

A molecular reappraisal of the taxonomic status of *Eptesicus serotinus turcomanus*

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Abstract. The taxonomic status of the Turanian serotine *Eptesicus serotinus turcomanus* is still debatable. To examine the pattern of genetic variation in *E. s. turcomanus* and *E. s. serotinus* from SE Europe we analyzed sequence data on mitochondrial *cytb* gene and introns of THY and SPTBN genes. The *cytb* results do not reveal any substantial difference between *E. s. turcomanus* and *E. s. serotinus* from southern Russia. In contrast to that, the data on both nuclear genes indicate moderate differentiation between eastern and western populations and, at the same time, suggest the existence of gene flow between them. Several population history hypotheses can be proposed to explain the pattern. However, regardless of the scenario, our results demonstrate the lack of genetic isolation between *E. s. serotinus* and *E. s. turcomanus* and, therefore, contradict the species status for the latter.

***Eptesicus*, taxonomy, serotine, East Europe, Central Asia, geographic variation, Chiroptera**

Introduction

The common serotine, *Eptesicus serotinus* (Schreber, 1774), is one of the most widely distributed Palaearctic bat species, occurring from westernmost Europe to northern Indochina and Taiwan (Bobrinskij et al. 1965, Corbet 1978, Corbet & Hill 1992, Simmons 2005, Smith & Xie 2008). Such a large distribution range correlates with pronounced geographical variation, with about ten subspecies being currently recognized (Simmons 2005); presumably two of them are present in the Russian fauna (Strelkov & Iljin 1992).

However, recent studies, and primarily those based on molecular data, demonstrated significant heterogeneity within *E. serotinus* sensu lato suggesting that this name might correspond to a complex of cryptic species (Ibáñez et al. 2006, Mayer et al. 2007). The separate species status of one of these forms – *E. isabellinus* (Temminck, 1840) – has been confirmed by several studies and accepted by most specialists (Benda et al. 2004, Garcia-Mudarra et al. 2009, Ibáñez et al. 2006).

The so-called Turanian serotine, *E. s. turcomanus* (Eversmann, 1840) was described from the territory of Kazakhstan, “in between the Aral and Caspian seas” (cf. Ognev 1928, Pavlinov & Rosolimo 1987), probably, from the northern slopes of the Ustyurt Plateau (see Sokolov & Shishkin 2005). Considered a full species in the original description, it is now regarded as a subspecies of *E. serotinus*. Traditionally *E. s. turcomanus* was distinguished from the nominotypical subspecies by smaller skull size and paler skin and fur coloration (Bobrinskij et al. 1965, Ognev 1928, Gaisler 1970). Both subspecies occur in the European part of Russia, their distribution being parapatric in the Lower Volga region (Iljin et al. 2002, Strelkov & Iljin 1992). Benda et al. (2006) analyzed craniodental measurements of West Asian serotines and confirmed that *E. s. turcomanus* is a well-

differentiated subspecies of *E. serotinus*. Smirnov & Yanyaeva (2003) conducted a colorimetric analysis of fur color; according to these authors, populations assigned to *E. s. turcomanus* are significantly different from the typical serotine despite high color variation within certain populations. Finally, based on the results of inter-SINE PCR analysis, it was suggested to elevate the Turanian serotine to the species rank (Mateveev 2003); the same conclusion was supported by some mtDNA studies (Juste et al. 2010, Benda et al. 2011). Contrary to that, our previous molecular results (Artyushin et al. 2009) did not reveal any significant difference between *E. s. serotinus* and *E. s. turcomanus* in southern Russia, this being in sharp contrast to huge mtDNA differentiation between Russian and West European *E. serotinus* s.str. The latter phenomenon was explained by mtDNA introgression from a different species – *E. nilssonii* (Keyserling et Blasius, 1839) – which affected only the European populations of *E. serotinus* (Artyushin et al. 2009).

Given this background, one has to conclude that the status of the Turanian serotine still requires clarification. In our study we addressed this issue based on the sequence data for one mitochondrial and two nuclear genes.

Material and Methods

In our analyses we used 67 *Eptesicus serotinus* s.l. tissue samples from museum vouchers and our tissue sample collection. We also used our previously obtained sequences deposited in the GeneBank. For sampling localities, voucher and GeneBank accession numbers see Appendix.

Genomic DNA was isolated from ethanol-fixed liver, kidney or muscles by proteinase K digestion, phenol-chloroform deproteinization and isopropanol precipitation (Sambrook et al. 1989). The whole of the mitochondrial *cytb* gene (1140 bp) was amplified by polymerase chain reaction (PCR) with the forward/reverse primer combination L14734/H15395_pip; in cases when DNA was degraded, fragments of *cytb* were amplified using the internal primers Ept_L486 and Ept_H602 (Artyushin et al. 2009). For amplification of intron 2 and exon 3 of THY gene and intron 13 of SPTBN gene we used primers designed by Eick et al (2005).

The conditions of the double-stranded PCR for *cytb* and introns amplification included the initial denaturation at 94 °C for 3 min, 35 cycles of 94 °C for 30 s, annealing at 57 °C for *cytb*, 59 °C for THY and 71 °C for SPTBN during 1 min, and extension at 72 °C for 1 min, followed by a final extension at 72 °C for 10 min, and indefinite storage at 4 °C. PCR products were visualized on 1% agarose gel and then purified using DEAE Watman or NH₄EtOH.

Approximately 10–40 ng of the purified PCR product were used for sequencing with each primer by the ABI 3100-Avant autosequencing system using ABI PRISM®BigDyeTM Terminator v. 3.1.

Sequences were aligned by eye in Bioedit (v. 7.0.9; Hall 1999). For allelic phase reconstruction, Phase 2.1 (Stephens et al. 2001, Stephens & Donnelly 2003) was used. Total alignment length for THY and SPTBN genes was 498 bp and 540 bp, respectively.

