.* 2011). The full understanding of the impact of these changes on the evolution of the Palaearctic *Eptesicus* and the relative contribution of the possible shaping forces (e.g. maternal phylopatry, local selection, etc.) need a more inclusive sampling (at the population level) as well as complementary information provided by additional molecular markers (e.g. Turmelle *et al.* 2011). Still, our integrative approach of morphological and molecular data has allowed us (Table 1), in this most entangled group of bats, to recover the genus *Rhyneptesicus*, and redefine *E. serotinus* and *E. bottae*. We also confirmed the species rank for *E. isabellinus* and *E. pachyomus*, within a 'serotinus group' and *E. ognevi* and *E. anatolicus* within the 'bottae group'.

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References

Artyushin, I. V., Bannikova, A. A., Lebedev, V. S. & Kruskop, S. V. (2009). Mitochondrial DNA relationships among North Palaearctic *Eptesicus* (Vespertilionidae, Chiroptera) and past hybridization between common Serotine and Northern bat. *Zootaxa*, *2262*, 40–52.

Artyushin, I., Lebedev, V., Bannikova, A. & Kruskop, S. (2012). A molecular reappraisal of the taxonomic status of Eptesicus serotinus turcomanus. *Vespertilio*, *16*, 31–42.

Baker, R. J., Porter, C. A., Patton, J. C. & Van Den Bussche, R. A. (2000). Systematics of bats of the family Phyllostomidae based on *RAG2* DNA sequences. *Occasional Papers, Museum Texas Tech University*, *202*, 1–16.

Ballard, J. W. & Whitlock, M. C. (2003). The incomplete natural history of mitochondria. *Molecular Ecology*, *13*, 729–744.

Benda, P. & Gvoždík, V. (2010). Taxonomy of the genus *Otonycteris* (Chiroptera: Vespertilionidae: Plecotini) as inferred from morphological and mtDNA data. *Acta Chiropterologica*, *12*, 83–102.

Benda, P., Ruedi, M. & Aulagnier, S. (2004). New data on the distribution of bats (Chiroptera) in Morocco. *Vespertilio*, *8*, 13–44.

Benda, P., Andreas, M., Kock, D., Lučan, R. K., Munclinger, P., Nová, P., Obuch, J., Ochman, K., Reiter, A., Uhrin, M. & Weinfurtová, D. (2006). Bats (Mammalia: Chiroptera) of the Eastern Mediterranean. Part 4. Bat fauna of Syria: distribution, systematics, ecology. *Acta Societatis Zoologicae Bohemicae*, *70*, 1–329.

Benda, P., Hanák, V., Horáček, I., Hulva, P., Lučan, R. & Ruedi, M. (2007). Bats (Mammalia: Chiroptera) of the Eastern Mediterranean. Part 5. Bat fauna of Cyprus: review of records with confirmation of six species new for the island and description of a new subspecies. *Acta Societatis Zoologicae Bohemicae*, *71*, 71–130.

Benda, P., Lučan, R. K., Obuch, J., Reiter, A., Andreas, M., Kock, D., Bačkor, P., Bohnenstengel, T., Eid, E. K., Ševčík, M., Vallo, P. & Amr, Z. S. (2010). Bats (Mammalia: Chiroptera) of the Eastern Mediterranean. Part 8. Bat fauna of Jordan: fauna, ecology, echolocation, ectoparasites. *Acta Societatis Zoologicae Bohemicae*, *74*, 185–353.

Benda, P., Hanák, V. & Červeny, J. (2011). Bats (Mammalia: Chiroptera) of the Eastern Mediterranean. Part 9. Bats from Transcaucasia and West Turkestan in collection of the National Museum, Prague. *Acta Societatis Zoologicae Bohemicae*, *75*, 159–222.

Berthier, P., Excoffier, L. & Ruedi, M. (2006). Recurrent replacement of mtDNA and cryptic hybridization between two sibling bat species *Myotis myotis* and *Myotis blythii*. *Proceedings of the Royal Society of London B*, *273*, 3101–3109.

Bianchi, V. (1917). Predvaritel'nyja zametki o letučih' myšah' (Chiroptera) Rossii [Notes préliminaires sur les chauve-souries ou Chiroptères de la Russie]. *Ežegodnik Zoologičeskogo Muzeja Akademii Nauk*, *21*, 73–82 (in Russian, French subtitle).

Bickford, D., Lohman, D. J., Sodhi, N. S., Ng, P. K. L., Meier, R., Winker, K., Ingram, K. K. & Das, I. (2007). Cryptic species as a window on diversity and conservation. *Trends in Ecology and Evolution*, *22*, 148–155.

Carrión, J. S., Rose, J. & Stringer, C. (2011). Early human evolution in the Western Palaearctic: ecological scenarios. *Quaternary Science Review*, *30*, 1281–1295.

Currat, M., Ruedi, M., Petit, R. J. & Excoffier, L. (2008). The Hidden side of invasions: massive introgression by local genes. *Evolution*, *62*, 1908–1920.

Czaplewski, N. J. & Morgan, G. S. (2003). Fossils bats of the Americas. Available via: <http://www.snomnh.ou.edu/collections-research/cr-sub/ vertpaleo/fossil_bats/index.shtml>.

