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Intraspecific phylogeny and nucleotide diversity of the least shrews, the *Sorex minutissimus-S. yukonicus* complex, based on nucleotide sequences of the mitochondrial cytochrome *b* gene and the control region

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Abstract. Phylogenetic analysis was conducted for various populations of the *Sorex minutissimus-S. yukonicus* complex based on mitochondrial gene (cytochrome *b* and/or the control region) sequences. *Sorex minutissimus* was divided into some monophyletic groups in Eurasia; it was divided into 2 main groups, eastern and western Eurasian clades, based on combined data of the cytochrome *b* and the control region. Monophyly of shrews from Hokkaido-Sakhalin, Primorye, Mongolia-Transbaikalia, southeastern Finland was strongly supported respectively in most analyses. *Sorex yukonicus* was phylogenetically close to *S. minutissimus* in eastern Siberia. Some shrews from western and central Siberia were included in the clade of southeastern Finland. Also, most shrews from central-northern Finland and Norway made a clade close to but different from the southeastern Finland clade. This finding suggests that Fennoscandian shrews might consist of individuals which were recolonised from various refugia after the Last Glacial Maximum. Nucleotide diversity of shrews from Hokkaido and Alaska was low. Three regional groups in Kamchatka-Sakha, Sakhalin, and Mongolia-Transbaikalia tended to have medium nucleotide diversity. In contrast, shrews from Cisbaikalia-western Siberia and Fennoscandia had high nucleotide diversity. The *S. minutissimus-S. hosonoi* group appears to have experienceed a quit different biogeographic history from two shrews with similar ranges, the *S. caecutiens-S. hosonoi* group and *S. tundrensis*.

Key words: cytochrome *b*, intraspecific phylogeny, nucleotide diversity, phylogeography, the mitochondrial control region.

Sorex minutissimus Zimmermann, 1780 is distributed in a very wide range in Eurasia from Chukotka in the easternmost edge to Norway in the westernmost one (Hutterer 2005; Ohdachi et al. 2010). In addition, a species similar to *S. minutissimus* was reported from Alaska and it was nominated as a new species *Sorex yukonicus* Dokuchaev, 1997 (Dokuchaev 1994, 1997). However, it was recently suggested based on molecular phylogenetics that *S. yukonicus* might be a local population of *S. minutissimus* in Alaska (Hope et al. 2010). Thus, including *S. yukonicus* in *S. minutissimus* (hereafter, called the "*S. minutissimus-S. yukonicus* complex"), the range of the shrew species covers in a wide area of the northern parts of the Holarctic region.

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Although the shrew of the *S. minutissimus-S. yukonicus* complex is famous as one of the smallest terrestrial mammal species of the world, its ecological information is scarce (Ohdachi et al. 2010). *Sorex minutissimus* has a wide distributional range but is generally rare in most habitats in Eurasia (Sheftel 1994; Ohdachi et al. 2010). Therefore, it is a fascinating subject to reveal how they are maintaining population under such sparse density. To infer the mechanism for recruitment of a population, the genetic structure of the population is basic information. In *S. minutissimus*, Ohdachi (2007, 2008) revealed intraspecific phylogeny among individuals from Hokkaido, Sakhalin, Primorye, and Fennoscandia, based on mitochondrial gene sequences. Further, Ohdachi (2007, 2008) demonstrated that the population in southeastern Finland had greater genetic diversity than that in Hokkaido, although sampling area is almost the same between the two regions. However, Ohdachi (2007, 2008) did not analyse most of the other parts of the range of *S. minutissimus*.

Recently, Hope et al. (2010) investigated intraspecific phylogeny of the *S. minutissimus-S. yukonicus* complex from various regions and demonstrated higher genetic diversity in Fennoscandia population than those from eastern Eurasia, based on the mitochondrial cytochrome *b* and 2 nuclear genes, as in Ohdachi (2007). However, in the analysis of Hope et al. (2010), the number of samples from central part of Eurasia is not enough and the sequence data used were of genes of slow evolutionary rate, which may be insufficient for full understanding of intraspecific relationships.