The SPTBN alignment contained informative gaps which were recoded and treated as nucleotide substitutions in subsequent analysis. For haplotype frequency analysis we divided our sample into 8 geographical groups (Fig. 1). Three eastern samples (ASTR, KAZ and UZB) include specimens which are identified as *E. s. turcomanus* based on morphological criteria. Four other geographical samples (KRSN, SAM, BRYA, UKR) represent the nominotypical subspecies. Most of specimens from the Volgograd region (VOLG) belong to *E. s. serotinus*; however, the attribution of several specimens is ambiguous.

Statistical parsimony networks were drawn by TCS v1.21 software (Clement et al. 2000) using default settings.

NJ trees and inter-population net-distance matrices were calculated in MEGA version 5 (Tamura et al. 2011) based on uncorrected p-distances between haplotypes. The matrix of net-distances between geographical populations was factored using the principal coordinate method as implemented in NTSYS 2.0 (Rohlf 1998)

Results and Discussion

Haplotype network analysis for both genes yielded distinct groupings: two groups for THY (TA, TB) and three groups for SPTBN (SA, SB, SC) (Fig. 2). The NJ trees also contain most of these groupings, but with moderate to low support (Fig. 3).

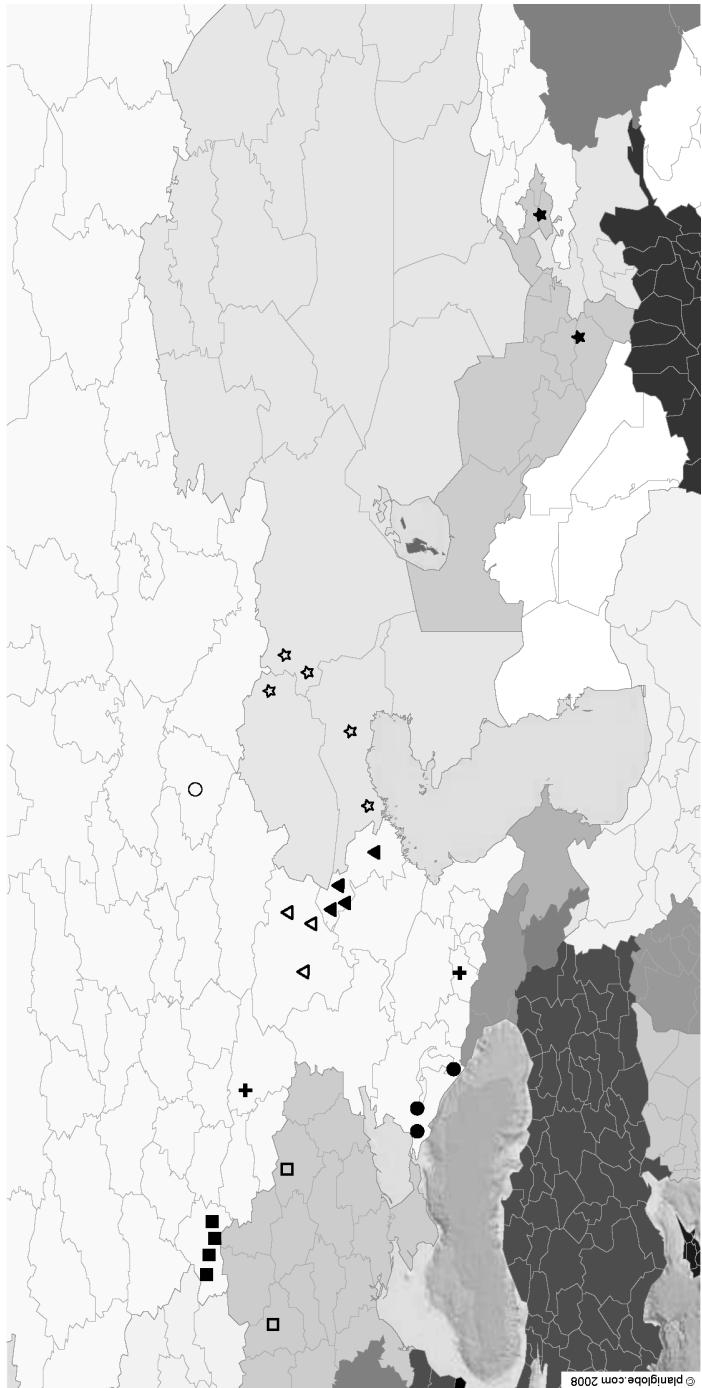


Fig. 1. Sampling localities and geographical groups. Samples belonging to different geographical groups are denoted by the following symbols: open square – UKR (Ukraine), filled square – BRYA (Bryansk region), open circle – SAM (Samara region), filled circle – KRSN (Krasnoyarsk), open triangle – VOLG (Volgograd region), filled triangle – ASTR (Astrakhan), open star – KAZ (Kazakhstan), filled star – UZB (Uzbekistan), cross – ungrouped samples.

