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Holarctic phylogeography of the tundra shrew (*Sorex tundrensis*) based on mitochondrial genes

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A range-wide phylogeographic study of the tundra shrew (*Sorex tundrensis*) was performed using cytochrome *b* and cytochrome oxidase I (COI) mitochondrial genes. The results based on 121 specimens from 42 localities demonstrate that the tundra shrew is divided into five main mitochondrial DNA phylogenetic lineages with largely parapatric distribution. In addition to a single Nearctic clade (Alaska) four Palearctic clades are identified: Western (Northen Urals, Kazakhstan, South-West Siberia), Eastern (from East Transbaikalia and the Middle Amur to Chukotka), South Central (Central Siberia, the Altai, the Dzhungarian Alatau) and North Central (Northern Siberia, Central Yakutia). Date estimates obtained by use of a molecular clock corrected for potential rate decay suggest Late Pleistocene age for the most recent common ancestor of all contemporary tundra shrew populations. Relatively high genetic divergence between phylogroups (0.95–1.6%) indicates that the observed phylogeographic structure was initiated by historical events that predated the Last Glacial Maximum. We assume that, being more cold- and arid-tolerant, tundra shrew underwent expansion during an early cold phase of the Last Glacial and spread through its recent range earlier than most of other Siberian red-toothed shrews. Comparative phylogeographic analysis of Siberian shrews and rodents suggests that evolutionary histories of species associated with azonal or open habitats show important differences compared to forest species.

ADDITIONAL KEYWORDS: Alaska – Eurasia – cytochrome *b* – molecular clock – Soricinae.

INTRODUCTION

Phylogeographic studies of the last decades have considerably contributed to our understanding of the Quaternary history of West European plants and animals (Bilton *et al*., 1998; Taberlet *et al*., 1998; Hewitt, 1999). The phylogeography of East Palearctic animals and especially Holarctic species is still less known, particularly as a result of difficulties of sampling across such an extensive area. Correspondingly, the Quaternary history of the species associated with temperate deciduous forests (Arbogast & Kenagy, 2001; Deffontaine *et al*., 2005; Runck & Cook, 2005; Kotlik *et al*., 2006; Rowe, Edward & Paige, 2006), as well as Eurasian taiga zone (Kvist *et al*., 2001, 2003; Zink, Drovetski & Rohwer, 2002; Zink *et al*., 2002; Goropashnaya *et al*., 2004; Oshida *et al*., 2005), are relatively well studied, whereas the impact of the ice ages on the species inhabiting open and semi-open habitats in the temperate zone is only at the beginning (Brunhoff *et al*., 2003; Sorokin & Kholodova, 2006; Fedorov *et al*., 2003; 2008). Meanwhile, species with extensive distribution and different landscape

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preferences may have had rather different range dynamics through the major climatic shifts.

In the present study, we examined the genetic structure of the tundra shrew *Sorex tundrensis* Merriam, 1900, which is one of the few extant Holarctic small mammal species. Another specific feature of the tundra shrew is its ecological preferences; being less habitat specific and more arid-tolerant than most of other Palearctic red-toothed shrew, it is rather common in open landscapes including steppes and is associated with tundra-like or azonal habitats (Stroganov, 1957; Dolgov, 1985; Yudin, 1989). By contrast to the majority of forest species of *Sorex*, the tundra shrew extends its range far to the north over the tundra zone to the Arctic coast, as well as to the south penetrating the steppe zone of Kazakhstan, Mongolia, and Transbaikalia.

Sorex tundrensis demonstrates relatively high level of morphological polymorphism across its geographical range (Stroganov, 1957; Dolgov, 1985), as a consequence of which eight to 11 valid subspecies have been described from Siberia and Far East of Russia (Stroganov, 1957; Yudin, 1989). The chromosomal variability is also high, although, in contrast to *Sorex araneus*, no chromosome races can be identified as the spatial structure of chromosome variation is much less pronounced (Lukáčová, Zima & Volobuev, 1996; Zima, Lukáčová & Macholán, 1998). The spectacular morphological and karyological variability of the tundra shrew prompted the hypothesis that *S. tundrensis* represents a complex of cryptic vicariant species (Kozlovsky, 1971; Okhotina, 1976; Ivanitskaya & Malygin, 1985).

Interspecific phylogeny and phylogeography of the tundra shrew may supply essential information for understanding the evolutionary history of this wide-range species and provide new insight into phylogeography of Siberian mammals. In the present study, we sequenced the complete mitochondrial cytochrome *b* (*cytb*) gene and a segment of the cytochrome oxidase subunit I gene (*COI*) to elucidate the genetic structure and evolutionary history of this species.

MATERIAL AND METHODS

Sampling, DNA extraction, amplification, and sequencing

The sample includes a total of 121 tundra shrews from 42 localities across the species range in Asia and North America (Fig. 1, see also the Appendix, Table A1). Several specimens of other species of the genus *Sorex* (Appendix, Table A2) were used as outgroup. Total DNA was extracted from ethanol preserved tissues from liver, kidney, tail ends, and toe clippings, or from dried muscles, using standard protocol of proteinase K digestion, phenol–chloroform deproteinization, and isopropanol precipitation (Sambrook, Fritsch & Maniatis, 1989).

Mitochondrial DNA sequences were obtained from a total of 119 individuals from 41 localities and two sequences were retrieved from GenBank. The entire cytochrome *b* gene (1140 bp) was amplified in either one polymerase chain reaction (PCR) with the forward/reverse primer combination L14734/ H15985 (Ohdachi *et al*., 2001) and Sorsat_glu/Sorsat_thr, or, when DNA was degraded, in two PCR reactions that produced overlapping fragments using the internal primers L373st and H589st, or L395 (Bannikova & Lebedev, 2010).

Typical conditions for *cytb* amplification included the initial denaturation at 94 °C for 3 min, 35 cycles of 94 °C for 30 s, annealing at 57–60 °C for 1 min, and extension at 72 °C for 1 min, followed by a final extension at 72 °C for 10 min and an indefinite hold at 4 °C. The partial of cytochrome oxidase subunit I (657 bp) was amplified using VF1_t1/ VR1_t1 and the PCR thermocycle program developed in the Canadian Centre for DNA Barcoding (Ivanova *et al*., 2007). 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 was used for sequencing with each primer by the autosequencing system ABI 3100-Avant in conjunction with ABI PRISM®BigDyeTM Terminator, version 3.1.

Sequence and phylogenetic analysis

Cytb and *COI* gene sequences were aligned by eye. The final alignment of the mitochondrial regions included 1140 bp for *cytb* and 657 bp for *COI*.

All tree reconstructions were performed separately for the *cytb* alignment (116 specimens) and for the concatenated data (94 specimens). Phylogenetic Neighbour-joining (NJ) and maximum parsimony (MP) analyses were performed using PAUP* 4.0b10 (Swofford, 2000). The NJ tree was reconstructed using the uncorrected *p*-distance. Unweighted MP analysis was performed using heuristic search starting with stepwise addition trees (random addition sequence, 10000 replicates) and employing tree bisection–reconnection branch-swapping with 'multrees' option turned off. To assess clade stability in the MP and NJ trees, 1000 bootstrap pseudoreplicates were analyzed using the same options (but with 20 randoms addition sequences for parsimony analysis). In the maximum likelihood (ML) reconstruction, the appropriate models of sequence evolution were chosen for each of the codon positions on the basis of Bayesian information criterion using MODELTEST, version 3.04 (Posada & Crandall, 1998). The ML tree was reconstructed using TREEFINDER (Jobb, 2008). Bootstrap analysis employed 1000 replicates.

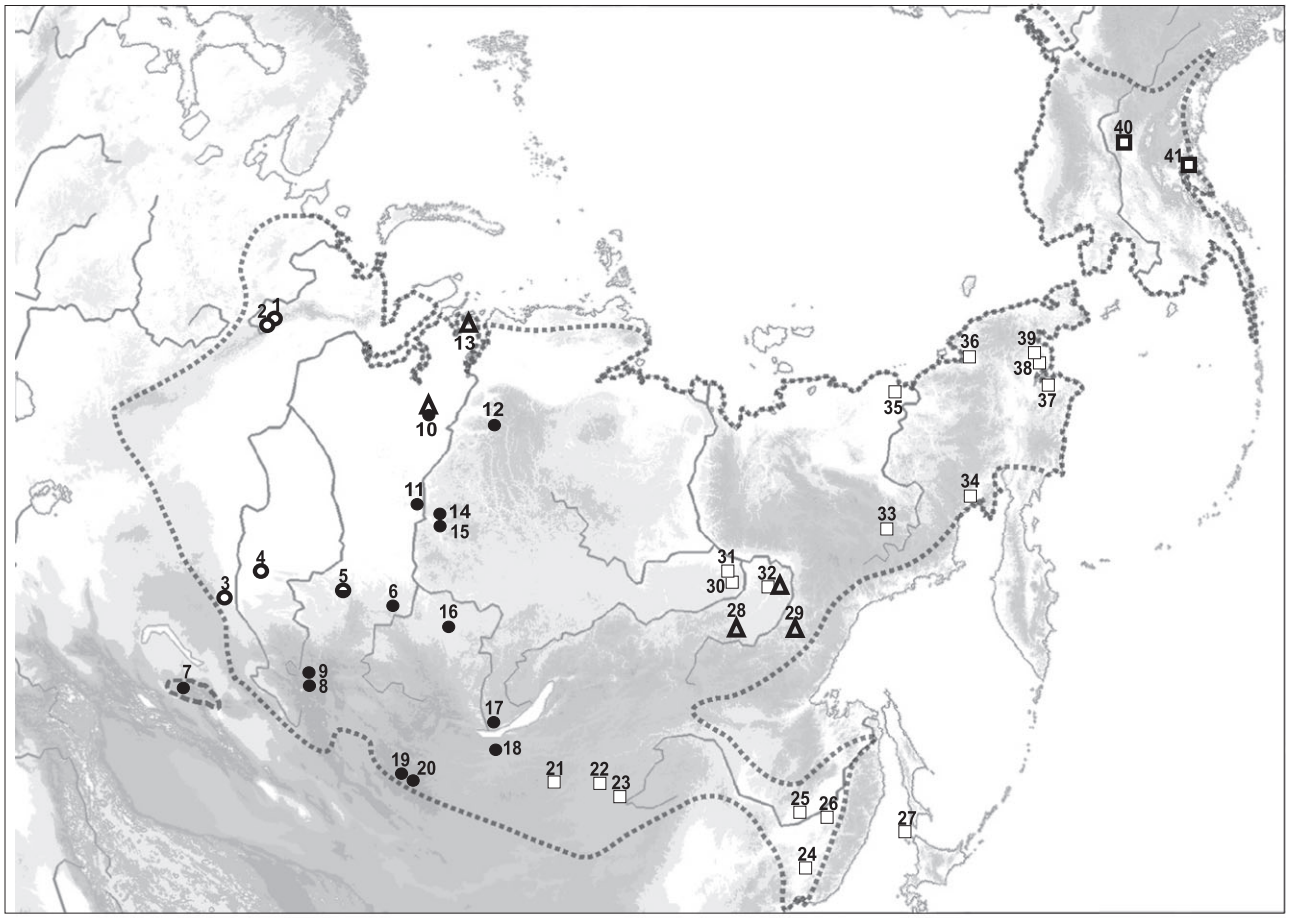

Figure 1. The distribution range of the tundra shrew (after Dolgov, 1985; Dokuchaev, 1999; the approximate limits are indicated by dotted line), the sampling localities and the distribution of mtDNA lineages. Numbers correspond to the localities in Table 1. The five haplotypic groups are represented: ○, Western; ●, South Central; △, North Central; ■, Eastern; □, Nearctic.

The species *Sorex asper*, *Sorex daphaenodon*, *S. araneus*, *Sorex granarius*, *Sorex antinorii*, and *Sorex coronatus* were used as outgroups in all analyses; more distant *Sorex arcticus* and *Sorex samniticus* were included only in molecular clock analysis.

Relationships among haplotypes were also examined using a median-joining network constructed for the *cytb* data using NETWORK, version 4.5.0.0 (Bandelt, Forster & Röhl, 1999) using default options but with $\varepsilon = 20$.