In this paper, we investigated intraspecific phylogeny of the *S. minutissimus-yukonicus* complex, using samples from more regions in Eurasia, based on nucleotide sequences of mitochondrial cytochrome *b* gene and the control region (D-loop). Evolutionary rate of the control region is higher than that of the cytochrome *b* gene in general since it includes hypervariable area and tandem repeats (Stewart and Baker 1994). However, because the data of *S. yukonicus* in Alaska were cited from Hope et al. (2010), no sequences of the control region for *S. yukonicus* were included in the present analysis (Appendix). Then, we discussed the diversification process of the *S. minutissimus-S. yukonicus* complex in the Holarctic region. Phylogeographic pattern was also compared between the *S. minutissimus-S. yukonicus* complex (or *S. minutissimus-S. hosonoi* group) and other Holarctic or trans-Eurasian species (or species group), the *Sorex caecutiens-S. shinto* group and *Sorex tundrensis*, to investigate the difference in biogeographic history among these wide-ranged shrews.

Materials and methods

Shrews examined

Seventy one individuals of *Sorex minutissimus* were collected from various locations of Eurasia in the present study (Fig. 1 and Appendix). In addition, 69 individuals of *S. minutissimus* and *S. yukonicus* used in Ohdachi et al. (2001) and Hope et al. (2010) were included in the present analysis. Totally, 140 individuals of the *S. minutissimus-yukonicus* complex were used (note that numbers of individuals examined varies depending on data set). These shrews were collected from 72 localities in northern Eurasia and Alaska (Fig. 1 and Appendix) (note that capture location of FiKo and FiNa from

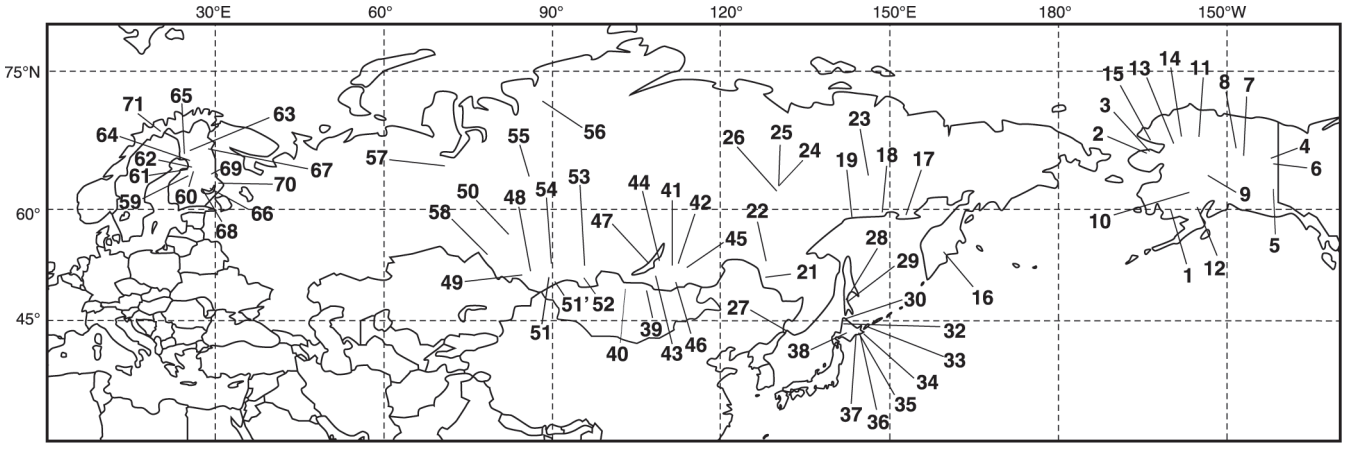

Fig. 1. Locations where individuals analysed were captured. Numbers corresponds with those of Appendix.

Kuivaniemi, Finland was regarded as the same because the sampling locations are very close). One individual of *Sorex hosonoi* Imaizumi, 1954 was used for an outgroup, when needed. Most specimens used were preserved in Botanical Garden Museum, Hokkaido University (Sapporo, Japan), NPO En-no-mori (Hamanaka-cho, Japan), University of Helsinki (Helsinki, Finland), Institute of Biological Problems of the North (Magadan, Russia), Zoological Institute Russian Academy of Sciences (Saint-Petersburg, Russia), and Institute of General and Experimental Biology (Ulan-Ude, Russia).