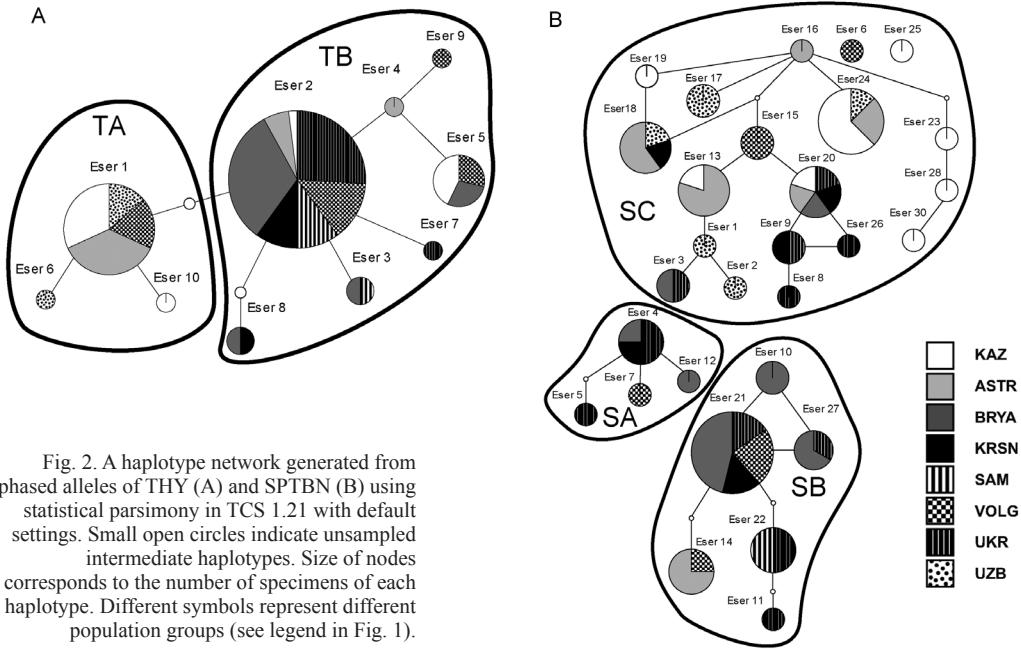


Fig. 2. A haplotype network generated from phased alleles of THY (A) and SPTBN (B) using statistical parsimony in TCS 1.21 with default settings. Small open circles indicate unsampled intermediate haplotypes. Size of nodes corresponds to the number of specimens of each haplotype. Different symbols represent different population groups (see legend in Fig. 1).

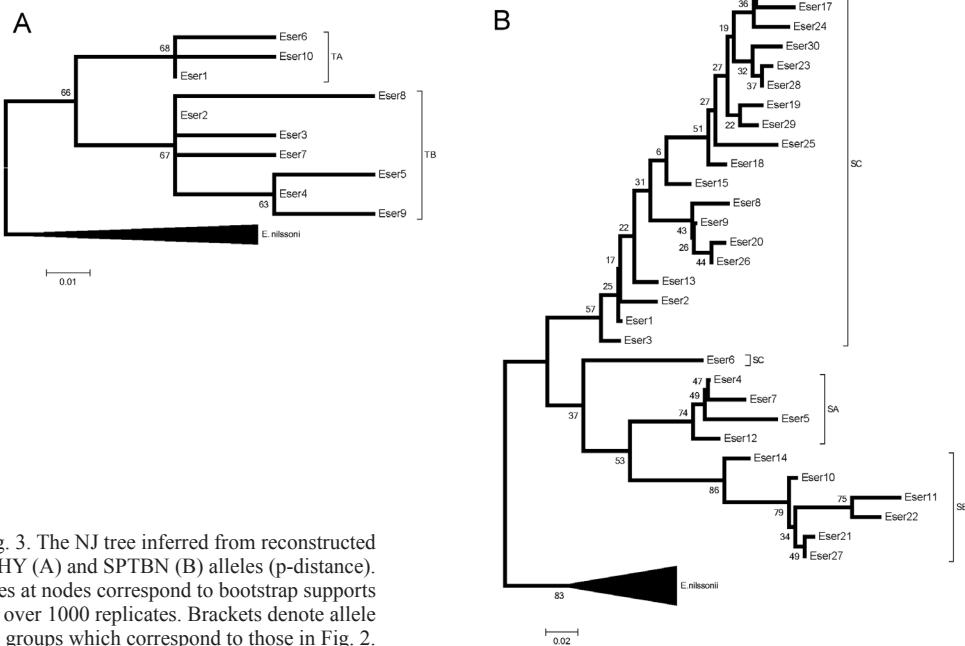


Fig. 3. The NJ tree inferred from reconstructed THY (A) and SPTBN (B) alleles (p-distance). Values at nodes correspond to bootstrap supports over 1000 replicates. Brackets denote allele groups which correspond to those in Fig. 2.

Table 1. Net-distance between different species and populations of *Eptesicus*. Distance values in rows 1 and 2 were calculated as the weighed mean of interpopulation net-distances with weights corresponding to sample sizes

net distance (%)	THY	SPTBN	<i>cyt b</i>
<i>E. s. serotinus</i> – <i>E. s. turcomanus</i>	0.13	0.70	0.150
<i>E. serotinus</i> – <i>E. nilssonii</i>	0.43	1.58	5.770
within <i>E. nilssonii</i> (western – eastern populations)	0.00	0.00	1.700
(<i>E. s. serotinus</i> – <i>E. s. turcomanus</i>) / (<i>E. serotinus</i> – <i>E. nilssonii</i>)	0.31	0.44	0.026

As can be seen in Fig. 2, neither of the two loci can be used to diagnose between *E. s. serotinus* and *E. s. turcomanus*. The most common allele groups such as TB and SC are distributed throughout the examined range. Moreover, a number of alleles (THY-Eser2, SPTBN-Eser20, SPTBN-Eser18) are shared by geographically distant eastern and western populations. For example, the haplotype THY-Eser2 is found in Ukraine, in the Bryansk, Volgograd and Krasnodar regions as well as in the Astrakhan region and Kazakhstan.

At the same time, the data indicate a certain level of differentiation between eastern (ASTR, KAZ and UZB) and western (KRSN, VOLG, SAM, BRYA, UKR) populations manifested primarily as the difference in haplotype group frequencies (Fig. 4). Thus, the SC and TB groups are obviously more frequent in eastern and western populations, respectively, while the SC group is restricted to the latter.

The same pattern of inter-population variation is evident from the results of the principle coordinate analysis (Fig. 5). The eastern populations (ASTR, KAZ, UZB) are clearly separated from most of the western ones (SAM, BRYA, UKR) with VOLG (in case of THY) or KRSN (in case of SPTBN) occupying intermediate positions. It can be concluded that genetic distances between populations roughly correspond to geographical distances, with the distance between adjacent VOLG and ASTR being, however, disproportionately large.