Degnan, J. H. & Rosenberg, N. A. (2009). Gene tree discordance, phylogenetic inference and the multispecies coalescent. *Trends in Ecology and Evolution*, *24*, 332–340.

Dool, S. E., Puechmaille, S. J., Dietz, C., Juste, J., Ibáñez, C., Hulva, P., Roué, S. G., Petit, E. J., Jones, G., Russo, D., Toffoli, R., Viglino, A., Martinoli, A., Rossiter, S. J. & Teeling, E. C. (in press). Phylogeography and postglacial recolonisation of Europe by Rhinolophus hipposideros: evidence from multiple genetic markers. *Molecular Ecology*.

Edwards, S. V. (2008). Is a new and general theory of molecular systematics emerging? *Evolution*, *63*, 1–19.

Edwards, S. V. & Bensch, S. (2009). Looking forwards or looking backwards in avian phylogeography? A comment on Zink and Barrowclough 2008 *Molecular Ecology*, *18*, 2930–2933.

Edwards, S. V., Kingan, S. B., Calkins, J. D., Balakrishnan, C. N., Jennings, W. B., Swanson, W. J. & Sorenson, M. D. (2005). Speciation in birds: genes, geography, and sexual selection. *Proceedings of the National Academy of Sciences*, *102*, 6550–6557.

Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution*, *39*, 783–791.

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

García-Mudarra, J. L., Juste, J. & Ibáñez, C. (2009). The Straits of Gibraltar: barrier or bridge to the Ibero-Marrocan bats. *Biological Journal of the Linnean Society*, *96*, 434–450.

Guindon, S. & Gascuel, O. (2003). A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Systematic Biology*, *52*, 696–704.

Hanák, V. & Gaisler, J. (1971). The status of *Eptesicus ognevi* Bobrinskij, 1918, and remarks on some other species of this genus (Mammalia: Chiroptera). *Věstník Československé Společnosti Zoologické*, *35*, 11–24.

Hanák, V., Benda, P., Ruedi, M., Horáček, I. & Sofianidou, T. S. (2001). Bats (Mammalia: Chiroptera) of the Eastern Mediterranean. Part 2. New records and review of distribution of bats in Greece. *Acta Societatis Zoologicae Bohemicae*, *65*, 279–346.

Harrison, D. L. (1975). Scientific results of the Oman Flora and fauna survey, 1975. Description of a new subspecies of Botta's

serotine (Eptesicus bottae Peters, 1869, Chiroptera: Vespertilionidae) from Oman. *Mammalia*, *39*, 415–418.

Heller, K. G. & Volleth, M. (1984). Taxonomic position of *Pipistrellus societatis* Hill, 1972 and the karyological characteristics of the genus *Eptesicus* (Chiroptera: Vespertilionidae). *Zeitschrift für Zoologische Systematik und Evolutionsforschung*, *22*, 65–77.

von Helversen, OV (1998). *Eptesicus bottae* (Mammalia, Chiroptera) auf der Insel Rhodos. *Bonner Zoologische Beiträge*, *48*, 113–121.

Higuchi, R., von Beroldingen, C. H., Sensabaugh, G. F. & Erlich, H. A. (1988). DNA typing from hairs. *Nature*, *332*, 543–546.

Hill, J. E. & Harrison, D. L. (1987). The *baculum* in the Vespertilioninae (Chiroptera: Vespertilionidae) with a systematic review, a synopsis of *Pipistrellus* and *Eptesicus*, and the descriptions of a new genus and subgenus. *Bulletin of the British Museum (Natural History)*, *Zoology*, *52*, 225–305.

Hill, J. E. & Smith, J. D. (1984). *Bats. A Natural History* (pp. 243). London: The British Museum (Natural History).

Holderied, M. W., Korine, C., Fenton, M. B., Parsons, S., Robson, S. & Jones, G. (2005). Echolocation call intensity in the aerial hawking bat *E. bottae* (Vespertilionidae) studied using stereo videogrammetry. *Journal of Experimental Biology*, *208*, 1321–1327.

Hoofer, S. R. & Van Den Bussche, R. A. (2003). Molecular phylogenetics of the chiropteran family Vespertilionidae. *Acta Chiropterologica*, *5*, 1–63.

Hoofer, S. R., Van Den Bussche, R. A. & Horáček, I. (2006). Generic status of the American pipistrelles (Vespertilionidae) with description of a new genus. *Journal of Mammalogy*, *87*, 981–992.

Horáček, I. & Hanák, V. (1986). Generic status of *Pipistrellus savii* and comments on classification of the genus *Pipistrellus* (Chiroptera, Vespertilionidae). *Myotis*, *23–24*, 9–16.

Horáček, I., Hanák, V. & Gaisler, J. (2000). Bats of the Palearctic region: a taxonomic and biogeographic review. In B. W. Wołoszyn (Ed.) *Proceedings of the VIIIth EBRS. Vol. 1. Approaches to Biogeography and Ecology* (pp. 11–157). Kraków: Platan Publication House.