DNA sequencing

Sequencing was conducted following Ohdachi et al. (2006) for the mitochondrial cytochrome *b* gene (*cytb*). In addition to Ohdachi et al. (2006), new primers were designed: 5'-CAACTACAGAAACATTTA-3' (position, L14747) and 5'-CACGAAACTGGCTCTAACAA-3' (L15366) for forward primers; 5'-AATTTTGTCTGCG TCTGAGGATA-3' (H15406) and 5'-TTACAAGACCA GTATAATGGTTATA-3' (H15927) for reverse primers. Here, position numbers are those corresponding for the 3' end of L and H strands of human mitochondrial genome (Anderson et al. 1981). A whole 1,140 bp region of *cytb* was sequenced. Some sequence data in Ohdachi et al. (2001) were renewed in the present study.

The control region (D-loop) of mitochondrial genome was also sequenced. The primers for PCR (L16517 and H651) were cited from Fumagalli et al. (1996). Schematic diagram of the amplified region of the control region of *Sorex* shrews was indicated in Fig. 2. The sequencing condition and procedure were basically the same as in the mitochondrial cytochrome *b* gene by Ohdachi et al. (2006). The total length of the PCR amplified region is approximately 1,100–1,200 bp, which largely varied among individuals depending on the number of the repeat motifs.

Phylogenetic analysis

Phylogenetic analysis was conducted using sequence data which were revealed in the present analysis and those cited from published elsewhere. See Appendix for sources of the nucleotide sequences. Note that the sequence of the control region for FiJo5 was completely different from others, and the repeat motif of the control region for FiJo14 was uncommon. Thus, the control region sequences of these two individuals were not used in the present analyses.

Three data sets were prepared: the control region (D-loop), *cytb*, and combined data of the control region + *cytb*. For the combined data set, only individuals which had both the control region and *cytb* sequences were included (i.e., no missing data for the data set). Alignments of *cytb* sequences were straightforward because of lack of insertions/deletions. Including data cited from DNA data banks (DDBJ/GenBank/EMBL), the total lengths of *cytb* sequence used for analysis were 960–1,140 bp. The control region sequences composed of conservative regions near 5' and 3' ends and highly-variable tandem repeat region in the middle part (Fig. 2). Numbers of repeats were highly variable among individuals, and homology of each unit of repeats could not be warranted confidently. Therefore, the tandemly repeated region (except the first and last terminal motifs of the repeated region) was omitted in the analysis. Sequences of the control region were initially aligned using MAFFT ver. 6.857b (Katoh et al. 2005) and edited by eye according to the similarity criterion (Simmons 2004). The region also includes indels, and poorly aligned regions were excluded from the analyses. As a result, the total length used in analysis of the control region was 751 bp including gaps.

We applied the maximum likelihood (ML) and Bayesian (BY) analyses to estimate the phylogenetic relationships. ML tree was searched using PAUP* 4.0b10 (Swofford 2002). Heuristic search with TBR (tree bisection reconnection) branch swapping was performed with a neighbor joining (NJ) tree as a starting tree. Bootstrap support values were calculated by 100 replications of NNI (nearest neighbor interchange) branch

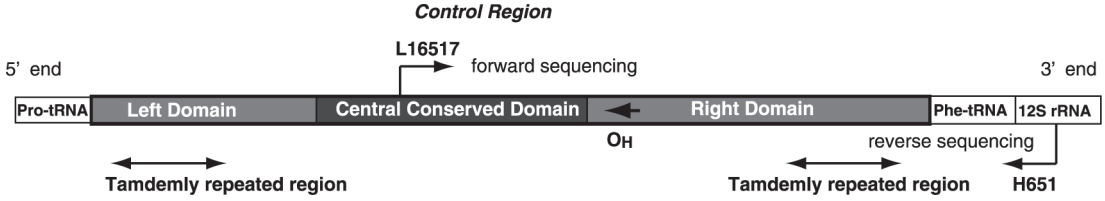

Fig. 2. A schematic diagram of the organization in the mitochondrial control region (D-loop) and positions of the PCR primers (L16517 and H651). O_H: the H-strand replication origin.