In contrast to nuclear genes, the mtDNA shows a little geographical variation. All native (non-introgressed) haplotypes from both western and eastern populations constitute a single shallow clade (Fig. 6). As evident from the values of net-distances (Table 1), there is an apparent

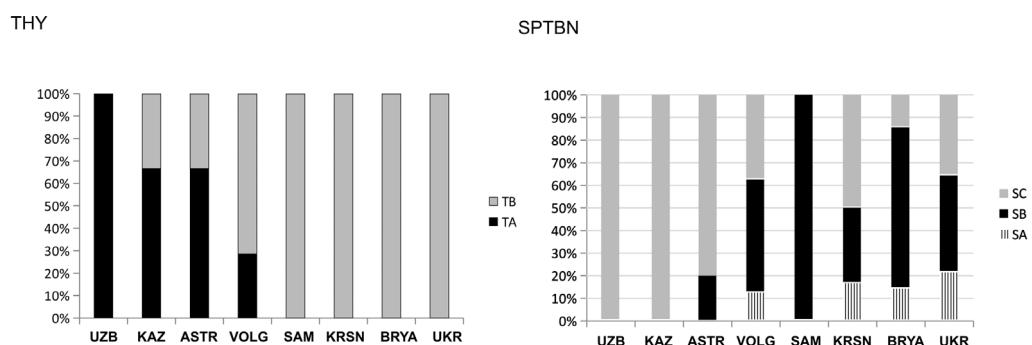


Fig. 4. Distribution of allele groups in different geographical samples. All designations follow Fig. 2.

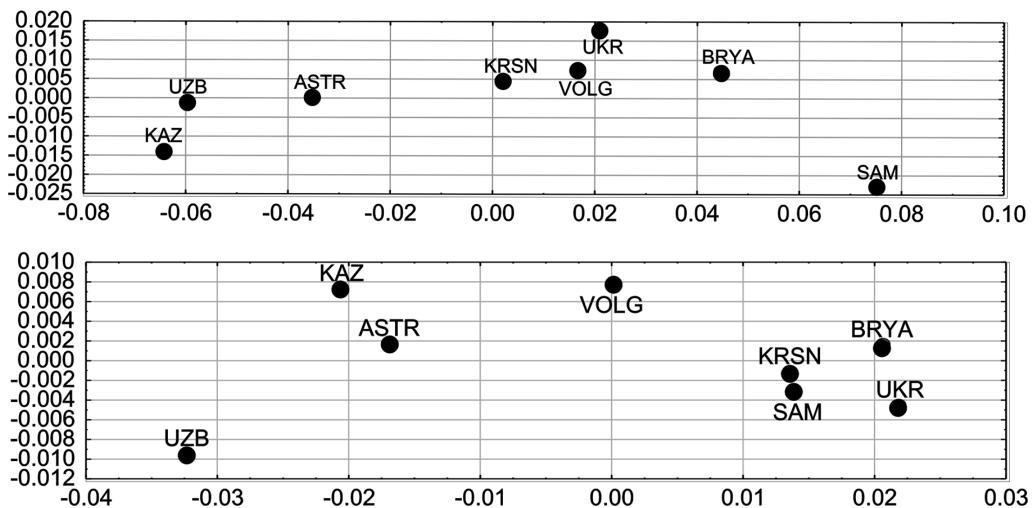


Fig. 5. The plot of the first two principal coordinates (% and %) produced by the analysis of net-distance matrix calculated from THY (above) and SPTBN (below) data.

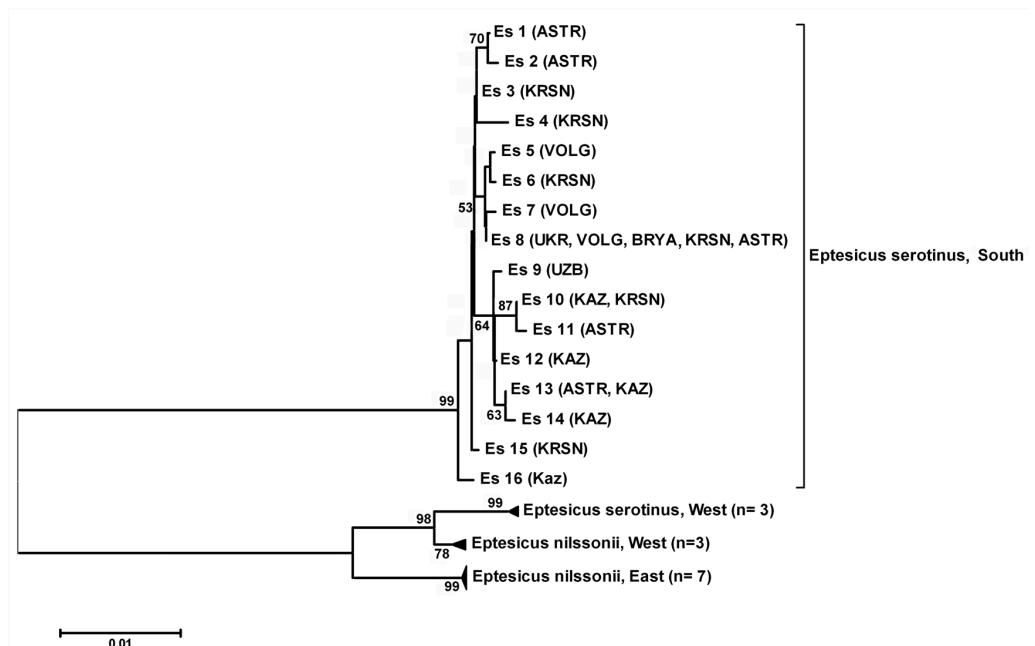


Fig. 6. The NJ tree inferred from reconstructed cytb haplotype data (p-distance). Values at nodes correspond to bootstrap supports over 1000 replicates.

discrepancy in the levels of genetic differentiation between *E. s. serotinus* and *E. s. turcomanus* as assessed from the nuclear versus mitochondrial data. Nuclear genes suggest a much higher divergence between the two forms relative to the distance between *E. serotinus* and *E. nilssonii* or within the latter.