Hoskin, C. J., Tonione, M., Higgie, M., MacKenzie, J. B., Williams, S. E., VanDerWal, J. & Craig Moritz, C. (2011). Persistence in peripheral refugia promotes phenotypic divergence and speciation in a rainforest frog. *The American Naturalist*, *178*, 561–578.

Huelsenbeck, J. P. & Ronquist, F. (2001). MrBayes: Bayesian inference of phylogenetic trees. *Bioinformatics*, *17*, 754–755.

Hulva, P., Fornusková, A., Chudárková, A., Evin, A., Allegrini, B., Benda, P. & Bryja, J. (2010). Mechanisms of radiation in a bat group from the genus *Pipistrellus* inferred by phylogeography, demography and population genetics. *Molecular Ecology*, *19*, 5417–5431.

Ibáñez, C., García-Mudarra, J. L., Ruedi, M., Stadelmann, B. & Juste, J. (2006). The Iberian contribution to cryptic diversity in European bats. *Acta Chiropterologica*, *8*, 277–297.

Juste, J., Bilgin, R., Muñoz, J. & Ibáñez, C. (2009). mtDNA signatures at different spatial scales: from the effects of the Strait of Gibraltar to population structure in the meridional serotine bat (*Eptesicus isabellinus*). *Heredity*, *103*, 178–187.

Kawai, K., Nikaido, M., Harada, M., Matsumura, S., Lin, L. K., Wu, Y., Hasegawa, M. & Okada, N. (2002). Intra- and interfamily relationships of Vespertilionidae inferred by various molecular markers including SINE insertion data. *Journal of Molecular Evolution*, *55*, 284–301.

Kearney, T. C., Volleth, M., Contrafatto, G. & Taylor, P. J. (2002). Systematic implications of chromosome GTG-band and bacula morphology for Southern African *Eptesicus* and *Pipistrellus* and several other species of Vespertilioninae (Chiroptera: Vespertilionidae). *Acta Chiropterologica*, *4*, 55–76.

Leroy, S. A. G., Arpe, K. & Mikolajewicz, U. (2011). Vegetation context and climatic limits of the Early Pleistocene hominin dispersal in Europe. *Quaternary Science Reviews*, *30*, 1448–1463.

Lyons, L. A., Laughlin, T. F., Copeland, N. G., Jenkins, N. A., Womack, J. E. & O'Brien, S. J. (1997). Comparative anchor tagged sequences (CATS) for integrative mapping of mammalian genomes. *Nature Genetics*, *15*, 47–56.

Maniatis, T., Fritsh, E. F. & Sambrook, J. (1989). *Molecular Cloning: A Laboratory Manual*, 2nd edn. Cold Spring Harbor: Cold Spring Harbor Laboratory Press.

Mao, X., Zhang, J., Zhang, S. & Rossiter, S. (2010). Historical male-mediated introgression in horseshoe bats revealed by multilocus DNA sequence data. *Molecular Ecology*, *19*, 1352–1366.

Mayer, F. & von Helversen, O. (2001). Cryptic diversity in European bats. *Proceedings of the Royal Society of London B*, *268*, 1825–1832.

Mayer, F., Dietz, C. & Kiefer, A. (2007). Molecular species identification boosts bat diversity. *Frontiers in Zoology*, *4*, 4.

Morales, J. C., Scott, C., Ballinger, V., Bickham, J. W., Greenbaum, I. F. & Schlitter, D. A. (1991). Genetic relationships among eight species of *Eptesicus* and *Pipistrellus* (Chiroptera: Vespertilionidae). *Journal of Mammalogy*, *72*, 286–291.

Moratelli, R., Peracchi, A. L., Dias, D. & de Oliveira, J. (2011). Geographic variation in South American populations of *Myotis nigricans* (Schinz, 1821) (Chiroptera, Vespertilionidae), with the description of two new species. *Mammalian Biology*, *76*, 592–607.

Nader, I. A. & Kock, D. (1990). *Eptesicus* (*Eptesicus*) *bottae* (Peters 1869) in Saudi Arabia with notes on its subspecies and distribution (Mammalia Chiroptera: Vespertilionidae). *Senckenbergiana Biologica*, *70*, 1–13.

Padial, J. M. & De la Riva, I. (2010). A response to recent proposals for integrative taxonomy. *Biological Journal of the Linnean Society*, *101*, 747–756.

Padial, J. M., Miralles, A., De la Riva, I. & Vences, M. (2010). The integrative future of taxonomy. *Frontiers in Zoology*, *7*, 16.

Pagel, M. & Meade, A. (2006). Bayesian analysis of correlated evolution of discrete characters by reversible-jump Markov chain Monte Carlo. *The American Naturalist*, *167*, 808–825.

Posada, D. (2008). ModelTest: phylogenetic model averaging. *Molecular Biology and Evolution*, *25*, 1253–1256.

Roehrs, Z. P., Lack, J. B. & Van Den Busshe, R. A. (2010). Tribal phylogenetic relationships within Vespertilioninae (Chiroptera: Vespertilionidae) based on mitochondrial and nuclear sequence data. *Journal of Mammalogy*, *91*, 1073–1092.