Genetic diversity

The number of haplotypes (H), haplotype diversity (h), nucleotide diversity (π), Tajima's D, and Fu's F_s neutrality tests and mismatch distributions of substitutional pairwise nucleotide differences were calculated for *cytb* data using ARLEQUIN, version 3.11 (Schneider, Roessli & Excoffier, 2000).

To analyse geographic diversity, the total data set ($N = 116$) was subdivided into 31 samples corresponding to local populations. In all analyses, geographically adjacent populations of Pechora valley (localities 1 and 2), Anadyr (localities 37, 38, 39), and Hangay (localities 18 and 19) were combined, which is justified by the absence of significant differences between the subsamples ($P < 0.05$ F_{ST} tests performed in Arlequin 3.11). Measures of intrapopulation variability were calculated for ten samples containing more than four individuals (Appendix, Table A1), as well as for pooled samples composed of several local samples representing the same large geographical region.

Analogous calculations were performed for the joint sample of mainland populations of *Sorex caecutiens* ($N = 31$, GenBank data AB119181–119189, AB119192, AB028547–028557, AB062730–062735, AB062720, and four original sequences GU929333–929336), which was used for comparison as an example of a widespread taiga shrew species whose range overlaps considerably with that of *S. tundrensis*.

Measures of interpopulation genetic differentiation (net distances and F_{ST}) among the tundra shrew populations were calculated using ARLEQUIN, version 3.11, and significance was tested by 1000 permutations.

To retrieve a group structure and to define maximally differentiated groups spatial analysis of molecular variance (SAMOVA; Dupanloup, Schneider & Excoffier, 2002) was applied to the *cytb* data with the number of a priori defined groups (K) varying in the range 2–15. To assess the significance of population structure of *cytb* variation, we used the analysis of molecular variance (AMOVA, Excoffier, Smouse & Quattro, 2002) based on the results of SAMOVA test.

To test the hypothesis that the observed pattern of geographic variation can be explained by isolation-by-distance, simple and partial Mantel tests were conducted in ARLEQUIN, version 3.11, using data on 13 samples with $N \geq 3$. The framework of this analysis was in accordance with that described by Telles & Diniz-Filho (2005).

Molecular dating

Methodological issues

In the case of *S. tundrensis* (as well as in many others), molecular time estimation is problematic as a result of to the paucity of fossils that could provide unambiguous internal calibrations within the *S. araneus* group in general and *S. tundrensis* in particular. Although *S. tundrensis* is represented in fossil faunas from early Late Pleistocene (Mezhzherin, 1972), these findings could be used only to impose the lower limit on its separation from its sister species *S. asper* but do not help us to determine the time of radiation of its recent lineages. Therefore, to obtain absolute time estimates, we have to rely on substitution rate estimates obtained from indirect evidence or from other groups of *Sorex*. Another important problem is the potential bias in estimated divergence times for recent events (< 1–2 Mya) as a result of rate decay (time dependency of rate estimates; Ho *et al*., 2005, 2007; Henn *et al*., 2009). As a consequence of rate decay, the phylogenetic rates obtained from the external ancient calibrations could be inapplicable for dating intraspecific splits. Given that most of the events pertaining to *S. tundrensis* radiation definitely lie within Pleistocene, this methodological complication should be taken into account.

Molecular clock analysis

Lack of rate heterogeneity among taxa was confirmed by use of hierarchical likelihood ratio tests as implemented in PAML, version 4.0 (Yang, 2007).

To obtain estimates of divergence times the concatenated *cytb* + *COI* alignment of the two mitochondrial genes was analyzed using BEAST, version 1.4.8 (Drummond & Rambaut, 2007). Because BEAST cannot explicitly handle the rate decay, we fixed the mean substitution rate at 1.0 and estimated node heights in units of substitution/site rather than absolute time. Next, a set of potential substitution rates was inferred from several calibrations of different age (see below). On this basis, we examined the pattern of rate decay and considered several approaches to transformation of node heights into divergence time estimates. Specifically, three potential rates were considered (see below). The first (population rate) was estimated by use of recent calibrations dating back to Holocene/Pleistocene boundary; the other two (phylogenetic rates) were inferred from early Upper Pleistocene and late Lower Pleistocene calibrations, respectively. To allow for the effect of rate decay, we evaluated the relationship between genetic differentiation and time by means of nonlinear regression. With the use of these procedures, we obtained several sets of estimates of times of the most recent common ancestor and calculated approximate confidence intervals for them. The details of BEAST and regression analyses are provided in the Supporting information (Table S1).

On the basis of the above, we aimed to estimate population and species divergence times, which are dependent also on the unknown level of polymorphism in the ancestral population. To model the expected ancestral coalescence time, the empirical distribution of coalescence times for pairs of sequences randomly drawn from contemporary local populations was used as a proxy.

Calibration points

To discuss the range of possible substitution rates in *Sorex* radiation, we considered several potentially informative calibration points:

1 The time of origin of Irish population of *Sorex minutus*, which was presumably introduced onto Ireland by humans in Late Mesolythic around 6 Kya (Mascheretti *et al*., 2003; McDevitt *et al*., 2009), which is based on both fossil and molecular estimates (McDevitt *et al*., 2009). The Irish sample of this species (14 specimens, *cytb* sequence, 1110 bp; Mascheretti *et al*., 2003) is monophyletic and demonstrates relatively low levels of nucleotide diversity and significantly negative Tajima's D, indicating plausible past demographic expansion. If we assume that τ parameter of the expansion (1.37 as estimated in ARLEQUIN, version 3.11) refers to the time of the initial colonization, the calculated 2μ (divergence rate per site) is close to 21% per My (generation time of 1 year).

2 The times of expansion of Northern haplogroup of *Sorex monticolus* and north-western haplogroup of *Sorex cinereus*, which both currently occur only within the area which was covered by continental glaciation during the Last Glacial (Demboski & Cook, 2001, 2003). In both cases, pronounced signals of population expansion are observed. Accepting the scenario discussed in Demboski & Cook (2001), one can attribute this expansion to the onset of warming at approximately 12 Kya, which translates into the divergence rate of 37% and 31%, respectively.

3 The time of expansion of 'central' clade of British *S. minutus*. The time of expansion of British population should refer to postglacial or late glacial time (McDevitt *et al*., 2009). At present, two groups of haplotypes are found there (peripheral and central), which most probably correspond to two ancestral populations with different colonization histories. The mismatch distribution for the latter group perfectly fits the hypothesis of sudden expansion with $\tau = 6.1$ substitutions. It was suggested that the central group arrived in Britain after the Young Dryas cooling (11.5 Kya) but not later than 8.5 Kya when the English channel reappeared (Searle *et al*., 2009). However, this haplogroup includes also some haplotypes from Central and Western Europe, which suggests that its expansion could have taken place on the continent and predated the colonization event. Assuming that the expansion time corresponds rather to the post-Last Glacial Maximum (LGM) warming (not earlier than 18 Kya), we obtain a minimum estimate of divergence rate equal to 30.5% substitutions/site per Myr. With some reservation, we can apply the same line of reasoning to the dating of demographic events in the'peripheral' group of British pygmy shrew (Searle *et al*., 2009) and mainland Scotish populations of *S. araneus* (White & Searle, 2008). In these cases, the rate is estimated at 23.2% and 27.5% per Myr.

4 The time of origin of island population of *S. araneus* inhabiting archipelagoes west off Scotland. According to the hypothesis discussed by White & Searle (2008), most of these populations could have originated during a rather short time period, approximately 11.5 Kya. Expansion times (τ) of nine individual island populations range between 0.56 and 4.15. Taking into account that: (1) error of τ is high as a result of the small sample size ($N = 5$ in most cases) and (2) that, in some cases, expansion time can refer not to the population origin but to the end of some recent bottleneck, we estimated the time of the original expansion based on the mean τ for the five more variable samples. It was equal to 3.52 (range 2.47 – 4.15), which corresponds to substitution rate of 27.8% substitutions/site per Myr.

5 Separation of *S. granarius* from *S. araneus*. It was suggested that this event might be attributed to the Eemian Interglacial (110–130 Kya, Taberlet, Fumagalli & Hausser, 1994; Hewitt, 2001). Taking into account that the split between *S. araneus* and the somewhat more divergent *S. antinorii* was as well referred to the Last Interglacial (Hausser, Dannelid & Catzeflis, 1986; Hewitt, 1996), we conducted additional analysis assuming that all three species separated approximately 120 Kya.

6 *Sorex daphaenodon*/*S. araneus* dichotomy. To estimate the approximate age of this split, we considered both fossil and molecular evidence. First, the earliest fossil of *S. daphaenodon* found in the horizon II of the Olyorian formation dating back to 0.8–0.95 Mya (Sher, 1974; Virina, Zazhigin & Sher, 1984) provides the upper bound for the separation of *S. araneus* and *S. daphaenodon* lineages. Second, the molecular clock analysis of the *cytb* data (unpublished data by Bannikova A. A. & Lebedev V. S.) based on the assumption that the split between subgenera *Sorex* s.str. and *Otisorex* ocurred 9–11 Mya (Harris, 1998) suggests that the time of divergence between *S. araneus* and *S. daphaenodon* clades is approximately 1 Mya. Thus, this molecular date is consistent with the fossil evidence and therefore we consider it a valid secondary calibration point for the present analysis.

It should be noted that the relevance of all the above calibration points critically depends on the validity of certain additional assumptions (such as adequacy of paleogeographic scenario) and, hence, our molecular clock results should be treated as preliminary.

RESULTS

The complete (1140 bp) or almost complete (more than 900 bp) sequences of *cytb* gene of 116 specimens from 39 localities resulting in 71 unique haplotypes were used in phylogenetic analysis. The length of five sequences was much shorter; thus, they were not included in the alignment used for tree reconstruction. However, these partial sequences were compared with the corresponding parts of the complete sequence. The fragment obtained for the specimens Ala434/2008 from Alaska was 547 bp long; it showed no change compared to the complete sequence of the specimens from this locality. Only one specimen from the isolated population of Dzungarian Alatau (locality 7) was available; as a result of a shortage of DNA, we could only sequence a small part of *cytb* (477 bp). The length of the fragment sequenced for the only specimen from the Bolshoy Kemchug (locality 6), was

500 bp. Both sequences were similar to the corresponding part of the complete sequences of specimens from the Central Siberia, as well as the short sequence D85371 (407 bp) retrived from GeneBank. The region of *COI* (657 bp) was sequenced in 94 specimens and resulted in 74 haplotypes. Sequences were deposited in GenBank under the accession numbers: *cytb* – GU564723–564853; COI – GU929218–929332.

Phylogenetic and phylogeographic analysis

The results of NJ, ML, and MP analyses of the dataset are presented in Figures 2, 3.

In accordance with the recommendations of MODELTEST in the ML analysis, we used TN + G, HKY + G and TN + G model for the first, second, and third codon positions, respectively.

The NJ phylogenetic analysis of the *cytb* data set (Fig. 2) based on 116 complete or near complete sequences of tundra shrews revealed five distinctive haplogroups with high or moderate bootstrap values, which correspond to five geographical parts of the species range: (1) Western Palearctic group (W), South Central Palearctic group (SC), North Central Palearctic group (NC), Eastern Palearctic group (E), and Nearctic group (NA). We put forward a provisional hypothesis suggesting that these haplogroups correspond to groups of populations (further phylogroups), the distribution of which is shown on the Figure 1 and in the Appendix (Table A1).

The NJ phylogenetic analysis of the combined *cytb* + *COI* data set (Fig. 3) retrieved the same five distinctive *cytb* + *COI* lineages, however, with higher bootstrap support. (ML – TN + G, HKY and TN + G model for the first, second, and third codon positions, respectively) Indeed, in the *cytb* tree, the E group was poorly supported (Fig. 2); however, in the combined tree of two mitochondrial genes, the support for it increased, thus suggesting that this group is not an artefact.

The median-joining network (Fig. 4) was reconstructed based on the whole data set (121 sequences). It revealed the same five distinctive *cytb* lineages and demonstrated the tendency to the affiliation of E/W/NA haplogroups on the one hand and SC/NC groups on the other.