swapping, each using an NJ tree as starting tree. Branch support values were also calculated using the parsimony-based bootstrapping, with TBR branch swapping. Thus, bootstrap values were calculated by ML and parsimony (PA) methods. For *cytb* and the combined data sets, number of maxtree was set to 1,000 for parsimony bootstrapping because of huge numbers of equally parsimonious trees were estimated from these data sets. Bayesian trees were searched using MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003). We performed 2 runs each with 4 chains for 10,000,000 generations and trees were sampled every 1,000 generations. First 5,000 trees were excluded for burn-in, and a 50% majority consensus tree was computed using the rest of trees to obtain Bayesian posterior probabilities. Best fit models for ML and BY searches were selected using the hierarchical likelihood ratio test as implemented in Modeltest ver. 3.7 (Posada and Crandall 1998) and MrModeltest ver. 2.3 (Nylander 2004), respectively. For Bayesian analyses, data were subdivided into the following four categories (first, second and third codon positions of *cytb* and the control region), and different substitution model was applied for each category. Aligned sequences and list of the estimated models are available at http://insect3.agr.hokudai.ac.jp/psoco-web/sorex_minutissimus/.

In addition, we also estimated median joining (MJ) networks from the control region and *cytb* data set using SplitTree 4 (Huson and Bryant 2006). No outgroup was used. Default setting was adopted to obtain the network with "Ignore Gaps setting". Thus, the total lengths of sequences used for the network were 960 bp for *cytb* and 723 bp excluding gaps for the control region.

Initial preliminary searches of all data set provided highly congruent tree concerning the ingroup topology. In contrast, position of the root was very unstable among the trees estimated from different data sets (root attached to Nad3 by analysis of control region and SahS3 by *cytb* analysis: original trees available online). Because of the following reasons, we considered the rooting position obtained from the combined data to be the most suitable, and this rooting position was forced to the analyses of the control region and *cytb* data sets: 1) the best tree obtained from the unconstrained analyses of the control region and *cytb* data provided an extremely asymmetrical tree with large differences in branch lengths (see online supplement); 2) mid-point rooting of the independent analyses of the ingroup trees placed roots at the exactly the same (*cytb*) or very close (i.e., next branch: the control region) positions with that estimated from the combined data set; 3) no significant decreases in -ln value were detected between unconstrained and constrained trees by AU-test (Shimodaira 2002) using CONSEL ver. 0.20 (Shimodaira and Hasegawa 2001) (P = 0.182 for *cytb* and 0.265 for the control region).

Genetic diversity

Haplotype diversity, nucleotide diversity π, and other related statics were calculated by DnaSP ver. 5 (Librado and Rozas 2009). Three data sets, the mitochondrial control region, *cytb*, and combined data were separately used. Genetic diversity was calculated for 8 regional groups: *S. yukonicus* in Alaska (1–15), *S. minutissimus* from Kamchatka to Sakha (16–26), *S. minutissimus* in Primorye (27), *S. minutissimus* in Sakhalin (28–29), *S. minutissimus* in Hokkaido (30–38), *S. minutissimus* in Mongolia-Transbaikalia (39–46), *S. minutissimus* from Cisbaikalia to western Siberia (47–58), and *S. minutissimus* in Fennoscandia (59–71). Numbers in parentheses are location numbers (Fig. 1). Demarkations of these groups were arbitrarily determined. Thus, note that these regional groups do not always correspond with those of clustering group in phylogenetic trees.

Results

Control region

The ML analysis of the control region data sets yielded a single most likely tree (-ln = 1973.65; root fixed). Note that no samples from *S. yukonicus* in Alaska were included in this analysis because sequence data for the control region were not available for the species (Appendix).

The trees reconstructed by the ML and BY methods were fundamentally the same, except for topologies of terminal nodes. Therefore, the description of tree topology was based only on the ML tree (Fig. 3).

Shrews from Hokkaido were grouped in a clade showing a little genetic diversity with high confidence in ML, BY and MP methods (Fig. 3). Then, Hokkaido shrews clustered with those in Sakhalin with high confidence. All individuals from Primorye clustered together (Primorye Clade). Shrews from Central Siberia to Kamchatka Peninsula made a clade (Central Siberia-Kamchatka Clade) with relatively high confidence (Fig. 3). The shrews from Mongolia and Transbaikalia were not phylogenetically closed to Central Siberia-Kamchatka Clade (Fig. 3), although Mongolia-Transbaikalia is geographically close to Central Siberia-Kamchatka. Shrews