Ruedi, M. & Mayer, F. (2001). Molecular systematics of bats of the genus *Myotis* (Vespertilionidae) suggests deterministic ecomorphological convergences. *Molecular Phylogenetics and Evolution*, *21*, 436–448.

Salgueiro, P., Ruedi, M., Coelho, M. M. & Palmeirim, J. M. (2007). Genetic divergence and phylogeography in the genus *Nyctalus*

(Mammalia, Chiroptera): implications for population history of the insular bat *Nyctalus azoreum*. *Genetica*, *130*, 169–181.

Schlitter, D. A. & Aggundey, I. R. (1986). Systematics of African bats of the genus *Eptesicus* (Mammalia: Vespertilionidae). 1. Taxonomic status of the large serotines of eastern and southern Africa. *Cimbebasia*, *Ser A*, *8*, 167–174.

Simmons, N. B. (2005). Order chiroptera. In D. E. Wilson & M. Reeder (Eds) *Mammal Species of the World: A Taxonomic and Geographic Reference* (pp. 312–529). Baltimore: The Johns Hopkins University Press.

Smith, M. F. & Patton, J. L. (1993). Diversification of South American muroid rodents: evidence from mitochondrial DNA sequence data for the Akodontine tribe. *Biological Journal of the Linnean Society*, *50*, 149–177.

Spitzenberger, F. (1994). The genus *Eptesicus* (Mammalia: Chiroptera) in southern Anatolia. *Folia Zoologica*, *43*, 437–454.

Stadelmann, B., Herrera, L. G., Arroyo-Cabrales, J., Flores-Martínez, J. J., May, B. P. & Ruedi, M. (2004). Molecular systematics of the fishing bat *Myotis* (*Pizonyx*) *vivesi*. *Journal of Mammalogy*, *85*, 133–139.

Stadelmann, B., Lin, K. L., Kunz, T. H. & Ruedi, M. (2007). Molecular phylogeny of New World Myotis (Chiroptera, Vespertilionidae) inferred from mitochondrial and nuclear DNA genes. *Molecular Phylogenetics and Evolution*, *43*, 32–48.

Strelkov, P. P. & Iljin, V. J. (1992). Bats of easternmost Europe: distribution and faunal status. In I. Horáček & V. Vohralík (Eds) *Prague Studies in Mammalogy* (pp. 193–205). Praha: Charles University Press.

Swofford, D. L. (2001). *PAUP*. Phylogenetic Analysis Using Parsimony (*and other methods) Version 4.0b10*. Sunderland, MA, USA: Sinauer Associates.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. (2011). MEGA5: molecular evolutionary genetics analysis using maximum likelihood, Evolutionary Distance, and Maximum Parsimony Methods. *Molecular Biology and Evolution*, *28*, 2731–2739.

Toews, D. & Brelsford, A. (2012). The biogeography of mitochondrial and nuclear discordance in animals. *Molecular Ecology*, *21*, 3907–3930.

Turmelle, A. S., Kunz, T. H. & Sorenson, M. D. (2011). A tale of two genomes: contrasting patterns of phylogeographic structure in a widely distributed bat. *Molecular Ecology*, *20*, 357–375.

Vallo, P., Benda, P., Červeny, J. & Koubek, P. (2012). Conflicting mitochondrial and nuclear paraphyly in small-size West African house bats (Vespertilionidae). *Zoologica Scripta*, *42*, 1–12.

Volleth, M. & Tideman, C. R. (1989). Chromosome studies in three genera of Australian vespertilionid bats and their systematic implications. *Zeitschrift für Säugetierkunde*, *54*, 215–222.

Volleth, M. & Tidemann, C. R. (1991). The origin of the Australian Vespertilioninae bats, as indicated by chromosomal studies. *Zeitschrift für Säugetierkunde*, *56*, 321–330.

Volleth, M., Bronner, G., Göpfert, M. C., Heller, K. G., von Helversen, O. & Yong, H. S. (2001). Karyotype comparison and phylogenetic relationships of *Pipistrellus*-like bats (Vespertilionidae; Chiroptera; Mammalia). *Chromosome Research*, *9*, 25–46.

Wiens, J. J. (1998). Combining data with different phylogenetic stories. *Systematic Biology*, *47*, 568–581.

Wiens, J. J., Kuckzynski, C. A. & Stephens, P. R. (2010). Discordant mitochondrial and nuclear gene phylogenies in emydid turtles: implications for speciation and conservation. *Biological Journal of the Linnean Society*, *99*, 445–461.

Zhang, D.-X. & Hewitt, G. M. (2003). Nuclear DNA analyses in genetic studies of populations: practice, problems and prospects. *Molecular Ecology*, *12*, 563–584.

Zhang, J. S., Han, N. J., Jones, G., Lin, L. K., Zhang, J. P., Zhu, G. J., Huang, D. W. & Zhang, S. Y. (2007). A new species of *Barbastella* (Chiroptera: Vespertilionidae) from north China. *Journal of Mammalogy*, *88*, 1393–1403.