The level of genetic divergence (*p*-distance) among the five mtDNA (*cytb*) haplogroups (Fig. 1, Table 1) was in the range 0.95–1.6% (mean 1.3%). The E and NA groups were the least divergent, whereas the largest distance was observed between the W/SC and NA/SC phylogroups.

Thus, almost all samples can be easily ascribed to a certain phylogroup. This pattern of non-overlapping geographical distributions is violated by haplotypes of the NC lineage because they were found in the Yamalo-Nenets region (localities 10, 13) along with the haplotypes of SC phylogroup and in the locality 32 in the Lena River basin where haplotypes of E Palearctic phylogroup are also present. Another locality where haplotypes belonging to two distinct mtDNA lineages are found is the Middle Tom (locality 5). Although four of the five specimens from this sample belong to the W phylogroup the fifth one produced a SC haplotype.

Differentiation within phylogroups

At the next step, the genetic differentiation within phylogroups can be considered. In all groups except for SC and NC, a subdivision into two to five subgroups with high or moderate bootstrap support is observed.

The W clade consists of three well supported clusters, one of which corresponds to the tundra shrews from northern Kazakhstan and West Siberia and the other two are composed of specimens from the Ural Mountains (Pechora valley).

Based on the *cytb* + *COI* data set, five well supported allopatric mitochondrial lineages are found within the E clade; they correspond to the geographical areas: (1) Chukotka and adjacent regions of the North-East Asia; (2) Central Yakutia; (3) Moneron Island; (4) South of the Russian Far East (Lower Amur region); and (5) Eastern Transbaikalia and

Table 1. Mean interhaplotype and net p-distances (below and above the diagonal, respectively) among phylogroups of *Sorex tundrensis* based on cytochome *b* (*cytb*) sequences

p-distance (%) ± SE	South Central	Western	Eastern	North Central	Nearctic
South Central		1.1 ± 0.29	0.96 ± 0.28	0.84 ± 0.26	1.1 ± 0.30
Western	1.60 ± 0.30		0.61 ± 0.21	1.0 ± 0.28	0.68 ± 0.22
Eastern	1.55 ± 0.30	1.09 ± 0.23		0.87 ± 0.27	0.47 ± 0.18
North Central	1.30 ± 0.28	1.36 ± 0.29	1.32 ± 0.29		0.98 ± 0.28
Nearctic	1.60 ± 0.31	1.06 ± 0.24	0.95 ± 0.19	1.32 ± 0.30	

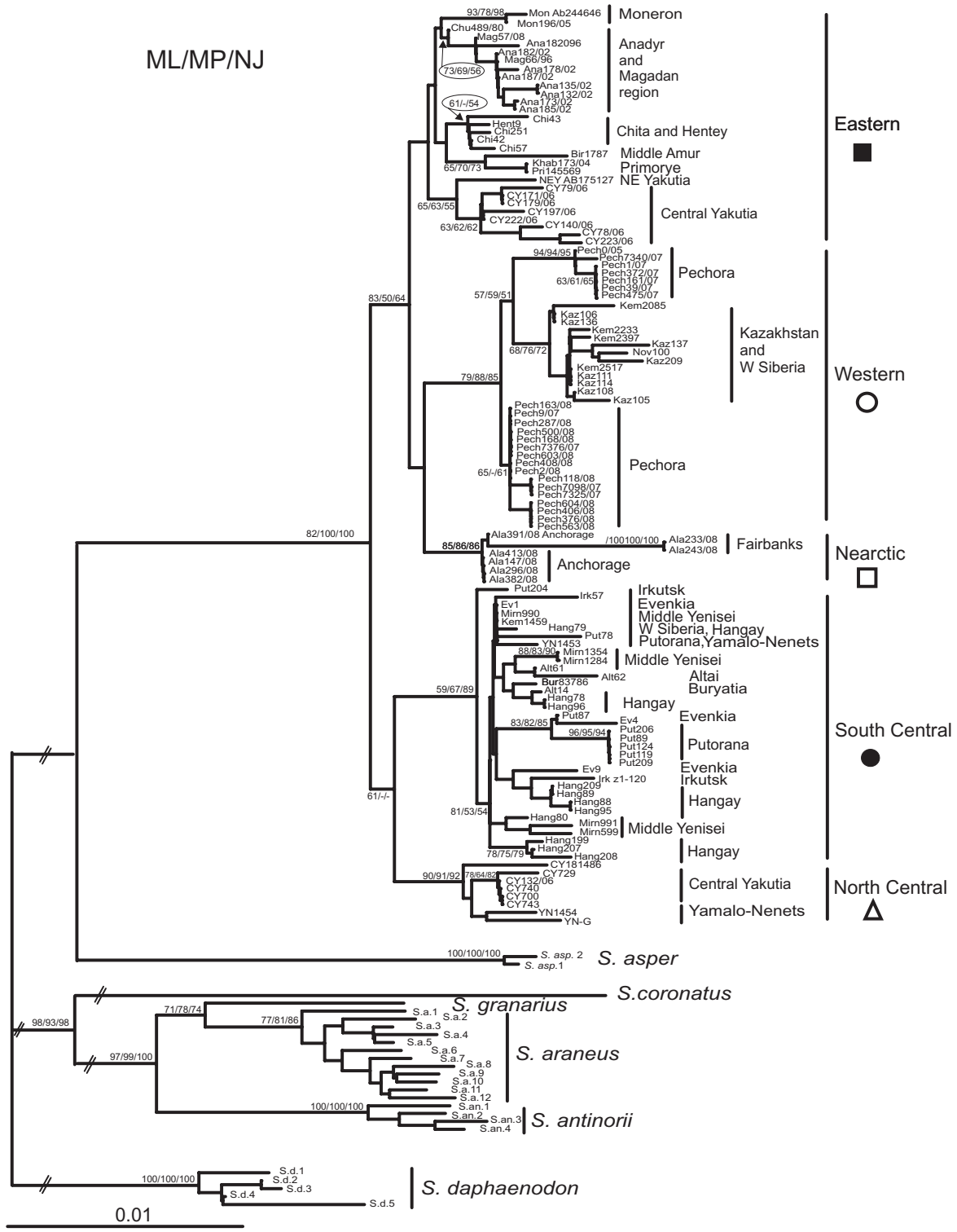

Figure 2. Neighbour-joining (NJ) tree illustrating the phylogenetic relationships among the cytochrome *b* gene haplotypes in the 116 specimens of the tundra shrew (*Sorex tundrensis*). The tree is based on the uncorrected *p*-distances. The percentage bootstrap resampling support (1000 iterations) > 50% obtained in maximum likelihood (ML), maximum parsinomy (MP) and NJ analysis is listed for major lineages in the order ML/MP/NJ. *Sorex asper*, *Sorex daphaenodon*, *Sorex araneus*, *Sorex granarius*, *Sorex antinorii*, and *Sorex coronatus* were used as outgroups. The specimen designations are the same as in the Appendix (Tables A1, A2).

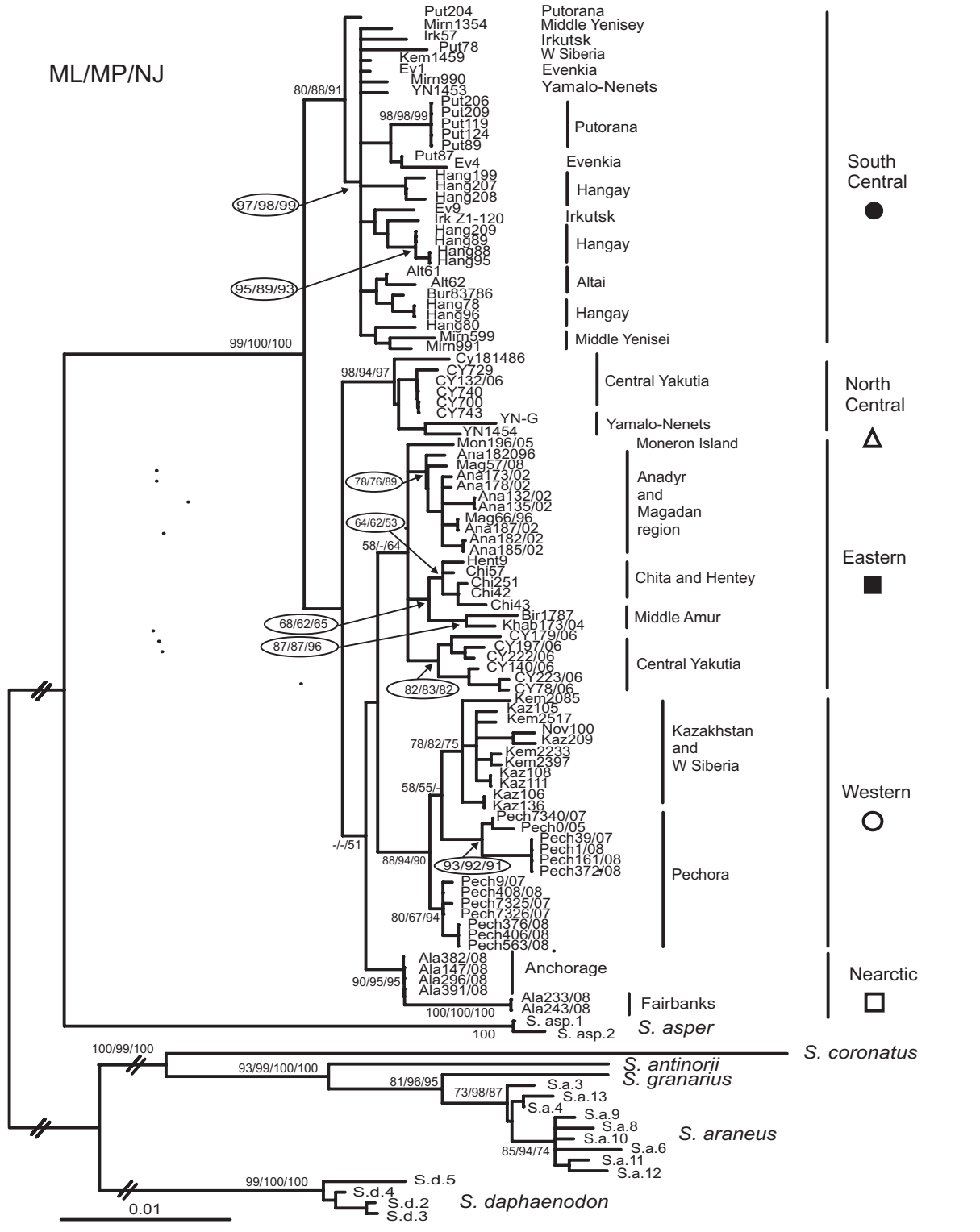

Figure 3. Maximum-likelihood tree illustrating the phylogenetic relationships among the mitochondrial haplotypes (*cytb* + COI) in the 94 specimens of tundra shrew (*Sorex tundrensis*). The percentage bootstrap resampling support (1000 iterations) > 50% obtained in maximum likelihood (ML), maximum parsinomy (MP) and Neighbour-joining (NJ) analysis is listed for major lineages in the order ML/MP/NJ. *Sorex asper*, *Sorex daphaenodon*, *Sorex araneus*, *Sorex granarius*, *Sorex antinorii*, and *Sorex coronatus* were used as outgroups. The specimen designations are the same as in the Appendix (Tables A1, A2).