The observed discordance between nuclear and mitochondrial gene markers can be explained by several scenarios. One of them implies that, in correspondence to the mtDNA data pattern, the two forms have diverged quite recently. Then, the apparently excessive variability in nuclear genes might be explained by high ancestral polymorphism. This hypothesis is in agreement with the fact that *E. s. serotinus* and *E. s. turcomanus* are very similar morphologically with size, skull shape and pelage coloration being the main discriminative features.

At the same time, nuclear data tentatively support subdivision into two distinct groups of populations (despite the fact that their gene pools share many common alleles). Based on that, one can hypothesize that the ancestors of *E. s. serotinus* and *E. s. turcomanus* were once separated and, for some time, the two lineages evolved in isolation from each other having differentiated finally to the level of distinct subspecies. At a later stage, a secondary contact zone was formed, presumably as a result of postglacial range expansion. According to this scenario, the pattern of spatial variation in nuclear genes (a steep cline in the Lower Volga region) is best explained by the effect of recent and/or on-going hybridization between the two forms. In this case, the low level of mtDNA differentiation can be accounted for by mtDNA introgression from one subspecies to the other followed by rapid fixation of alien haplotypes (say, due to a selective sweep).

Both scenarios remain speculative and should be tested based on a larger number of genes and sampling localities. At the same time, regardless of which hypothesis is correct, the observed pattern of genetic variation indicates a lack of genetic isolation between *E. s. serotinus* and *E. s. turcomanus* and, hence, does not support species status for the latter taxon.

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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.
- BENDA P., RUEDI M. & AULAGNIER S., 2004: New data on the distribution of bats (Chiroptera) in Morocco. *Vesperilio*, **8**: 13–44.
- BENDA P., ANDREAS M., KOCK D., LUČAN R. K., MUNCLINGER P., NOVÁ P., OBUCH J., OCHMAN K., REITER A., UHRIN M. & WIENFURTOVÁ D., 2006: Bats (Mammalia: Chiroptera) of the Eastern Mediterranean. Part 4. Bat fauna of Syria: distribution, systematics, ecology. *Acta Societas Zoologicae Bohemicae*, **70**: 1–329.
- BENDA P., HANÁK V. & ČERVENÝ J., 2011: Bats (Mammalia: Chiroptera) of the Eastern Mediterranean and Middle East. Part 9. Bats from Transcaucasia and West Turkestan in collection of the National Museum, Prague. *Acta Societas Zoologicae Bohemicae*, **75**: 159–222.
- BOBRINSKIJ N. A., KUZNECOV B. A. & KUZÁKIN A. P., 1965: *Opredelitel' mlekopitaûsih SSSR [Identification Guide to the Mammals of the USSR]*. Prosvešenie, Moskva, 382 pp (in Russian).

- CLEMENT M., POSADA D. & CRANDALL K., 2000: TCS: a computer program to estimate gene genealogies. *Molecular Ecology*, **9**: 1657–1660.
- CORBET G. B., 1978: *The Mammals of the Palaearctic Region: A Taxonomic Review*. Cornell University Press, London & Ithaca, 314 pp.
- CORBET G. B. & HILL J. E., 1992: *The Mammals of the Indomalayan Region*. Oxford University Press, Oxford, 488 pp.
- EICK G. N., JACOBS D. S. & MATTHEE C. A., 2005: A nuclear DNA phylogenetic perspective on the evolution of echolocation and historical biogeography of extant bats (Chiroptera). *Molecular Biology and Evolution*, **22**: 1869–1886.
- GAISLER J., 1970: The bats (Chiroptera) collected in Afghanistan by the Czechoslovak Expeditions of 1965–1967. *Acta Scientiarum Naturalium Academiae Scientiarum Bohemoslovacae Brno*, s. n., **4**(6): 1–56.
- GARCIA-MUDARRA J. L., IBÁÑEZ C. & JUSTE J., 2009: The Straits of Gibraltar: barrier or bridge to Ibero-Moroccan bat diversity? *Biological Journal of the Linnaean Society*, **96**: 434–450.
- HALL T. A., 1999: BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nuclear Acids Symposium Series*, **41**: 95–98.
- IBÁÑEZ C., GARCIA-MUDARRA J. L., RUEDI M., STADELMANN B. & JUSTE J., 2006: The Iberian contribution to cryptic diversity in European bats. *Acta Chiropterologica*, **8**: 227–297.
- ILJIN V. Y., SMIRNOV D. G., KRASILNIKOV D. B. & YANYAEVA N. M., 2002: *Materialy k kadastru rukokrylyh (Chiroptera) Evropejskoj Rossii i smežnyh regionov [Materials to the Cadastre of Bats (Chiroptera) in European Russia and Neighbouring Regions]*. Penza State Pedagogical University, Penza, 64 pp (in Russian).
- JUSTE J., BENDA P., GARCIA-MUDARRA J. L. & IBÁÑEZ C., 2010: Molecular phylogeny and systematics of Old World serotine bats (*Eptesicus*, *Vespertilionidae*). Pp.: 189–190. In: HORÁČEK I. & BENDA P. (eds.): *15th IBRC – the Conference Manual. Programme, Abstracts, List of Participants. Volume of Abstracts of the 15th International Bat Research Conference, held in Prague, 23–27 August 2010*. Lesnická práce s.r.o., Kostelec nad Černými lesy, 381 pp.
- MAYER F., DIETZ C. & KIEFER A., 2007: Molecular species identification boosts bat diversity. *Frontiers in Zoology*, **4**(4).
- OGNEV S. I., 1928: *Zveri vostočnoj Evropy i severnoj Azii. Tom I [The Mammals of East Europe and North Asia. Volume I]*. Gosudarstvennoe Izdatel'stvo, Moskva & Leningrad, 631 pp (in Russian).
- MATVEEV V. A., 2003: *Systematika rukokrylyh Starogo Sveta po rezul'tatam issledovaniâ dispergirovannyh povtorov DNK [The Systematics of the Old World Bats as Inferred from the Examination of the Interspersed DNA Repeats]*. Unpubl. PhD Thesis. Moskovskij Gosudarstvennyj Universitet, Moskva, 247 pp (in Russian).
- PAVLINOV I. Y. & ROSSOLIMO O. L., 1987: Sistematička mlekopitaúših SSSR [Systematics of mammals of the USSR]. *Sbornik Trudov Zooložičeskogo Muzeá MGU*, **25**: 1–285 (in Russian).
- ROHLF F. J., 1998: *NTSYSpc. Numerical Taxonomy and Multivariate Analysis System Version 2.0 User Guide*. Applied Biostatistics Inc. Setauket, New York, 37 pp.
- SAMBROOK J., FRITSCH E. F. & MANIATIS T., 1989: *Molecular Cloning. A Laboratory Manual. 2nd Edition*. II Cold Spring Harbor Laboratory Press, Cold Spring Harbor, USA, 1659 pp.
- SIMMONS N. B., 2005: Order Chiroptera. Pp.: 312–529. In: WILSON D. E. & REEDER D. M. (eds.): *Mammal Species of the World: A Taxonomic and Geographic Reference. Third Edition*. Johns Hopkins University Press, Baltimore, 2142 pp.
- SMIRNOV D. G. & YANYAEVA N. M., 2003: Izmenčivost' okraski pozdnogo kožana (*Eptesicus serotinus*) v central'noj časti areala [To the variability of coloration of the serotine bat (*Eptesicus serotinus*) from a central part of its distribution range]. Pp.: 205–208. In: AVERIANOV A. O. & ABRAMSON N. I. (eds.): *Sistematička, filogeniá i paleontologiá melkikh mlekopitaúših [Systematics, Phylogeny and Paleontology of Small Mammals]*. ZISP RAS, St. Peterburg, 246 pp (in Russian, with a summary in English).