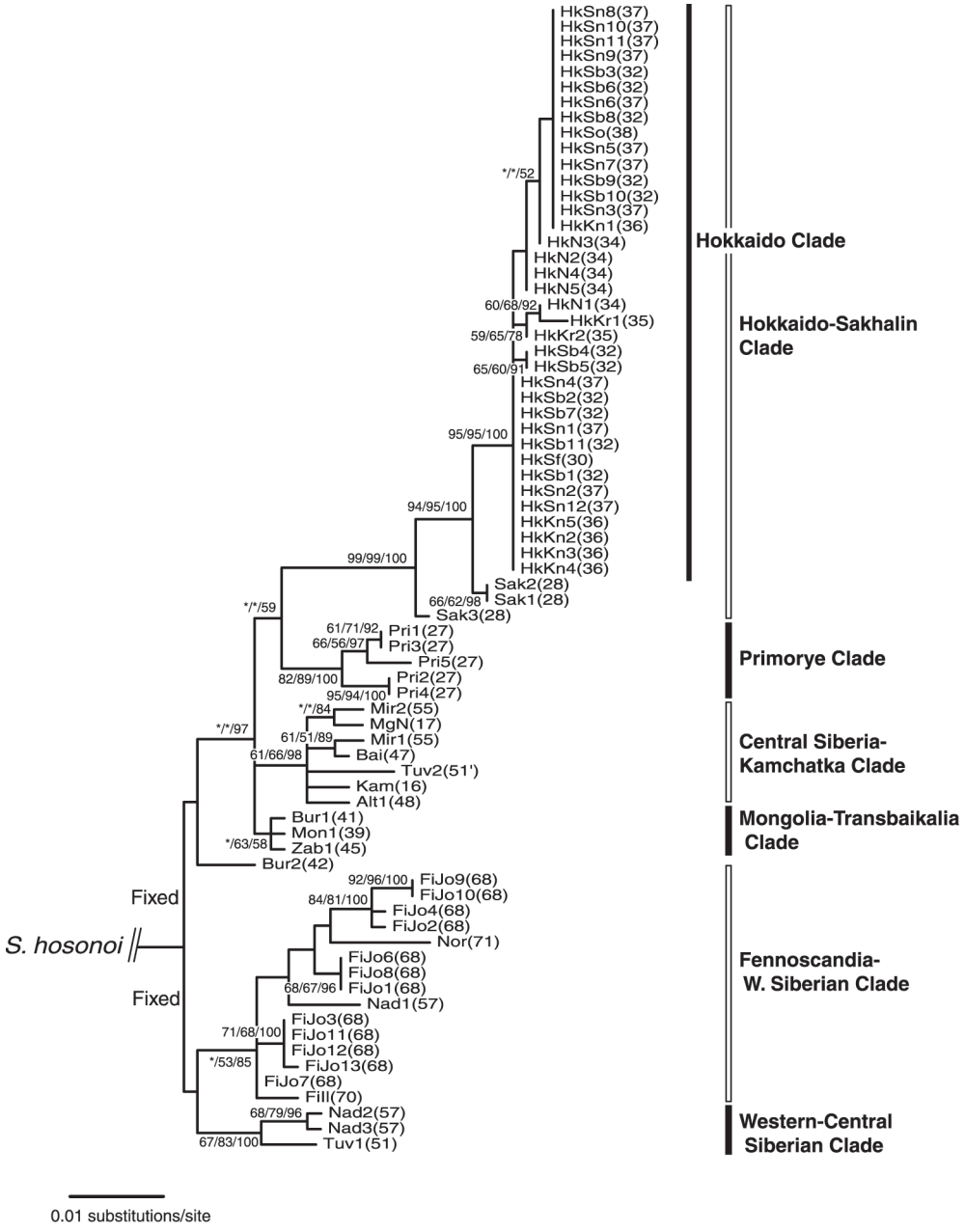

Fig. 3. Maximum likelihood tree of *Sorex minutissimus* based on the mitochondrial control region gene nucleotide sequences (751 bp including gaps). Numbers near nodes indicate bootstrap values (>50%) for the maximum likelihood analysis, the maximum parsimony analysis, and Baysian posterior probabilities, respectively. Bootstrap values were calculated by 100 replications. *Sorex hosonoi* is the outgroup and its root position was fixed expediently. See text for details for the fixation of rooting. See Appendix for the codes of OTUs. *: nodes with confidence less than 50%.

from Finland and Norway clustered in a clade, but the confidence of the ML method was less than 50% (Fig. 3). In this clade, one individual from northern western Siberia (Nad1: location #57) was also included (Fennoscandia-Western Siberia Clade). One individual from Tuva Republic (Tuv1; location #51) was closely posited with two western Siberian shrews (Nad2 and Nad3), while another one from Tuva Republic (Tuv2; location #51') was included in Central Siberia-Kamchatka Clade (Fig. 3).