Appendix 1 List of specimen acronyms, taxonomic considerations, localities, GenBank accession numbers (*Cytb*, *ND1*, *RAG2* and *BGN*) and source of the samples used for the study

			GenBank accession no.				
Specimen	Proposed taxonomy	Locality	Cyt b	ND1	RAG2	BGN	Voucher/source
1Ean IR	*Eptesicus anatolicus*	Bisotun, Kermanshah, Iran	EU786802	EU786926	–	–	
2Ean IR	*Eptesicus anatolicus*	Bavineh, Lorestan, Iran	EU786803	EU786927	–	–	NMP 48192
3Ean IR	*Eptesicus anatolicus*	Qasr-e-Shirin, Kermashah, Iran	EU786804	EU786928	–	–	NMP 48193
4Ean SY	*Eptesicus anatolicus*	Qala'at Sheisar, Hama, Syria	EU786805	EU786929	–	–	NMP 48893
5Ean SY	*Eptesicus anatolicus*	Qala'at Sheisar, Hama, Syria	EU786806	EU786930	–	–	NMP 48894
6Ean IR	*Eptesicus anatolicus*	Deh Bakri, Kerman, Iran	EU786807	EU786931	FJ841977	KF018958	NMP 48363
7Ean SY	*Eptesicus anatolicus*	Baniyas, Hama, Syria	EU786808	EU786932	EU786878	KF018959	NMP 48900
8Ean SY	*Eptesicus anatolicus*	Baniyas, Hama, Syria	EU786809	EU786933	EU786879	KF018960	NMP 48901
9Ean SY	*Eptesicus anatolicus*	Qala'at Marqab, Hama, Syria	EU786810	EU786934	–	–	NMP 48918
10Ean TK	*Eptesicus anatolicus*	Silifke, Içel, Turkey	EU786811	EU786935	EU786880	KF018961	Karataş, A.
11Ean TK	*Eptesicus anatolicus*	Silifke, Astim Caves, Içel, Turkey	EU786812	EU786936	EU786881	KF018962	Karataş, A.
12Ebo IR	*Eptesicus bottae taftanimontis*	Bam, Kerman, Iran	EU786813	EU786937	FJ841978	KF018963	NMP 48114
13Ebo IR	*Eptesicus bottae taftanimontis*	Bam, Kerman, Iran	EU786814	EU786938	–	–	NMP 48115
14Ebo JO	*Eptesicus bottae innesi*	Wadi Rum, Jordan	EU786815	EU786939	EU786882	KF018964	NMP 92100
15Ebo SY	*Eptesicus bottae hingstoni*	Balis, Halab, Syria	EU786816	EU786940	–	–	NMP 48770
16Ebo SY	*Eptesicus bottae hingstoni*	Rasafah, Raqqa, Syria	EU786817	EU786941	EU786883	–	NMP 48771
17Ebo SY	*Eptesicus bottae hingstoni*	Rasafah, Raqqa, Syria	EU786818	EU786942	–	–	NMP 48772