- SMITH A. T. & XIE Y. (eds.), 2008: *A Guide to the Mammals of China*. Princeton University Press, Princeton, 544 pp.
- SOKOLOV V. E. & SHISHKIN V. S., 2005: *Razvitiye otečestvennoj teriologii v XIX veke [Development of the Theriology of Our Country in the XIX Century]*. Nauka, Moskva, 324 pp (in Russian).
- STEPHENS M. & DONNELLY P., 2003: A comparison of Bayesian methods for haplotype reconstruction from population genotype data. *American Journal of Human Genetics*, **73**: 1162–1169.
- STEPHENS M., SMITH N. J. & DONNELL Y. P., 2001: A new statistical method for haplotype reconstruction from population data. *American Journal of Human Genetics*, **68**: 978–989.
- STRELKOV P. P. & ILJIN V. Y., 1992: Bats of easternmost Europe: distribution and faunal status. Pp.: 193–205. In: HORÁČEK I. & VOHRALÍK V. (eds.): *Prague Studies in Mammalogy*. Karolinum – Charles University Press, Praha, 245 pp.
- 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.

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Appendix

Sampling localities, vouchers and GB accession numbers

Abbreviations: AN – accession number; D – district (okres); GN – group number; K – region (krai); NR – natural reserve (zapovednik); R – region (oblast'); r – river; SPTB – SPTB presence; TS – tissue sample (biopsy); ZIN – Zoological Institute RAS, Sankt Petersburg; ZMMU – Zoological Museum of the Moscow State University, Moskva

species	coll. ID	site	THY AN	CYTB AN	SPTB	GN
1 <i>E. nilssonii</i>	ZISP 70784	Russia, Hakassia	JX902575			
2 <i>E. nilssonii</i>	ZMMU S-170719	Russia, Tverskaâ R, Starickij D, Ordino	JX902517			
3 <i>E. nilssonii</i>	ZMMU S-171265	Russia, Brânskaâ R, Zubcovskij D, Mozgovo	JX902515	JX902463	+++	
4 <i>E. nilssonii</i>	ZMMU S-180219	Russia, Brânskaâ R, Suzemka D, Nerussa	JX902568			
5 <i>E. nilssonii</i>	TS K1106	Russia, Čelâbinskaâ R, 30 km NW of Kyšym	JX902553			
6 <i>E. nilssonii</i>	TS 6CK1	Russia, Čitinskaâ R, Aleksandrovskozavodskij D, Kirkira river	JX902450	JX902450	+++	
7 <i>E. nilssonii</i>	ZMMU S-175949	Russia, Čitinskaâ R, Aleksandrovskozavodskij D, Kirkira river	JX902563			
8 <i>E. nilssonii</i>	ZMMU S-175950	Russia, Čitinskaâ R, Aleksandrovskozavodskij D, Kirkira river	JX902516			
9 <i>E. nilssonii</i>	ZMMU S-175350	Russia, Čitinskaâ R, Mogočskij D, Šilka river	JX902482			
10 <i>E. nilssonii</i>	ZMMU S-180233	Russia, Kaliningradskaa R, Zelenogradskij D, Romanovo	JX902534			
11 <i>E. nilssonii</i>	TS P11_40	Russia, Hanty-Mansijskâ R, Kondinskij D, Kuminsky, Kuma r	JX902506			
12 <i>E. nilssonii</i>	TS P11_41	Russia, Hanty-Mansijskâ R, Kondinskij D, Kuminsky, Kuma r	JX902507			
13 <i>E. nilssonii</i>	TS P11_42	Russia, Hanty-Mansijskâ R, Kondinskij D, Kuminsky, Kuma r	JX902508			
14 <i>E. nilssonii</i>	TS P11_35	Russia, Hanty-Mansijskâ R, Nižnevartovskij D, Koniki, Maleye Korlikî r	JX902500			
15 <i>E. nilssonii</i>	TS P11_37	Russia, Hanty-Mansijskâ R, Sovietskij D, Verhne-Kondinskij NR	JX902503			
16 <i>E. nilssonii</i>	TS P11_38	Russia, Hanty-Mansijskâ R, Sovietskij D, Verhne-Kondinskij NR	JX902504			
17 <i>E. nilssonii</i>	TS P11_39	Russia, Hanty-Mansijskâ R, Sovietskij D, Verhne-Kondinskij NR	JX902505			
18 <i>E. nilssonii</i>	TS P11_36	Russia, Hanty-Mansijskâ R, Surgut	JX902502			
19 <i>E. nilssonii</i>	ZMMU S-181985	Russia, Krasnoâriskaâ R, Enisej r, Mirnoe	JX902486			
20 <i>E. nilssonii</i>	TS M6	Russia, Krasnoâriskaâ R, Enisej r, Mirnoe	JX902547			
21 <i>E. nilssonii</i>	TS M4	Russia, Krasnoâriskaâ R, Enisej r, Mirnoe	JX902562			
22 <i>E. nilssonii</i>	TS P11_47	Russia, Samarskaâ R, Samarskaâ Luka, Širâevo	JX902509			
23 <i>E. nilssonii</i>	TS P11_48	Russia, Samarskaâ R, Samarskaâ Luka, Širâevo	JX902510			
24 <i>E. nilssonii</i>	TS P11_49	Russia, Samarskaâ R, Samarskaâ Luka, Širâevo	JX902511	JX902451	+++	
25 <i>E. nilssonii</i>	TS CK14	Russia, Sverdlovskaâ R, Severouralsk				
26 <i>E. nilssonii</i>	TS CK1	Russia, Sverdlovskaâ R, Severouralsk				
27 <i>E. nilssonii</i>	TS CK5	Russia, Sverdlovskaâ R, Severouralsk				
28 <i>E. serotinus</i>	ZMMU 1023L-74/10	Kazakhstan, Aktobe, Kobda D, Žarsaj	JX902542			
29 <i>E. serotinus</i>	ZMMU 1034L-74/10	Kazakhstan, Aktobe, Kobda D, Žarsaj	JX902527	JX902444	+++	1
30 <i>E. serotinus</i>	TS P11_18	Kazakhstan, Aktobe, 100 km S of Sol – Iletsk road	JX902556			1
31 <i>E. serotinus</i>	ZMMU 1046L-74/10	Kazakhstan, Atyrauskâ R, Inder D, Beket	JX902535	JX902448	+++	1