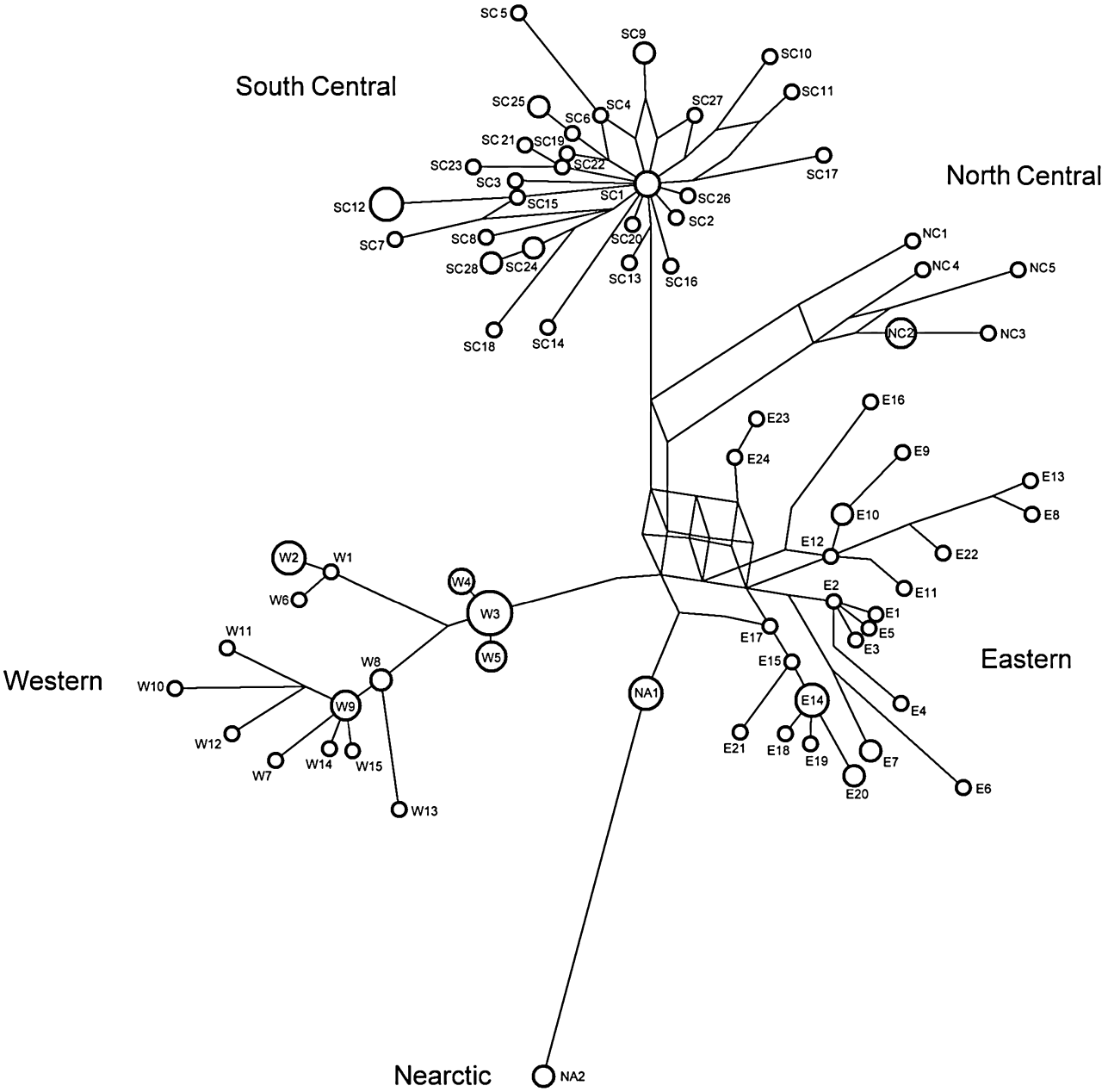

Figure 4. Median-joining network showing phylogenetics relationships among haplotypes in the tundra shrew. Haplotype and specimen codes refer to the Appendix (Table A1). The size of circles corresponds to the number of specimens with identical haplotype.

Hentey. Among these, the latter two demonstrate clear tendency to cluster together; the relationships among the others remain unresolved. On the basis of the c*ytb* data, the existence of additional lineages may be assumed, such as represented by the Kolyma haplotype (locality 35), which is not related to other haplotypes found east of the Lena river. Nearctic phylogroup appears to contain two well differentiated clusters corresponding to the samples from Anchorage and Fairbanks each producing different haplotypes, although there is almost no variability within these clusters. At the same time, within the SC clade, haplotypes do not show any clear tendency to group according to their geographic origin and the branching order remains mostly unresolved (Figs 2, 3).

The mean level of the genetic differentiation (*p*-distance) within major phylogroups based on *cytb* sequencing are 0.44 ± 0.1%, with the largest distance

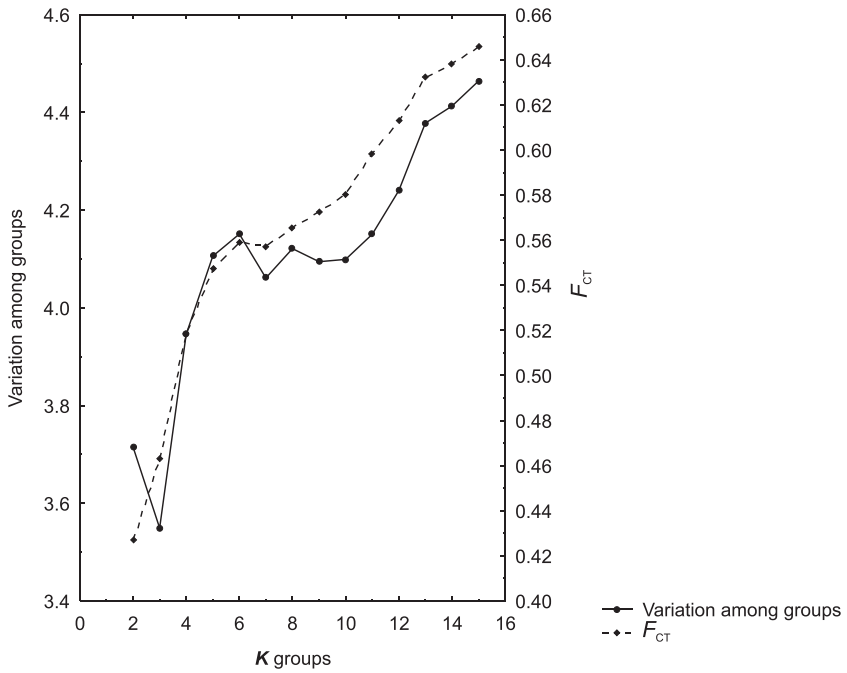

Figure 5. The results of the spatial analysis of molecular variance (SAMOVA) based upon *cytb* haplotype data. The proportion (F_{CT}) and the amount of total genetic variance accounted for by among group differentiation as inferred for the optimal structure recovered in SAMOVA are plotted against the a priori defined number of groups (K). Local maximum corresponds to $K = 6$.

observed within SC phylogroup (0.58 ± 0.1%) and the lowest found for NC and NA phylogroups (0.3 ± 0.1%).

Spatial structure of tundra shrew inferred by SAMOVA

The results of the SAMOVA analysis (Fig. 5) indicate that the proportion of variation among groups (F_{CT}) is increasing with the number of groups (K) and, hence, in our case, provides poor basis for unambiguous identification of the optimum value of K, as it was suggested in Dupanloup *et al*. (1992). However, the among group variance component (*Va*) demonstrates local maxima corresponding to $K = 6$ and $K = 8$. The former solution is just slightly better than that for $K = 5$, which is fully coherent with partitioning into five main phylogroups (W, SC, NC, E, and NA). At $K = 6$ the inferred spatial structure is still consistent with the regional subdivision into five groups but with the Middle/Lower Amur and Primorye (Khanka Lake) samples recognized as a distinct Far-Eastern group separate from the remainder of the E phylogroup. However, this configuration is unstable because, for $K = 7$, 10 or 11, the Far-Eastern specimens form an aggregation together with shrews from the Hentey and Chita region. At $K = 8$, four phylogroups (W, SC, NC, and NA) retain their composition, however, within the Eastern group, four subgroups are supported (Yakutia + Magadan + Moneron, Anadyr + Kolyma, Chita + Hentey, and the above mentioned Far-Eastern subgroup). Thus, the results obtained for $K > 5$ indicate a high level of geographic variation within the Eastern group relative to the others. Given that the optimum values of K selected based on F_{CT} or *Va* criteria are evidently biased upwards and that the configurations recovered for $K > 5$ are not mutually compatible, we might assume that the strongest 'true' structure corresponds to $K = 5$ as a plausible optimum. The subdivision of all haplotypes from Europe, Asia, Siberia, and Alaska into five different groups (Fig. 5) is also consistent with the results suggested by the median-joining network and the phylogenetic trees.

Population genetic structure inferred by AMOVA

To assess the distribution of *cytb* variation, we used AMOVA (Excoffier *et al*., 2002) based on the results of the SAMOVA test. We found that more than half of the overall population variation falls to the differentiation among five main phylogroups (54.62%). The divergence among geographical populations within phylogroups amounts to 21.34% of the overall population variation, and the differentiation within geographical populations (individual variation) forms 24.04%.

Three of five examined phylogroups are characterized by large range and high structuring inside each of them. Thus, we performed the AMOVA test separately for these three phylogroups (W, SC, and E) with the exclusion of presumably admixed haplotypes from Middle Tom (locality 5) and Central Yakutia (locality 32) populations. It appears that, for the SC phylogroup, the divergence among geographical populations (28.47%) is lower than differentiation within populations (71.53%), whereas for the E phylogroup, the situation is inverse (60.36% and 39.64%, accordingly). Differentiation within populations of the W phylogroup (48.15%) is the same as of the E phylogroup but lower than in SC phylogroup. Thus, the AMOVA test reveals the increased intrapopulation and lower interpopulation variation in SC phylogroup.

The analysis of the interpopulation differentiation using F_{ST} statistics and average interpopulation pairwise difference (*p*-distance) demonstrate that although the proportion of variation (*Vb*) explained by interdeme differences is rather small, there are significant differences between populations within groups. Thus, even within the central group for which the lowest level of *Vb* is observed we find significant difference between the Putorana sample and samples from Hangay, Altai, and Middle Yenisei ($P < 0.05$). As expected, Pechora region sample is significantly different from Kazakh Upland and Middle Tom samples, and Central Yakutia sample is significantly different from Anadyr ($P < 0.05$). A simple Mantel test demonstrates significant correlation between geographic distance and F_{ST} ($r^2 = 0.23$; $P < 0.001$), suggesting the possible role of isolation-by-distance. However, this result can be biased as a result of the effect of long-term historical divergence between allopatrically distributed phylogroups. Partial Mantel tests testing significance of correlation of genetic distance and geographic distance with the effect of phylogroup membership controlled (and vice versa) indicate that only 3.6% of variance can be attributed to isolation-by-distance within phylogroups, in contrast to that long-term divergence explains 49% of variation of genetic distance. However, both tests are significant ($P = 0.002$ and $P < 0.001$, respectively).

Diversity measures and neutrality tests

Haplotype diversity (*h*) and nucleotide diversity (π) of *cytb* was calculated for each of geographic region where the size of sample was more than two specimens (Table 2). Overall nucleotide diversity was estimated as 1.09 ± 0.55%. Nucleotide diversity within the phylogroups was estimated at 0.23–0.58%. Nucleotide diversity in the local populations was in the range 0.2–0.8% and was the highest for the localities

Table 2. Measures of intrapopulation and intraphylogroup variability, Tajima's *D*, and τ-values for five main mitochondrial (mt)DNA genetic lineages of *Sorex tundrensis* based on *cytb* haplotypes

Population or phylogroup	*n* specimens	*N* haplotypes	Haplotype diversity (*h*) (% ± SE)	Nucleotide diversity (π) (% ± SE)	Tajima's *D*	Tajima's *D* *P*-value	τ
South Central, total	34	32	0.99 ± 0.01	0.58 ± 0.31	–1.76	0.016	7.2
Putorana Plateau	8	8	1.00 ± 0.06	0.38 ± 0.24	–0.25	0.428	
Middle Yenisei	5	5	1.00 ± 0.126	0.40 ± 0.28	0.46	0.690	
Hangay	10	9	0.98 ± 0.05	0.41 ± 0.24	–0.11	0.460	
Western Palearctic, total	36	26	0.96 ± 0.02	0.38 ± 0.21	–1.09	0.136	5.9
Pechora valley	23	15	0.90 ± 0.05	0.24 ± 0.01	0.92	0.730	
Middle Tom	5	5	1.00 ± 0.18	0.72 ± 0.20	–0.61	0.212	
Kazakh Upland	8	7	0.96 ± 0.08	0.21 ± 0.15	–1.47	0.062	
Eastern Palearctic, total	26	26	1.00 ± 0.01	0.55 ± 0.30	–1.51	0.045	7.6
Anadyr	8	8	1.00 ± 0.06	0.17 ± 0.13	–1.23	0.12	
Central Yakutia	7	7	1.00 ± 0.07	0.37 ± 0.24	–0.36	0.38	
North Central, total	7	7	1.00 ± 0.07	0.32 ± 0.21	–1.41	0.06	7.2
Nearctic, total	7	2	0.95 ± 0.09	0.36 ± 0.23	0.61	0.747	
Anchorage	5	1	0.90 ± 0.16	0.00	0.00	1.00	

Only the values for the localities containing no less than five individuals are presented; values for the mtDNA phylogroups (indicated in bold) are based on pooled samples.

where two different haplogroups were found: Middle Tom (locality 5), Yamalo-Nenetsky region (locality 10), and Central Yakutia (locality 32). If geographical populations with no admixture are considered, the nucleotide diversity ranges from very low at the north-eastern part of the range (0.17% in Anadyr or even 0.00 in Anchorage) to relatively high at the central-south part (0.41% in Hangay sample).