Appendix 1. Continued

Specimen	Proposed taxonomy	Locality	GenBank accession no.				Voucher/source
			Cyt b	ND1	RAG2	BGN	
18Ebo SY	*Eptesicus bottae hingstoni*	Dura Europos, Deir ez-Zur, Syria	EU786819	EU786943	–	–	NMP 48805
19Ebo SY	*Eptesicus bottae hingstoni*	Khazneh, Hassake, Syria	EU786820	EU786944	EU786884	KF018965	NMP 48818
20Ebo SY	*Eptesicus bottae hingstoni*	Khazneh, Hassake, Syria	EU786821	EU786945	–	–	NMP 48819
21Eho SA	*Eptesicus hottentotus hottentotus*	Algeria Natal Forestry St. South Africa	AJ841963	EU786946	EU786885	KF018966	Ruedi, M.
22Epa SA	*Eptesicus hottentotus pallidior*	Goodhouse, South Africa	EU786823	EU786947	EU786886	KF018967	Ruedi, M.
23Eis LI	*Eptesicus isabellinus isabellinus*	Leptis Magna, Libya	EU786824	EU786948	EU786887	KF018968	NMP 49940
24Eis LI	*Eptesicus isabellinus isabellinus*	Leptis Magna, Libya	EU786825	EU786949	–	–	NMP 49941
25Eis LI	*Eptesicus isabellinus isabellinus*	Ar Sharsharah, Tarhunah, Libya	EU786826	EU786950	–	–	NMP 49950
26Eis LI	*Eptesicus isabellinus isabellinus*	Ar Sharsharah, Tarhunah, Libya	EU786827	EU786951	–	–	NMP 49951
27Eis LI	*Eptesicus isabellinus isabellinus*	Nanatalah, Libya	EU786828	EU786952	–	–	NMP 49961
28Eis LI	*Eptesicus isabellinus isabellinus*	Sabratah, Libya	EU786829	EU786953	EU786888	KF018969	NMP 49976
29Eis LI	*Eptesicus isabellinus isabellinus*	Sabratah, Libya	EU786830	EU786954	–	–	NMP 49977
30Eis LI	*Eptesicus isabellinus isabellinus*	Sabratah, Libya	EU786831	EU786955	–	–	NMP 49979
31Eis MO	*Eptesicus isabellinus boscai*	Berkane, Gorge du Zegzel, Morocco	EU786832	EU786956	–	–	NMP 90086
32Eis MO	*Eptesicus isabellinus boscai*	Berkane, Gorge du Zegzel, Morocco	EU786833	EU786957	–	–	NMP 90087
33Eis MO	*Eptesicus isabellinus boscai*	Ez Zarka, Yarrhite, Tetouan, Morocco	EU786834	EU786958	EU786889	KF018970	This paper
34Eis MO	*Eptesicus isabellinus boscai*	Oued Massa, Morocco	EU786835	EU786959	EU786890	KF018971	This paper
35Eis SP	*Eptesicus isabellinus boscai*	Túnel del Picote, Huelva, Spain	EU786836	EU786960	EU786891	KF018972	This paper
36Eis SP	*Eptesicus isabellinus boscai*	Puente Cañaveroso, Sevilla, Spain	EU786837	EU786961	EU786892	KF018973	This paper
37Eis SP	*Eptesicus isabellinus boscai*	Cádiz, Spain	EU786838	EU786962	–	–	This paper
38Rna IR	*Rhyneptesicus nasutus nasutus*	Pir Sohrab, Baluchestan, Iran	FJ841980	FJ841982	–	–	This paper
39Rna IR	*Rhyneptesicus nasutus nasutus*	Pir Sohrab, Baluchestan, Iran	EU786839	EU786963	EU786893	KF018974	NMP 48405
40Rna IR	*Rhyneptesicus nasutus nasutus*	Dehbarez, Hormozgan, Iran	EU786840	EU786964	EU786894	KF018975	NMP 48437
41Rna IR	*Rhyneptesicus nasutus nasutus*	Dehbarez, Hormozgan, Iran	FJ841981	FJ841983	–	–	This paper
42Ead CH	*Eptesicus pachyomus pallens*	Daguping, nr Foping, Shaanxi, China	EU786841	EU786965	EU786895	KF018976	NMP 90554
43Etu CY	*Eptesicus serotinus mirza*	Troodos Forest, Kalidonia Trail, Cyprus	EU786842	EU786966	EU786896	KF018977	NMP 90409