32	<i>E. serotinus</i>	ZMMU 1041L-74/10	Kazakhstan, Atyrauskaâ R, Inder Lake	JX902445	+++	1
33	<i>E. serotinus</i>	ZMMU 1042L-74/10	Kazakhstan, Atyrauskaâ R, Inder Lake	JX902446	+++	1
34	<i>E. serotinus</i>	ZMMU 1043L-74/10	Kazakhstan, Atyrauskaâ R, Inder Lake	JX902447	+++	1
35	<i>E. serotinus</i>	ZMMU 1019L-74/10	Kazakhstan, Zapadno-Kazahstanskâ R, Syrymskij D, Mirgorodka	JX902440	+++	1
36	<i>E. serotinus</i>	TS Russ117	Russia, Astrahan'skaâ R	JX902512	+++	2
37	<i>E. serotinus</i>	ZIN 70095	Russia, Astrahan'skaâ R, Baskunčak	JX902581	+++	2
38	<i>E. serotinus</i>	TS EIK-E.s.t.	Russia, Astrahan'skaâ R, Baskunčak	JX902454	+++	2
39	<i>E. serotinus</i>	ZIN 70096	Russia, Astrahan'skaâ R, Černobyl'ny	JX902584	+++	2
40	<i>E. serotinus</i>	ZMMU S-1866653	Russia, Astrahan'skaâ R, Černoârskij D, Solodniki	JX902468	+++	2
41	<i>E. serotinus</i>	ZMMU S-1866654	Russia, Astrahan'skaâ R, Černoârskij D, Solodniki	JX902469	+++	2
42	<i>E. serotinus</i>	ZMMU S-1866655	Russia, Astrahan'skaâ R, Černoârskij D, Solodniki	JX902470	+++	2
43	<i>E. serotinus</i>	TS P0913	Russia, Astrahan'skaâ R, Černoârskij D, Solodniki	JX902460	+++	2
44	<i>E. serotinus</i>	ZMMU S-191983	Russia, Astrahan'skaâ R, Černoârskij D, Solodniki	JX902492	+++	2
45	<i>E. serotinus</i>	ZMMU S-190384	Russia, Brânskaâ R, Brasovskij D	JX902558	+++	3
46	<i>E. serotinus</i>	TS K1105	Russia, Brânskaâ R, Pogarskij D, Pogar	JX902551	+++	3
47	<i>E. serotinus</i>	ZMMU S-180210	Russia, Brânskaâ R, Suzemskij D, Berezovka	JX902480	+++	3
48	<i>E. serotinus</i>	ZMMU S-190385	Russia, Brânskaâ R, Suzemskij D, Berezovka	JX902559	+++	3
49	<i>E. serotinus</i>	TS K1101	Russia, Brânskaâ R, Suzemskij D, Nerussa	JX902445	+++	3
50	<i>E. serotinus</i>	TS K1102	Russia, Brânskaâ R, Suzemskij D, Nerussa	JX902446	+++	3
51	<i>E. serotinus</i>	TS K1103	Russia, Brânskaâ R, Suzemskij D, Nerussa	JX902448	+++	3
52	<i>E. serotinus</i>	TS K1104	Russia, Brânskaâ R, Suzemskij D, Nerussa	JX902550	+++	3
53	<i>E. serotinus</i>	ZMMU S-183027	Russia, Brânskaâ R, Suražskij D, Lâliči	JX902665	+++	3
54	<i>E. serotinus</i>	ZMMU S-183028	Russia, Brânskaâ R, Suražskij D, Lâliči	JX902664	+++	3
55	<i>E. serotinus</i>	ZMMU S-183029	Russia, Brânskaâ R, Suražskij D, Lâliči	JX902557	+++	3
56	<i>E. serotinus</i>	ZMMU S-186650	Russia, Brânskaâ R, Suražskij D, Lâliči	JX902443	+++	3
57	<i>E. serotinus</i>	ZMMU S-186651	Russia, Brânskaâ R, Suražskij D, Lâliči	JX902466	+++	3
58	<i>E. serotinus</i>	TS SG21.06.05	Russia, Dagestan'skaâ R, Bulkazmaâr	JX902560	+++	3
59	<i>E. serotinus</i>	TS Russ72	Russia, Kabardino-Balkarskaâ R	JX902567	+++	3
60	<i>E. serotinus</i>	TS AAB-Nalchik	Russia, Kabardino-Balkarskaâ R, Nalčik	JX902514	+++	3
61	<i>E. serotinus</i>	ZISP 82433	Russia, Krasnodarskij K, Krasnaja Polâna	JX902577	+++	4
62	<i>E. serotinus</i>	ZMMU S-167376	Russia, Krasnodarskij K, Krasnaja Polâna	JX902484	+++	4
63	<i>E. serotinus</i>	ZMMU S-186656	Russia, Krasnodarskij K, Krymskij D, Nižnebakanskij	JX902386	+++	4
64	<i>E. serotinus</i>	ZMMU S-186657	Russia, Krasnodarskij K, Krymskij D, Nižnebakanskij	JX902554	+++	4
65	<i>E. serotinus</i>	ZMMU S-186849	Russia, Krasnodarskij K, Krymskij D, Nižnebakanskij	JX902544	+++	4
66	<i>E. serotinus</i>	ZMMU S-180234	Russia, Kaliningradskâ R, Zelenogradskij D, Romanovo	JX902336	+++	4
67	<i>E. serotinus</i>	TS P11_5	Russia, Samarskaâ R, Samarskaâ Luka, Krestovâ Polâna	JX902487	+++	5
68	<i>E. serotinus</i>	TS P11_3	Russia, Samarskaâ R, Samarskaâ Luka, Širâevo	JX902485	+++	5
69	<i>E. serotinus</i>	TS P11_4	Russia, Samarskaâ R, Samarskaâ Luka, Širâevo	JX902483	+++	5
70	<i>E. serotinus</i>	TS P11_9	Russia, Samarskaâ R, Samarskaâ Luka, Širâevo	JX902570	+++	5
71	<i>E. serotinus</i>	TS P11_11	Russia, Tatarsian, Kamsko-Ustinskij D, Kamskoe Uste	JX902495	+++	5
72	<i>E. serotinus</i>	TS P11_6	Russia, Vologradskâ R, Dubovskij D, Gornaa Prolejka	JX902488	+++	6