We used Tajima's D and Fu's F_s statistics (Tajima, 1989; Fu, 1997) to test the deviation from the standard neutral model. On the basis of these statistics, none of the geographical populations shows significant departures from neutral equilibrium expectations ($P < 5\%$). At the same time, Tajima's D-test shows the signs of demographic expansion for the pooled samples of SC and E but not W phylogroups ($P = 0.016$, 0.045, and 0.11, respectively) and bell-shaped mismatch distributions were detected within the former two phylogroups, which suggests population expansions in the past (not shown).

The finding that Tajima's D and Fu's F_s statistics are more negative in the pooled samples than in the samples of the individual populations, is not unexpected and was previously mentioned in population genetic studies (Hammer *et al*., 2003; Arunyawat, Stephan & Städler, 2007) and simulations (Städler *et al*., 2009). Less negative Tajima's D statistics in the individual populations could be related with several processes such as range expansion under a low migrant rate (Ray, Currat & Excoffier, 2003) or population subdivision under constant size (Hammer *et al*., 2003). In the former case, global expansion occurs only as a result of an increase in the number of demes with deme size remaining constant. Under these conditions, local samples will not deviate from neutrality; however, scattered sampling (a single randomly chosen sequence per deme) will result in a negative Tajima's D, with behaviour of pooled samples being somewhat intermediate (Städler *et al*., 2009).

In our case, the results of scattered sampling are concordant with global expansion in the SC group but are inconclusive for the E group. In both cases, Tajima D's are negative (for SC, mean $D = 1.75$ $\tau = 6.1$; for E, mean $D = 1.22$, $\tau = 7.4$); however, significance is achieved only for the former one ($P = 0.025$ and 0.11 for SC and E groups, respectively). Additional data are necessary to reveal the role of different demographic factors in the structuring of genetic variation in *S. tundrensis*.

The mismatch distribution of substitutional differences between pairs of haplotypes was calculated within the main genetic lineages (W, SC, NC, E, and NA clades). The sudden expansion model is appropriate for all Palearctic phylogroups but inconsistent with Nearctic phylogroup, perhaps, because of the reduced sample size. The τ-values for the four main mitochondrial (mt)DNA genetic lineages are presented in Table 2.

When compared with the results for main phylogroups of *S. tundrensis*, those for the pooled sample of *S. caecutiens* are characterized by relatively more negative Tajima's ($D = -2.3$; $P = 0.003$) and smaller τ (4.9), suggesting more pronounced and more recent expansion in the latter species.

The rate of molecular evolution and molecular dating

Rate constancy could not be rejected for the concatenated data including sequences of *S. tundrensis*, *S. daphaenodon*, *S. araneus*, *S. granarius*, *S. antinorii*, and *S. coronatus* ($\chi^2 = 132$, d.f. = 110, $P = 0.07$).

The inspection of the output produced by BEAST, version 1.4.8, performed using TRACER, confirmed adequate mixing and convergence in the two independent runs after the burn-in period of three million generation. The summary statistics for relative divergence times among haplotypic lineages (measured in substitutions per site) are presented in the Table 3.

As can be concluded from our preliminary analysis (see Material and methods; see also Supporting information, Table S1) the estimates of substitution rate for most recent events are rather high ($2\mu = 21$–37%). By contrast, phylogenetic rates, which are inferred based on the evidence for splits dating 100–1000 Kya, are lower; thus, the divergence rate calculated from *S. araneus*/*S. granarius* dichotomy is 13.6% and that for *S. daphaenodon*/*S. araneus* is 8.6%. Although the present data are insufficient to address the problem of time dependency of inferred rate in whole detail, it appears inadequate to apply the same rate to all events in radiation of *S. tundrensis*. The range of date estimates obtained by use of population (27.8%, calculated as the mean for seven recent most calibrations) and phylogenetic rates is given in Table 3 (columns 1–3). The simplest approach would be to apply each rate value exclusively within some time interval around the corresponding calibration date (see dates given in bold in Table 3 as a variant). However, for precision dating, this would require an extensive set of additional calibrations, which is now unavailable. Another approach would be to model the pattern of rate decay explicitly (Ho *et al*. 2005). To obtain time estimates in the present analysis, we considered nonlinear regression of time (t) upon the level of genetic divergence [d, which is either expansion parameter (τ) or ultrametric tree node height] (for results, see Table 3, columns 4–5; for technical details, see Supporting information, Table S1). It should be noted that the resultant errors are quite high and, hence, the calculated estimates should be

Table 3. The estimates of divergence times on the base of cytochrome *b* (*cytb*) and cytochrome oxidase I (*COI*)

	Time of most common recent ancestor (Kya)				Time of basal interpopulation split within a clade (rate decay correction)
	Discrete rate			Rate decay model	
	1	2	3	4	5
Species, phylogroups, populations	27.8%	13.6%	8.6%	Expected values ± SE (CI)	Expected values ± SE (CI)
Sorex tundrensis Chukotka	**16.6**	33.8	53.6	18.3 ± 5.3 (10.9–28.2)	
Sorex tundrensis Northern Kazakhstan + Western Siberia	**19.1**	38.9	61.7	21.4 ± 5.1 (13.8–30.3)	
Sorex tundrensis Yakutia	**20.7**	42.3	67.1	23.6 ± 5.9 (14.7–34.0)	
Sorex tundrensis Transbaikalia/Middle Amur	**22.5**	45.9	72.8	25.9 ± 6.2 (16.5–37.0)	13.9 ± 11.8 (0.0–31.4)
Sorex tundrensis Alaska	**25.6**	52.2	82.7	29.9 ± 9.1 (16.8–46.3)	18.0 ± 13.5 (0.0–39.4)
Sorex tundrensis Western clade	**28.7**	58.5	92.7	34.2 ± 7.2 (23.5–47)	22.2 ± 12.1 (1.9–41.3)
Sorex tundrensis North Central clade	**32.0**	59.3	94.0	34.8 ± 8.6 (22.5–50.2)	22.8 ± 13.0 (1.2–43.7)
Sorex tundrensis Hangay	**30.3**	61.8	98.0	36.6 ± 7.7 (25.6–50.8)	
Sorex tundrensis Putorana	**30.6**	62.4	98.9	36.9 ± 8.0 (25.5–51.9)	
Sorex tundrensis South Central clade	**29.0**	65.3	103.6	39.0 ± 7.9 (27.8–53.5)	27.1 ± 12.6 (6.8–47.4)
Sorex tundrensis East clade	**32.9**	67.2	106.4	40.3 ± 7.6 (28.7–53.6)	28.4 ± 12.4 (7.4–47.2)
Sorex granarius + *Sorex araneus*	58.8	**120.0**	190.2	83.3 ± 19.3 (54.9–117.9)	71.3 ± 21.3 (38.1–107.9)
Sorex tundrensis	62.7	**128.0**	202.8	90.6 ± 15.2 (68.0–117.4)	78.6 ± 17.8 (50.3–109.1)
Sorex antinorii + *Sorex granarius* + *Sorex araneus*	98.1	**200.2**	317.3	167.4 ± 34.1 (116.1–227.5)	155.4 ± 35.4 (100.8–217.0)
Sorex tundrensis + *Sorex asper*	165.6	**337.9**	**535.5**	364.7 ± 80.3 (244.7–507.8)	352.7 ± 80.8 (232.0–496.1)
Sorex tundrensis + *Sorex araneus* + *Sorex daphaenodon*	309.2	631.0	**1000.0**	1008.2 ± 147.1 (774.7–1253.9)	996.2 ± 147.5 (760.1–1240.9)
Root (*Sorex samniticus*/all)	518.7	1058.5	**1677.6**	2491.9 ± 405.0 (1862.4–3171.2)	2479.9 ± 405.4 (1848.1–3157.4)

Dates estimates for which the rate coefficients are appropriate are given in bold. CI, confidence interval.

regarded as approximations. Nevertheless, it is evident that the initial pulse of radiation within *S. tundrensis* dates back to the first half of the Upper Pleistocene whereas the peak of diversification within the main phylogroups should be attributed to the second half of the Last Glacial. *Sores asper* and *S. tundrensis* lineages separated in the Middle Pleistocene. Inclusion of the additional calibration point (Supporting information, Table S1) has inconsiderably shifted the times of the most recent common ancestors of most groups towards the present; however, the general outcome remained principally unchanged.

DISCUSSION

General remarks

The pattern of mtDNA variation revealed in the present study suggests the existence of five distinct lineages in the tundra shrew; four of them appear to be distributed parapatrically (Fig. 1). Although there are no definite geographical barriers that could block gene exchange between Palearctic phylogroups, the observed level of introgression is low. Although few alien haplotypes are found in only three samples collected close to putative contact zones, populations located in central parts of the phylogroup ranges are free of any trace of admixture, thus suggesting a limited local effect of intergroup gene flow.

History of the intraspecific structure of the tundra shrew

The major phylogeographic discontinuities found in the tundra shrew across Eurasia and North America associated with significant reciprocal monophyly provides evidence for a relatively early colonization of Eurasian Continent and Alaska. Moreover, it is plausible that the range of the tundra shrew was broader in the past and occupied not only Asia and North America, but also a large part of Europe. According to Osipova, Rzebik-Kowalska & Zaitsev (2006), fossils identified as *Sorex runtonensis* from different Late Pleistocene (36–38 Kya) localities of Poland and Russia may belong to *S. tundrensis.* The Late Pleistocene (Glacial phase) remains of *S. tundrensis* (named as '*S. arcticus*') are known from Moldova, the sediments of Desna and Dnieper rivers, and from Chernigov region of Ukraine (Mezhzherin, 1972).

Various hypotheses could be suggested to explain the origin and age of the retrieved phylogeographical pattern, the most appropriate should take into account both the obtained genetic data and the life history of the tundra shrew in the context of paleoenvironmental changes in Eurasia during the late Quaternary. It was noted above that, in contrast to most of other Palearctic red-toothed shrews, tundra shrew prefers more open and arid landscapes, being widely spread in habitats with tundra and forest-steppe vegetation (Stroganov, 1957; Dolgov, 1985; Yudin, 1989). It is tempting to speculate that, similar to other cold-tolerant organisms and in contrast to forest-dwelling species, the tundra shrew could have found favourable habitat in large areas of Asia and North America dominated by steppe-tundra vegetation during cold and dry glacial phases of Late/Middle Pleistocene. By contrast, during interglacials, when boreal taiga and/or deciduous forests spread to its maximum extent, tundra shrew populations concentrated mainly in tundra or steppe refuges. The reason for this could not only be the climatic or ecological shift *per se* but also that *S. tundrensis* may have suffered from competition with other red-toothed shrews that reached higher densities in forest habitats (Dokuchaev, 1994; Sheftel, 2005), thus forcing the tundra shrew to avoid multispecies forest communities, which can include up to six to eight species (Sheftel, 1994; Dokuchaev, 2005). It should be emphasized that, although a huge part of the contemporary range of *S. tundrensis* lies in the taiga zone, the tundra shrew is almost absent from typical taiga, its actual habitat being restricted to floodplains. Compared to forest community dominants (*S. caecutiens*, *S. araneus*), populations of *S. tundrensis* are characterized by relatively low numbers and patchy distribution (Sheftel, 1994).