44Ese CZ	*Eptesicus serotinus serotinus*	Kolence, South Bohemia, Czech Republic	EU786843	EU786967	EU786897	KF018978	NMP 90182
45Ese CZ	*Eptesicus serotinus serotinus*	Třebíč, Příštpo, Moravia, Czech Republic	EU786844	EU786968	EU786898	KF018979	NMP 90183
46Ese DE	*Eptesicus serotinus serotinus*	Gredstedbro, Jutland, Denmark	EU786845	EU786969	EU786899	KF018980	Baagøe, H.
47Ese FR	*Eptesicus serotinus serotinus*	Châtelus, France	EU786846	EU786970	–	–	Noblet, J.F
48Ese GR	*Eptesicus serotinus serotinus*	Kombotades, Lamia, Greece	EU786847	EU786971	–	–	NMP 48723
49Ese GR	*Eptesicus serotinus serotinus*	Chalkidiki, Greece	AF376837	AY033950	–	–	GenBank
50Ese IT	*Eptesicus serotinus serotinus*	Modena, Italy	EU786848	EU786972	EU786900	KF018981	Scaravelli, D.
51Ead LA	*Eptesicus pachyomus andersoni*	Nam Chong River, Novaphan, Laos	EU786849	EU786973	EU786901	KF018982	EBD25698
52Ead LA	*Eptesicus pachyomus andersoni*	Bam Buaphath, Novaphan, Laos	EU786850	EU786974	EU786902	KF018983	ROM 118316
53Ese SL	*Eptesicus serotinus serotinus*	Dobrá Niva, Zvolen, Slovakia	EU786851	EU786975	–	–	NMP 9018
54Ese SP	*Eptesicus serotinus serotinus*	El Rasillo, La Rioja, Spain	EU786852	EU786976	EU786903	KF018984	This paper
55Ese SP	*Eptesicus serotinus serotinus*	Sima de San Pedro, Teruel, Spain	EU786853	EU786977	EU786904	KF018985	This paper
56Ese SP	*Eptesicus serotinus serotinus*	Ordesa, Huesca, Spain	EU786854	EU786978	–	–	This paper
57Etu SY	*Eptesicus serotinus mirza*	Slinfeh, Al Lataquieh, Syria	EU786855	EU786979	EU786905	KF018986	NMP 48058
58Etu SY	*Eptesicus serotinus mirza*	Slinfeh, Al Lataquieh, Syria	EU786856	EU786980	–	–	NMP 48059
59Etu SY	*Eptesicus serotinus mirza*	Safita, Hama, Syria	EU786857	EU786981	–	–	NMP 48875
60Etu SY	*Eptesicus serotinus mirza*	Hayalien, Hama, Syria	EU786858	EU786982	EU786906	KF018987	NMP 48924
61Etu SY	*Eptesicus serotinus mirza*	Hayalien, Hama, Syria	EU786859	EU786983	EU786907	KF018988	NMP 48925
62Ese TU	*Eptesicus serotinus serotinus*	Tuz Gölü, Turkey	EU786860	EU786984	–	–	NMP 90012
63Etu TU	*Eptesicus serotinus mirza*	Van Castle, Anakõz Gate, Turkey	EU786861	EU786985	–	–	Karataş, A.
64Ese UK	*Eptesicus serotinus serotinus*	Devon, United Kingdom	EU786862	EU786986	EU786908	KF018989	Rossiter, S.
65Ese UK	*Eptesicus serotinus serotinus*	Somerset, United Kingdom	EU786863	EU786987	EU786909	KF018990	Rossiter, S.
66Eni GE	*Eptesicus nilssonii*	Germany	AF376836	AY033987	DQ120811	KF018991	GenBank*
67Edi VE	*Eptesicus diminutus*	Guárico, Venezuela	EU786864	EU786988	EU786910	KF018992	TK15033
68Efr VE	*Eptesicus furinalis*	Guárico, Venezuela	EU786865	EU786989	EU786911	KF018993	TK15160
69Efs US	*Eptesicus fuscus*	Texas, USA	EU786866	EU786990	EU786912	KF018994	TK5893
70Efs US	*Eptesicus fuscus*	Massachussets, USA	EU786867	EU786991	EU786913	KF018995	TK13274
71Nbr GA	*Neoromicia bruneus*	Estuaire province, Gabon	EU786868	EU786992	EU786914	KF018996	TK21501
72Nso KE	*Neoromicia somalicus*	Coastal province, Kenya	EU786869	EU786993	EU786915	KF018997	TK33190