species	coll. ID	site	THY	AN	CYTB	AN	SPTB	GN
73 <i>E. serotinus</i>	TS P11_7	Russia, Volgogradskâ R, Dubovskij D, Gornâa Prolejka	JX902490					6
74 <i>E. serotinus</i>	ZMMU S-181981	Russia, Volgogradskâ R, Dubovskij D, Olene	JX902513				+++	6
75 <i>E. serotinus</i>	TS P11_13	Russia, Volgogradskâ R, Dubovskij D, Olene	JX902571					6
76 <i>E. serotinus</i>	TS P11_8	Russia, Volgogradskâ R, Dubovskij D, Strehoširokoe	JX902491				+++	6
77 <i>E. serotinus</i>	ZMMU S-186652	Russia, Volgogradskâ R, Illyinskij D, Ereckij	JX902555	JX902467				6
78 <i>E. serotinus</i>	TS DON1	Russia, Volgogradskâ R, Kamyshinskij D	JX902529	JX902452			+++	
79 <i>E. serotinus</i>	TS KL2	Russia, Volgogradskâ R, Serafimovičskij D, Kleckij	JX902531				+++	6
80 <i>E. serotinus</i>	ZMMU S-191978	Russia, Volgogradskâ R, Serafimovičskij D, Kleckij	JX902489				+++	
81 <i>E. serotinus</i>	TS EIK-E.s.s.	Russia, Voronež	JX902552	JX902453			+++	6
82 <i>E. serotinus</i>	TS P11_10	Russia, Voronežskâ R, Belgorod	JX902493					
83 <i>E. serotinus</i>	TS P11_14	Ukraine, Harkiv	JX902497				+++	7
84 <i>E. serotinus</i>	TS P11_15	Ukraine, Harkiv	JX902498				+++	7
85 <i>E. serotinus</i>	TS P11_16	Ukraine, Harkiv	JX902499				+++	
86 <i>E. serotinus</i>	TS K091L	Ukraine, Kyiv	JX902545				+++	7
87 <i>E. serotinus</i>	TS K092	Ukraine, Kyiv	JX902549	JX902456			+++	7
88 <i>E. serotinus</i>	TS K093	Ukraine, Kyiv	JX902528	JX902457			+++	7
89 <i>E. serotinus</i>	TS K0951	Ukraine, Kyiv	JX902524	JX902458			+++	7
90 <i>E. serotinus</i>	TS K096L	Ukraine, Kyiv	JX902524	JX902459			+++	7
91 <i>E. serotinus</i>	TS P11_17	Ukraine, Lviv	JX902501				+++	
92 <i>E. serotinus</i>	ZMMU T001L-58/10	Uzbekistan, Fergonskaâ R, Äzâvanskij D, Tal-Kuduk-Kum	JX902569				+++	7
93 <i>E. serotinus</i>	ZMMU 1009L-58/10	Uzbekistan, Kaškadarijskaâ R, Yakkabogskij D	JX902494	JX902439			+++	8
94 <i>E. serotinus</i>	TS, 1007L	Uzbekistan, Kaškadarijskaâ R, Yakkabogskij D	JX902583	JX902438			+++	8