Although the molecular dates inferred in the present study are rather imprecise and, hence, the results are compatible with a whole spectrum of potential scenarios, we consider the hypothesis specified below as the most plausible one. The underlying assumption is that divergence times correspond to the expansion phases and colonization events rather than to periods of range fragmentation (for justification see above). In the early Late Pleistocene during the warm and humid Kazantsevo (Eemian) interglacial (130–100 Kya, Svendsen *et al*., 2004), the formation of a continuous zone of deciduous and coniferous forests correlating with a retreat of tundra-steppe vegetation (Velichko, 2002) may have exerted negative impact on the species of open landscapes. Perhaps it was the time of the last common ancestor of all contemporary populations of tundra shrew that could have survived this period in a refuge with steppe and forest-steppe vegetation.

In the Early Zyryan stage (= Early Weichselian = Wisconsin, 100–50 Kya, Wetterich *et al*., 2008), a wide belt of tundra-steppe vegetation extended in Eurasia and North America (Frenzel, 1968; Vereschagin & Gromov, 1977; Frenzel, Pecsi & Velichko, 1992), thus creating favourable conditions for the first expansion of tundra shrews across the Palearctic. This view is

concordant with our results, according to which the tundra shrew may have colonized Eurasia 90–60 Kya. It could have penetrated North America over the Beringian land bridge that definitely existed during the cooler phases of the Late Quartenary until 15 Kya (Sher, 1974; Velichko *et al*., 1984). Neither morphological (Junge, Hoffmann & Debry, 1983; Okhotina 1983), nor cytogenetic (Meylan & Hausser, 1991; Lukáčová *et al*., 1996) evidence indicate a high level of divergence between Nearctic and Palearctic populations. At the same time, substantial mtDNA differentiation between populations of Alaska and North East Asia suggests that the tundra shrew could have colonized North America approximately at the same time (or at least not significantly later) as it expanded its range across Eurasia, presumably in the Early Zyryan.

It is not clear what events could provoke the formation of the five main phylogroups. It is considered that, during the somewhat milder and warmer stages of the Karganian time (40–32 Kya; Wetterich *et al*., 2008), the taiga forests spread across Siberia (Belova, 1985; Ukraintseva, 1996; Grichuk, 1989). Taking into account the affinity of the tundra shrew to open landscapes, it may be assumed that, at that time, the range could have split into few regional populations located predominantly in steppe or tundra refuges. On the other hand, it is possible that the maximum of Zyryan stage (77–59 Kya) was too harsh even for cold-tolerant species. If so, the climatic extremities of the Zyryan maximum could be responsible for range fragmentation and local extinction. Nevertheless, it is unlikely that the tundra shrew populations of North Siberia and North-East Asia (Chukotka) could have persisted through the coldest stages of the Last Glacial.

It is possible that the second wave of colonization of the extant range (including North-East Asia) via dispersal from five refugional populations occurred around the LGM (= Sartan glacial; Wetterich *et al*., 2008) 30–18 Kya (Table 3). At this time, populations within the phylogroups began to differentiate. It remains unclear which of the events dates back to the pre-LGM time or Late Sartan time, which was significantly warmer than the early Sartan (Andreev *et al*., 2002) and to what extent the harsh conditions of the LGM restricted the northward expansion of the tundra shrew. In any case, given a lack of haplotypic lineages shared by populations of Chukotka and Alaska, it might ne assumed that, during the Late Sartan, the tundra shrew did not penetrate North America for the second time.

West Palearctic phylogroup

The Western phylogroup includes three well supported clusters, two of them belong to the population of Pechora valley (Northwestern Urals). The coexistence of two separate mtDNA haplotypic lineages in the population Pechora valley can be explained either by the retention of ancestor polymorphism or by multiwave colonization of this region. The populations of West Siberia (Middle Tom) and Kazakhstan demonstrate a low level of genetic variation and, most probably, originated quite recently (post-LGM period). Taking into account that the species could hardly survive throughout the Last Glacial in the West Siberia because this territory was covered by large periglacial lakes and continental glaciers during the Zyryan stage (Borodin, 1996), it is more probable that the center of origin of the Western group was located somewhere in the Southern Urals. Some fossil remains of the tundra shrew from this area were attributed to the first half of the Late Pleistocene, whereas, at the Pleistocene/Holocene boundary, this species dominated the shrew community (Zaitsev, 1998).

Central Palearctic phylogroups (SC and NC)

Only shallow phylogeographic structure was detected for the SC phylogroup. However, total variation within it was rather high, being equal to that of the E clade in which five differentiated well-supported subclades were found. The nature of this phenomenon lies with high intrapopulation diversity observed in northern as well as southern populations (Hangay, Middle Yenisei, and Putorana). The actual reason for this pattern of variation remains obscure; however, it might be assumed that it could be explained by either rather recent divergence from a highly polymorphic common ancestor or by metapopulation effects such as high level of migration among demes not observed in other parts of the species range, or rapid deme turnover (multiple population extinction/recolonization cycles). The SC phylogroup is distributed mainly within the Yenisei drainage basin, and its riparian zone could act as a long-range dispersal corridor.

The haplotype found in an isolated population from the Dzungarian Alatau mountains in the eastern Kazakhstan also belongs to the SC group, suggesting that, in the past, its range extended significantly in southern and south-western directions.

The distribution of the NC group remains largely unexplored. We found NC haplotypes in northern parts of western Siberia and in central Yakutia; however, large areas of northern-central Siberia (the Lena–Yenisei watershed area, Taymyr, etc.), which could also be a part of the group range, remain unstudied. It may be assumed that, in the Karganian time, when the expansion of the taiga resulted in effective fragmentation of the tundra shrew range,

several populations might have survived in tundra terrains to the north of the taiga belt. In the Sartan glacial with the retreat of forests, the descendants of these northern refugial populations obviously dispersed southward until they contacted expanding SC and E populations, generating several populations of mixed origin as a result.

Alternatively, the pattern of distribution of SC and NC haplotypes might be explained by the retention of ancestral polymorphism. This scenario implies rather recent radiation of all central populations from a single polymorphic ancestral stock and incomplete lineage sorting in some peripheral populations. Finally, the distribution of the NC haplotypic lineage might be shaped by selective forces. Selective advantage of certain mitochondrial lineages contributing to adaptation to colder environment was previously demonstrated in white-toothed shrews (Fontanillas *et al*., 2005) and hypothesized in humans (Mishmar *et al*., 2003), hares (Melo-Ferreira *et al*., 2005), and the pygmy shrew (McDevitt *et al*., 2010).

Given the abovementioned considerations we have to conclude that the existence of NC phylogroup (i.e. the group of populations with common evolutionary history) requires confirmation with additional genetic data.

East Palearctic and Nearctic phylogroups and colonization of Beringia

The data from the present study indicate that *S. tundrensis* colonized Beringia several times. A hypothetical route of dispersal from Central Siberia can be suggested. Steppe areas on the left bank of the Lena River (Giterman *et al*., 1968) could serve as the ecological corridor through which tundra shrews rapidly advanced northwards. Then, it could penetrate Beringia, dispersing along the Arctic coastal shelf north of the Verkhoyansk ridge (Dokuchaev, 1999), which is otherwise an important barrier for small mammal migrations (Krivosheyev, 1973).

The first wave of colonization of the North-Eastern Asia and Alaska dates back to the initial stage of radiation among the contemporary lineages of the tundra shrew in Early Zyryan. However, at present, haplotypes that can be attributed to this wave are found only in the Nearctic. To explain this pattern, it should be acknowledged that *S. tundrensis* had disappeared from North-Eastern Asia during the Zyryan maximum, being unable to resist cold winters with low level of snow cover (Sher, 1974).

The second wave of colonization corresponds to separation of the Chukotka sub-lineage and refers most probably to the late Karganian time when climate became milder, although tundra (or steppe-tundra) landscapes still dominated in the north-eastern regions of Asia. The Chukotka population persisted through the LGM; however, because its level of nucleotide diversity is the lowest among all Asiatic populations, it can be concluded that it must have experienced a serious bottleneck at that time.

The existence of the Kolyma haplotype (locality 35) which is not related to Chukotka haplotypes but demonstrates some affinity to Central Yakutian sub-lineage enables us to hypothesize also the existence of the third colonization event which could be provisionally attributed to the post-LGM (Latest Sartan) time. However, to support this conclusion, additional sampling in the north-eastern Yakutia is required.

On the basis of the relatively high π of the sample of Central Yakutia in comparison to other branches of E clade, it may be assumed that Central Yakutia could harbour a stable population for most of the time from the LGM until recent.

The populations of the South of the Russian Far East and the Chita region diverged in the latest Pleistocene (after the LGM but no later than 10 Kya) when the continuous steppe belt still persisted far to the north from its contemporary location (Andreev *et al*., 2002; Alfimov, Berman & Sher, 2003).

The population of Moneron appears to be the only surviving descendant of a separate Far Eastern sub-lineage, which is now extinct elsewhere. The land-bridge between the Moneron Island and Sakhalin (or Hokkaido) existed during the LGM until approximately 17.5 Kya (Velizhanin, 1976), which is consistent with the inferred time of separation of the ancestor of Moneron tundra shrews at approximately 25–30 Kya. However, at present, *S. tundrensis* is absent from both Sakhalin and Hokkaido, as well as from the mainland coasts of the seas of Japan and Okhotsk. This pattern illustrates significant alterations to the distribution of the species that must have occurred in the early Holocene, when, probably as a result of increased competition with forest species of *Sorex*, the tundra shrew disappeared from a large part of its range in the Far East, including Sakhalin and Hokkaido (Ohdachi *et al*., 1997), as well from almost all of its European Pleistocene range.

The divergence between Anchorage and Fairbanks populations may be explained by the isolation effect of Alaskan mountain ridge, whereas the observed intrapopulation homogeneity might be a result of the LGM bottleneck.

Taxonomic notes

The level of divergence between the phylogroups (1.0–1.5%) lies bellow the lower limit of interspecific differentiation as presented in Bradley & Baker (2001); hence, mitochondrial data provide no evidence

Table 4. Geographical distribution of morphological subspecies according to Stroganov (1957) and Okhotina (1983) and its correlation with mitochondrial (mt)DNA structure

Morphological subspecies	Geographical distribution	mtDNA haplogroups
Sorex tundrensis tundrensis Merriam, 1900 – tricoloured coloration, short-tailed	Alaska	NA
Sorex tundrensis borealis Kastschenko, 1905 – conspicuously tricoloured, paler than the nominative form, tail very short	Tundra zone from Taymyr to Chukotka	SN?, SC?, E
Sorex tundrensis 'buxtoni' Allen, 1903* – coloration darker, bicoloured, short-tailed	Wood zone of East Siberia including the Lena basin and the NE Okhotsk coast	E, SN
Sorex tundrensis baikalensis Ognev, 1913 – similar to *S. t. 'buxtoni'* but smaller and with even shorter tail	Steppe zone from Central Mongolia (S Hangay) to Transbaikalia and the Middle Amur	E, SC
Sorex tundrensis sibiriensis Ognev, 1921 – relatively large, coloration darker than in *S. t. baikalensis* and *S. t. 'buxtoni'*, tail long	Taiga zone of Central and West Siberia east to the Baikal	SC, W?
Sorex tundrensis schnitnikovi Ognev, 1921 – the most long-tailed form, larger than the previous subspecies, coloration dark	isolated population of the Dzungarian Alatau	SC
Sorex tundrensis petschorae Ognev, 1921 – tricoloured coloration like in *S. t. borealis* but darker, tail longer than in the latter	Tundra zone of NE Europe and W Siberia east to the Yenisei	W, NC
Sorex tundrensis transrypheus Stroganov, 1956 – small, most specimens tricoloured, tail relatively long	Steppe zone of Kazakhstan and W Siberia	W
Sorex tundrensis parvicaudatus Okhotina, 1976 – large, short-tailed, tricoloured	Moneron Island	E
Sorex tundrensis khankae Baranova & Zaitsev, 2003 – similar to *S. t. 'buxtoni'* but tail longer	Russian Far East, Primorye	E

*The name *buxtoni* Allen, 1903 refers to *S. caecutiens* (Junge *et al*., 1983), no valid names for this subspecies are available.
E, Eastern Palearctic group; NA, Nearctic group; NC, North Central Palearctic group; SC, South Central Palearctic group; W, Western Palearctic group.

supporting existence of several sibling species within *S. tundrensis s.l.* as was assumed in several cytogenetic studies (Kozlovsky, 1971; Ivanitskaya & Malygin, 1985; Rausch & Rausch, 1993). However, it should be noted that the observed intergroup genetic distances are comparable to that found between closely-related species *S. araneus* and *S. granarius* (1.6%, *cytb*).