Appendix 1. Continued

Specimen	Proposed taxonomy	Locality	GenBank accession no.				Voucher/source
			Cyt b	ND1	RAG2	BGN	
73Vmu SW	*Vespertilio murinus*	Valais, Switzerland	AF376834	AY033964	EU786916	KF018998	GenBank*
74Hca LA	*Hypsugo cadornae*	Laos	DQ318883	DQ120797	DQ120828	KF018999	GenBank*
75Hsa SP	*Hypsugo savii*	Spain	DQ120861	DQ120798	DQ120825	KF019000	GenBank*
77Pku SP	*Pipistrellus kuhlii*	Spain	DQ120846	DQ120796	DQ120829	KF019001	GenBank*
79Ppi SP	*Pipistrellus pipistrellus*	Spain	DQ120854	DQ120794	DQ120831	KF019002	GenBank*
81Pau SW	*Plecotus auritus*	Switzerland	AF513758	–	DQ120821	KF019003	GenBank*
82Pau GE	*Plecotus auritus*	Germany	–	AF401374	–	–	GenBank
83Pma SP	*Plecotus macrobullaris*	Spain	AY306213	AY328904	DQ120822	KF019004	GenBank*
84Mmy GE	*Myotis myotis*	Germany	AF376860	AY033986	–	–	GenBank
85Mmy SP	*Myotis myotis*	Spain	–	–	DQ120812	KF019005	GenBank*
86Msh IR	*Myotis schaubi*	Choplu, West Azerbaijan, Iran	AF376868	AY033955	DQ120818	–	NMP48130
90Etu IR	*Eptesicus serotinus* (=*turcomanus*)	Sharaf Caravanserai, Khorasan Razni, Iran	EU786870	EU786994	EU786918	–	NMP90779
91Etu IR	*Eptesicus serotinus* (=*turcomanus*)	Sharaf Caravanserai, Khorasan Razni, Iran	EU786871	EU786995	EU786919	KF019006	NMP90780
92Etu IR	*Eptesicus serotinus* (=*turcomanus*)	Amir Abad, Khorasan Razni, Iran	EU786872	EU786996	EU786920	–	NMP90800
93Etu IR	*Eptesicus serotinus* (=*turcomanus*)	Amir Abad, Khorasan Razni, Iran	EU786873	EU786997	EU786921	KF019007	NMP90801
94Etu IR	*Eptesicus serotinus* (=*turcomanus*)	Korud Abad, SE Ali Abad, Golestan, Iran	EU786874	EU786998	EU786922	KF019008	NMP90865
95Etu IR	*Eptesicus serotinus* (=*turcomanus*)	Korud Abad, SE Ali Abad, Golestan, Iran	EU786875	EU786999	EU786923	KF019009	NMP90866
96Eog IR	*Eptesicus ognevi*	Shurlaq, Khorasan Razni, Iran	–	–	FJ8419779	KF019010	NMP90789
97Eog IR	*Eptesicus ognevi*	Amir Abad, Khorasan Razni, Iran	EU786876	EU787000	EU786924	KF019011	NMP90809
98Eog IR	*Eptesicus ognevi*	Amir Abad, Khorasan Razni, Iran	EU786877	EU787001	EU786925	KF019012	NMP90810
99EboOM	*Eptesicus bottae omanensis*	Misfat Al-Khawater, Oman	KF019039	KF019069	KF018930	KF019013	NMP 93783
100EboOM	*Eptesicus bottae omanensis*	5 km W of Rawdah, Oman	KF019040	KF019070	KF018931	KF019014	NMP 93793
101EboOM	*Eptesicus bottae omanensis*	Al-Khudayrah, Oman	KF019041	KF019071	KF018932	KF019015	NMP 93818
102RnaOM	*Rhyneptesicus nasutus matschiei*	Muntasar, Oman	KF019042	KF019072	KF018933	KF019016	NMP 93719
103RnaOM	*Rhyneptesicus nasutus matschiei*	2 km S of Al-Rumayliyah, Oman	KF019043	KF019073	KF018934	KF019017	NMP 93720
104RnaOM	*Rhyneptesicus nasutus matschiei*	Al-Ajal, Oman	KF019044	KF019074	KF018935	KF019018	NMP 93828
105EanIR	*Eptesicus anatolicus*	Tadavan, Iran	KF019045	KF019075	KF018936	KF019019	Aihartza, J. *et al.*
106EseIR	*Eptesicus serotinus serotinus*	Dashkasan, Iran	KF019046	KF019076	KF018937	KF019020	Aihartza, J. *et al.*
107EseGEO	*Eptesicus serotinus serotinus*	Abano, Tusheti, Georgia	KF019047	KF019077	KF018938	KF019021	Aihartza, J. *et al.*
108EseGEO	*Eptesicus serotinus serotinus*	Dartlo, Tusheti, Georgia	KF019048	KF019078	KF018939	KF019022	Aihartza, J. *et al.*
109EseGEO	*Eptesicus serotinus serotinus*	Kveda Chkepi, Tmereti, Georgia	KF019049	KF019079	KF018940	KF019023	Aihartza, J. *et al.*
110EpaIR	*Eptesicus pachyomus pachyomus*	Dehbarez, Hormozgan, Iran	KF019050	KF019080	KF018941	KF019024	NMP 48436
111NguYE	*Neoromicia guineensis*	Jebel Bura, Riqab, Al Hudaydah, Yemen	KF019051	KF019081	KF018942	KF019025	PB3124
112NguYE	*Neoromicia guineensis*	Jebel Bura, Riqab, Al Hudaydah, Yemen	KF019052	KF019082	KF018943	–	PB3125
113NscYE	*Nycticeinops schlieffeni*	Kadamat al 'Abali, Lahj, Yemen	KF019053	KF019083	KF018944	KF019026	PB3602
114NguYE	*Neoromicia guineensis*	Ash Shukayrah, Taiz, Yemen	KF019054	KF019084	KF018945	KF019027	PB3663
115NguYE	*Neoromicia guineensis*	Ash Shukayrah, Taiz, Yemen	KF019055	KF019085	KF018946	–	PB3664
116RnaYE	*Rhyneptesicus nasutus batinensis*	Al Mawkir, Al Hudaydah, Yemen	KF019056	KF019086	KF018947	KF019028	PB3708
117RnaYE	*Rhyneptesicus nasutus batinensis*	Al Mawkir, Al Hudaydah, Yemen	KF019057	KF019087	KF018948	KF019029	PB3714
118NscYE	*Nycticeinops schlieffeni*	Al Mawkir, Al Hudaydah, Yemen	KF019058	KF019088	KF018949	KF019030	PB3716
119NscYE	*Nycticeinops schlieffeni*	Ba Tays, Abyan, Yemen	KF019059	KF019089	KF018950	KF019031	PB3801
120EboJO	*Eptesicus bottae innesi*	Khirbet Feynan, Karak, Jordan	KF019060	KF019090	KF018951	KF019032	NMP 92426
121EboJO	*Eptesicus bottae innesi*	Al Ghal, Aqaba, Jordan	KF019061	KF019091	KF018952	KF019033	NMP 92477
122EboJO	*Eptesicus bottae innesi*	Al Ghal, Aqaba, Jordan	KF019062	KF019092	KF018953	KF019034	NMP 92479
123EseUKR	*Eptesicus serotinus serotinus*	Uzundja, Crimea, Ukraine	KF019063	KF019093	KF018954	KF019035	PB4298
124EseUKR	*Eptesicus serotinus serotinus*	General'skoe, Crimea, Ukraine	KF019064	KF019094	–	KF019036	PB4362
125EboOM	*Eptesicus bottae omanensis*	Al Aqar, Wakan, Oman	KF019065	KF019095	KF018955	–	NMP 92622
126EboOM	*Eptesicus bottae omanensis*	Dhahir Al Fawaris, Oman	KF019066	KF019096	KF018956	KF019037	NMP 92655
127EboOM	*Eptesicus bottae omanensis*	Al Nakhar, Oman	KF019067	KF019097	KF018957	KF019038	NMP 92664
128EboOM	*Eptesicus bottae omanensis*	Mansaft, Oman	KF019068	KF019098	–	–	NMP 92781

*GenBank – Genbank and this paper.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. Comparison between evolutionary hypotheses obtained under Bayesian posterior probabilities for the two mtDNA fragments and according to GTR substitution models.

Fig. S2. Comparison between evolutionary hypotheses obtained under Bayesian posterior probabilities for the two nuDNA fragments and according to GTR substitution models.

Table S1. Estimates of net divergence between the main taxonomic units studied and obtained using the Kimura 2-parameter model (lower semi-matrix) and number of base differences per site (*P*-value; upper semi-matrix) conducted in MEGA5 Tamura *et al.* 2011.