The relationship between the distribution of mtDNA lineages and currently accepted morphological subspecies is given in Table 4. It can be seen that there is some correspondence between the intraspecific taxonomy and the mtDNA structuring; however, this correlation is imperfect. Thus, the western populations *S. t. baikalensis* belong to the SC group, whereas eastern populations belong to the E group, *S. tundrensis* '*buxtoni*' includes populations with both E and NC haplotypes, the range of *S. tundrensis petschorae* intersects with those of W and NC (or SC) groups. To resolve the controversies and provide an up-to-date revision, a critical reassessment if previous morphological inference is required, along with a test of the pattern produced with mtDNA by use of nuclear markers.

Comparative phylogeography of Palearctic shrews and other small mammals of Siberia and Russian Far East

All small mammal species distributed in Siberian taiga zone can be divided, with respect to their origin and ecological affinities, into three groups: West Palearctic forest zone species, East Palearctic forest species, and species associated with azonal habitats. Species of the first group usually demonstrate strong phylogeographic structure in the West Europe and, at the same time, they may have only limited genetic subdivision in the East Europe and Asia (the bank vole *Clethrionomys glareolus*: Deffontaine *et al*., 2005; Kotlik *et al*., 2006, Abramson Rodchenkova & Kostygov, 2009; the field vole *Microtus agrestis*: Jaarola &

Searle, 2002; the pygmy shrew *S. minutus*: Mascheretti *et al*., 2003). Among the boreal forest species of Siberian or East Asian origin some species demonstrate strong phylogeographical structure in Siberia (*Clethrionomys rufocanus*: Petrova & Abramson, 2007; *Clethrionomys rutilus*: Iwasa *et al*., 2002); however, others do not (*Pteromys volans*: Oshida *et al*., 2005; *Myopus schisticolor*: Fedorov *et al*., 2008; *Tamias sibiricus*: Obolenskaya *et al*., 2009). Meanwhile, substantial phylogeographic structure is more common in species characterized by affinity not to boreal forests but to azonal habitats, steppe or tundra landscapes (*Microtus oeconomus*: Brunhoff *et al*., 2003; Iwasa *et al*., 2009; *Microtus gregalis*: Abramson, 2007; *Microtus middendorffii s.l.*: Bannikova *et al*., 2010; true lemmings *Lemmus* and *Dicrostonyx*: Fedorov, Fredga & Jarrell, 1999; Fedorov, Goropashnaya, Jarrell, 1999; Fedorov *et al*., 2003).

Accordingly, Palearctic species of red-toothed shrews (*Sorex*), which are associated mainly with mixed and boreal coniferous forests, demonstrate little phylogeographic structure. For example, no phylogeographic discontinuities associated with significant reciprocal monophyly were detected across the *S. caecutiens* range, which was sampled throughout the mainland part of its range, including the eastern and western extremes of the continent (Ohdachi *et al*., 2001). In comparison with the main lineages of *S. tundrensis* (SC and E) *S. caecutiens* demonstrates a clear signal of a more recent expansion. Thus, the observed pattern of genetic variation in the two species conforms to the scenario according to which the tundra shrew underwent expansion in colder and drier pre-LGM time, whereas, in the Laxmann's shrew, the expansion was delayed until a warmer period dating back to the latest Pleistocene (post-LGM) or even early Holocene and was probably associated with the spread of taiga vegetation. The comparison between *S. tundrensis* and *S. caecutiens* is of special interest because these two species are of similar size, and both have extensive and widely overlapping Palearctic ranges but appear to be rather different in their ecological affinities.

Among forest species of *Sorex* of European origin, the common shrew *S. araneus* is remarkable for a deficit of geographic differentiation in the West Europe (Ratkiewicz, Fedyk & Bonaszek, 2002; Wojcik, Ratkiewicz & Searle, 2002; Andersson, 2004), as well as across the Eurasian range (Bannikova *et al*., 2005). The pygmy shrew *S. minutus* demonstrates shallow mtDNA structure in the eastern part of the range, with some differentiation found only among the West European populations (Mascheretti *et al*., 2003; McDevitt *et al*., 2010). At the same time, unlike the continental populations of boreal *S. caecutiens*, the least shrew *Sorex minutissimus* shows pronounced west/east divergence of mtDNA haplotypes (Ohdachi *et al*., 2001). However, although the latter species is associated mainly with the forest zone, it is less habitat-specific and is often found in steppe and tundra landscapes.

Thus, none of the examined Siberian red-toothed shrews has substantial phylogeographical structure, with the exclusion of *S. tundrensis* and *S. minutissimus*. Hence, it is likely that the complex pattern of geographical variation revealed in these species is explained by their contrasting habitat preferences.

In conclusion, it should be noted that the phylogeographic pattern revealed in the present study is based exclusively on mtDNA data, and, although we consider that it reflects not only the mitochondrial genealogy, but also the evolutionary history of populations, all advanced hypotheses are to be tested with additional genetic evidence.

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SUPPORTING INFORMATION

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

Supporting information text. Beast analysis and nonlinear regression.

Table S1. The estimates of divergence times based on cytochrome *b* (*cytb*) and cytochrome oxidase I (*COI*) genes. For reference, see Table 3. The present table below differs from Table 3 in that the calibration set employed includes additional point suggesting that *Sorex antinorii* diverged from *Sorex araneus* during the Last Interglacial (approximately 120 Kya).

APPENDIX

Table A1. Geographic information, number and designation of specimens used in the present study

Locality (Fig. 1)	Collecting locality (region or closest city and coordinates)	*n*	Specimen code (Fig. 2, 3)	Collection and collection code	Haplotype code	GenBank accession number	
						Cytb	*COI*
1	**Upper Pechora (I)** West foothills of North Ural mountains, Pechoro-Ilychsky Reserve, Komi Republic, Russia 62°39′01″N, 58°52′25″E	19	Pech0/05	PISR	W1	GU564782	GU929310
			Pech39/07	PISR	W2	GU564786	GU929300
			Pech9/07	PISR	W3	GU564787	GU929301
			Pech1/08	PISR	W2	GU564796	GU929303
			Pech2/08	PISR	W3	GU564797	
			Pech118/08	PISR	W4	GU564798	
			Pech161/08		W2	GU564802	GU929304
			Pech163/08	PISR	W3	GU564799	
			Pech168/08	PISR	W3	GU564783	
			Pech287/08	PISR	W3	GU564793	
			Pech372/08	PISR	W2	GU564800	GU929305
			Pech376/08	PISR	W5	GU564801	GU929309
			Pech406/08	PISR	W5	GU564789	GU929306
			Pech408/08	PISR	W3	GU564803	GU929307
			Pech475/08	PISR	W2	GU564790	
			Pech500/08	PISR	W3	GU564791	
			Pech563/08	PISR	W5	GU564792	GU929308
			Pech603/08	PISR	W3	GU564794	
			Pech604/08	PISR	W5	GU564795	
2	**Upper Pechora (II)** West foothills of North Ural mountains, Pechoro-Ilychsky Reserve, Komi Republic, Russia 62°03′41″N, 58°28′12″E	4	Pech7340/07	ZMMU S-183258	W6	GU564785	GU929299
			Pech7376/07	ZMMU S-183259	W3	GU564784	GU929298
			Pech7098/07	ZMMU S-183234	W4	GU564788	
			Pech7325/07	ZMMU S-183256	W4	GU564804	GU929302
3	**Kazakh Upland** NE Kazakhstan, Bayan-Aul, Pavlodar Region, Kazakhstan 50°45′06″N, 75°41′50″E	8	Kaz105	SZMN	W7	GU564812	GU929312
			Kaz106	SZMN	W8	GU564808	GU929313
			Kaz108	SZMN	W9	GU564807	GU929314
			Kaz111	SZMN	W9	GU564809	GU929315
			Kaz114	SZMN	W9	GU564810	
			Kaz136	SZMN	W8	GU564806	GU929316
			Kaz137	SZMN	W10	GU564811	
			Kaz209	SZMN	W11	GU564813	GU929317
4	**Baraba steppe** Karasuk, Novosibirsk Region, Russia 53°43′41″N, 78°03′18″E	1	Nov100		W12	GU564805	GU929311
5	**Middle Tom** Kemerovo Region, Krapivinsk area, Azhendarovo, Russia 54°59′59″N, 86°48′41″E	5	Kem2085	ZMMU S-177788	W13	GU564758	GU929295
			Kem2233	ZMMU S-177789	W14	GU564760	GU929297
			Kem2397	ZMMU S-177790	W15	GU564761	GU929296
			Kem2517	ZMMU S-177791	W9	GU564762	GU929294
			Kem1459	ZMMU S-177787	C1	GU564759	GU929293
6	**Bolshoy Kemchug** South of Krasnoyarsk Region, Russia 56°10′53″N, 91°37′17″E	1	BKem8	ZMMU S-81674	C2	GU564851	
7	**Dzungarian Alatau** Taldy-Kurgan Region, Tekeli District, South-east Kazakhstan 44°45′19″N, 78°56′32″8°56′32″E	1	DA4	ZISP 66009	C3	GU564852	GU929260
8	**South Altai (I)** Ukok Plateau, Kosh-Agach District, Gorno-Altai Republic 49° 18′60″N, 87°26′04″E	2	Alt61	SZMN	C4	GU564779	GU929276
			Alt62	SZMN	C5	GU564780	GU929277
9	**South Altai (II)** Tyutea River, Kosh-Agach District, Gorno-Altai Republic, Russia, 50°08′25″N, 87° 53′22″E	1	Alt14	ZISP 98873	C6	GU564781	
10	**Middle Taz** Krasnoselkupsky district, East of Yamalo-Nenetsky Region, Russia 65°43′45″N, 82°30′32″E	2	YN1453	KZM 1453	C16	GU564840	GU929292
			YN1454	KZM 1454	CN4	GU564841	GU929324

Table A1. *Continued*

Locality (Fig. 1)	Collecting locality (region or closest city and coordinates)	*n*	Specimen code (Fig. 2, 3)	Collection and collection code	Haplotype code	GenBank accession number	
						Cytb	*COI*
11	**Middle Yenisei** Left bank of River, Ecological station 'Mirnoe', Turukhansk district, Krasnoyarsk Region, Russia 62°17′17″N, 88°58′13″E	5	Mirn1354	ZMMU S-184034	C9	GU564819	GU929273
			Mirn990	ZMMU S-186045	C1	GU564820	GU929279
			Mirn991	ZMMU S-186046	C10	GU564821	GU929280
			Mirn1284	ZMMU S-186047	C9	GU564822	GU929282
			Mirn599	ZMMU S-186044	C11	GU564823	GU929281
12	**Putorana Plateau** Dyupkun Lake, Taimyr district, North of Krasnoyarsk Region, Russia 68°10′N 92°47′E	8	Put206	ZMMU	C12	GU564744	GU929275
			Put209	ZMMU	C12	GU564745	GU929266
			Put204	ZMMU S-181908	C13	GU564743	GU929274
			Put78	ZMMU S-181835	C14	GU564738	GU929262
			Put87	ZMMU S-181837	C15	GU564739	GU929261
			Put89	ZMMU S-181838	C12	GU564740	GU929263
			Put124	ZMMU S-181856	C12	GU564742	GU929264
			Put119	ZMMU S-181852	C12	GU564741	GU929265
13	**Gydan Peninsulae** NE Yamalo-Nenetsky Region, Tazovsky District, Russia 72°20′07″N, 78°18′29″E	1	YN-G	ZMMU 05/10	CN5	GU564842	GU929325
14	**South-West Evenkia (I)** Stolbovaya River, Basin of Podkamennaya Tunguska, Krasnoyarsk Region, Russia 62°16′72″N, 91°26′09″E	1	Even1	ZMMU 54/09 1	C1	GU564837	GU929289
15	**South-West Evenkia (II)** Podkamennaya Tunguska River, Krasnoyarsk Region, Russia 61°36′34″N, 90°07′56″E	2	Even4	ZMMU 54/09 4	C7	GU564838	GU929290
			Even9	ZMMU 54/09 9	C8	GU564839	GU929291
16	**Biryusa River** Taishet District, Irkutsk Region, Russia, 56°00′N, 97°35′E	1	IrkZ1-120	SZMN	CS18	GU564736	GU929272
17	**SW Baikal** B. Koty, Irkutsk Region, Russia 52°19′27″N, 104 °21′20″E	1	Irk57	ZMMU S-181339	CS17	GU564737	GU929270
18	**Middle Selenga** Selenga District, Buryatya, Russia 51°11′44″N,106°16′29″E	1	Bur83786	ZISP 83786	CS19	GU564814	GU929278
19	**North Hangay (I)**, Orohyn Daba, Tarbagatai ridge, Tariat Somon, Arhangay Aymag, Mongolia 48°15′22″N, 99°23′14″N	7	Hang78	ZMMU 48/09 78	CS25	GU564750	GU929283
			Hang79	ZMMU 48/09 79	CS26	GU564751	
			Hang80	ZMMU 48/09 80	CS27	GU564752	GU929284
			Hang88	ZMMU 48/09 88	CS28	GU564753	GU929285
			Hang89	ZMMU 48/09 89	CS24	GU564754	GU929286
			Hang95	ZMMU 48/09 95	CS28	GU564755	GU929287
			Hang96	ZMMU 48/09 96	CS25	GU564756	GU929288
20	**North Hangay (II)**, Tarbagatai ridge, Tariat Somon, Arhangay Aymag, Mongolia 48°10′09″N, 99°54′09″E	4	Hang199	ZMMU S-181053	CS21	GU564746	GU929269
			Hang207	ZMMU S-181055	CS22	GU564747	GU929267
			Hang208	ZMMU S-181056	CS23	GU564748	GU929268
			Hang209	ZMMU S-181057	CS24	GU564749	GU929271
21	**West Hentey** Upper Eroo River, Selenga Aimag, Mongolia 48°39′46″N, 108°54′26″E	1	Hent9	ZMMU S-182209	E1	GU564757	GU929240
22	**Torei Lakes, Chita** Ononskiy District Chita Region, Russia 50°11′39″N, 115°43′25″E	3	Chi42	SZMN	E2	GU564816	GU929237
			Chi57	SZMN	E3	GU564818	GU929239
			Chi43	SZMN	E4	GU564817	GU929238
23	**Upper Argun, Chita** Zabaikalsky District Chita Region, Russia 49°32′57″N, 117°50′13″E	1	Chi251	ZMMU S-182064	E5	GU564815	GU929236
24	**Khanka Lake** Gayvaron vill., Spassky District, Primorsky Region, Russia 44°44′22″N, 132°48′11″E	1	Pri145569	ZMMU S-145569	E7	GU564773	
25	**Middle Amur River** Smidovich District, Jewish AutonomyRegion, Russia 48°22′49″E, 134°06′51″E	1	Bir1787		E6	GU564772	GU929235
26	**Khabarovsk, 50 km S** Khabarovsk Region, Lazo District, Russia 47°57′36″N, 135°16′02″E	1	Khab173/2004	IBPN	E7	GU564834	GU929234

Table A1. *Continued*

Locality (Fig. 1)	Collecting locality (region or closest city and coordinates)	*n*	Specimen code (Fig. 2, 3)	Collection and collection code	Haplotype code	GenBank accession number	
						Cytb	*COI*
27	**Moneron Island, Japan Sea** Russia 46°27′N, 141°24′E	2	MonAB244646	IBPN	E23	*AB244646*	
			Mon196/2005	IBPN	E24	GU564771	GU929241
28	**Middle Amga River, C Yakutia** Amga District, Republic Sakha (Yakutia), Russia 59°49′N, 128°56′E	1	CY181486	ZMMU S-181486	CN1	GU564774	GU929318
29	**Maya River,C Yakutia** Ust'-Maya District, Republic Sakha (Yakutia), Russia 59°46′37″N, 134°48′51″E	4	Yakutia700	ZMMU S-183445	CN2	GU564775	GU929319
			Yakutia729	ZMMU S-183447	CN3	GU564778	GU929322
			Yakutia740	ZMMU S-183448	CN2	GU564776	GU929320
			Yakutia743	ZMMU S-183449	CN2	GU564777	GU929321
30	**Middle Lena River, C Yakutia (I)** Russia 61°45′15″N, 129°31′32″E	2	CY78/2006	MSB 146504	E8	GU564825	GU929253
			CY79/2006	MSB 146505	E9	GU564824	
31	**Middle Lena River, C Yakutia (II)** Russia 62°04′12″N, 128°56′18″E	5	CY171/2006	MSB 148479	E10	GU564826	
			CY179/2006	MSB 148481	E10	GU564827	GU929255
			CY197/2006	MSB 148842	E11	GU564828	GU929256
			CY222/2006	MSB 148750	E12	GU564829	GU929257
			CY223/2006	MSB 148755	E13	GU564830	GU929258
32	**Lower Amga, C Yakutia (III)** Amga District, Republic Sakha (Yakutia), Russia, 61°14′46″N,132°42′53″E	2	CY132/2006	MSB 148552	CN2	GU564835	GU929323
			CY140/2006	MSB 148655	E22	GU564836	GU929259
33	**Upper Kolyma River, Magadan** Susumansky Distrikt, Magadan Region, Russia 62°46′23″N, 148°12′52″E	1	Mag66/1996	IBPN	E14	GU564770	GU929243
34	**NE Okhotsk coast, Magadan** Severo-Evensky District, Magadan Region, Russia 61°54′51″N; 159°15′03″E	1	Mag57/2008	IBPN	E15	GU564768	GU929249
35	**Lower Kolyma, N Yakutia** Chukochya River, Nizhne-Kolymsky District, Russia 70°02′23″N, 159°13′09″E	1	NEY AB175127	IBPN	E16	*AB175127*	
36	**Chukotka** Ust'-Chaun, Chaunsky Distrikt, Chukotka Region, Russia 68°45′29″N, 170°46′01″E	1	Chu489/1980	IBPN	E17	GU564833	
37	**Anadyr (I)** Dionisia, Anadyrsky District, Chukotka Region, Russia 64°34′04″N, 177°18′48″E	2	Ana135/2002	UAM 84247	E20	GU564766	GU929245
			Ana132/2002	UAM 84242	E20	GU564769	GU929244
38	**Anadyr (II)** Shakhtersky, Anadyrsky District, Chukotka Region, Russia 64°48′45″N, 177°32′54″E	6	Ana178/2002	UAM 84375	E18	GU564765	GU929247
			Ana182/2002	UAM 84378	E14	GU564831	GU929251
			Ana185/2002	UAM 84395	E14	GU564832	GU929252
			Ana173/2002	UAM 84362	E19	GU564764	GU929246
			Ana161/2002	UAM 84277	E14	GU564853	GU929250
			Ana187/2002	UAM 84388	E14	GU564767	GU929248
39	**Anadyr (III)** Anadyrsky District, Chukotka Region, Russia 64°44′N, 177°40′E	1	Ana182096	ZMMU S-182096	E21	GU564763	GU929242
40	**Alaska, Fairbanks** 64°55′02′N, 147°50′51′W	3	Ala243/2008	UAM 102213	NA2	GU564844	GU929327
			Ala233/2008	UAM 102208	NA2	GU564843	GU929326
41	**Alaska, Anchorage**, Eagle River 61°17′43″N, 149°32′10″W	5	Ala147/2008	IBPN	NA1	GU564845	GU929330
			Ala296/2008	IBPN	NA1	GU564846	GU929331
			Ala382/2008	IBPN	NA1	GU564849	GU929328
			Ala391/2008	IBPN	NA1	GU564847	GU929329
			Ala413/2008	IBPN	NA1	GU564848	
			Ala434/2008	IBPN	NA1	GU564850	GU929332
42 (not shown)	Russia, Siberia W, Tyumen	1	D85371		CS20	*D85371*	

Figure references correspond to the localities in Fig. 1 and specimen codes in Figs 2, 3; *n*, number of specimens analyzed. The sequences retrived from GenBank are given in italics.
IBPN, Institute of Biological Problems of the North FEB RAS, Magadan; MSB, Museum of Southwestern Biology, Mammals; PISR, Pechora-Ilych State Reserve; SZMN, Museum of Institute f Systematics and Ecology of Animals, SB RAS, Novosibirsk; UAM, University of Alaska Museum, Mammals; ZMMU, Zoological Museum of Moscow State University, Moscow; ZISP, Zoological Institute RAS, St.-Petersburg.

Table A2. The list of sequences used as outgroup

Species	Collecting locality	Museum catalogue number, tissue or field code	Tree (Figs2, 3) code	GenBank accession number	
				Cytb	*COI*
Sorex coronatus	Vaud, Switzerland			AJ000419	–
Sorex coronatus	Vaud, Switzerland			–	AY332665
Sorex granarius	Spain			AJ000417	–
Sorex granarius	Spain			–	EF636539
Sorex araneus	Hungary	IZEA6137	S.a.1	AJ312028	–
Sorex araneus	Yugoslavia	JZ835	S.a.2	AJ312031	–
Sorex araneus	Russia, Siberia, Kemerovo	Sa Kem2247	S.a.3	GU564723*	–
Sorex araneus	Russia, Middle Yenisei	ZMMU Sa Mirn58	S.a.4	GU564730*	GU929225*
Sorex araneus	Russia, Middle Yenisei	ZMMU Sa Mirn1178	S.a.5	GU564725*	–
Sorex araneus	Russia, Voronezh	ZMMU Sa Voronezh	S.a.6	GU564724*	GU929219*
Sorex araneus	Sweden		S.a.7	AJ245893	
Sorex araneus	Russia, Bryansk reg.	Sa N21	S.a.8	GU564726*	GU929221*
Sorex araneus	Russia, Tver reg.	ZMMU Sa Kru11	S.a.9	GU564729*	GU929224*
Sorex araneus	Russia, Pechora valley	PISR Pech5	S.a.10	GU564731*	–
Sorex araneus	Russia, Tver reg.	Sa M14	S.a.11	GU564727*	GU929222*
Sorex araneus	Russia, Tver reg.	Sa S15-1	S.a.12	GU564728*	GU929223*
Sorex araneus	Russia, Middle Yenisei	ZMMU Sa Mirn94	S.a.13	–	GU929220*
Sorex antinorii	Italy, Piacenca	IZEA7514	S.an.1	AB175125	–
Sorex antinorii	Swiss		S.an.2	AJ312039	–
Sorex antinorii	Italy		S.an.3	AJ312035	–
Sorex antinorii	Italy, Grand Sasso	IZEA1394	S.an.4	AB175124	–
Sorex antinorii	Switzerland, Valais			–	EF636545
Sorex asper	Kirghizstan, Arashan	JZ892	S.as.1	AJ000425	–
Sorex asper	Kirghizstan, Arashan	JZ895	S.as.2	AJ000426	–
Sorex daphaenodon	Russia, Magadan	SO-2Kmisc-49	S.d.1	AB175126	–
Sorex daphaenodon	Mongolia	Sd Mong3	S.d.2	GU564732*	GU929227*
Sorex daphaenodon	Mongolia	Sd Mong4	S.d.3	GU564733*	GU929228*
Sorex daphaenodon	Mongolia	Sd Mong7	S.d.4	GU564734*	GU929229*
Sorex daphaenodon	Russia, Birobidgan	Sd Bir1803F	S.d.5	GU564735*	GU929230*
Sorex arcticus	Canada, Alberta	BIOUG HLC-16331		–	GU929233*
Sorex samniticus	Abruzzo, Italy			–	EF636547
Sorex samniticus	Abruzzo, Italy			–	AJ000429

Accession numbers for the specimens sequenced in the present study are given in bold. Original sequences are indicated by asterisks.

BIOUG, Biodiversity Institute of Ontario, University of Guelph; PISR, Pechora-Ilych State Reserve; ZMMU, Zoological Museum of Moscow State University, Moscow.