The topology of the MJ network (Fig. 4) was basically congruent with that of the ML tree (Fig. 3). In the network, an individual from Transbaikalia (Zab1; location #45) was located in the center. Then, shrews from Mongolia and Buryatia Republic were connected to Zab1 (Fig. 4).

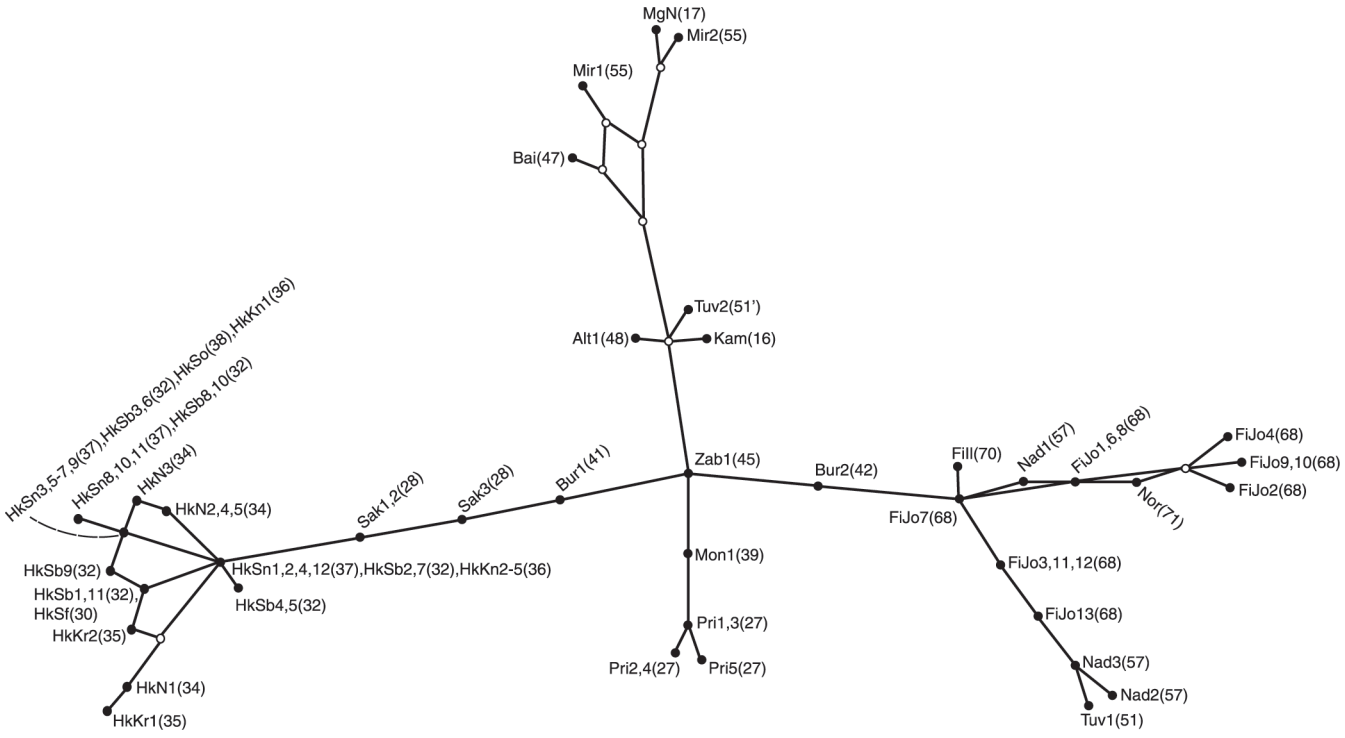

Fig. 4. Unrooted median joining network of *Sorex minutissimus* based on nucleotide sequences of the mitochondrial control region gene (723 bp including gaps). It was created by Spilts Tree 4 with "Ignore Gaps setting". Open circles denote hypothetical taxonomic units and solid circles substantial operational taxonomic units.

Cytochrome b gene

The ML analysis of *cytb* data set yielded 38 equally likely trees (–ln = 3269.74; root was fixed). However, they only differed in rearrangements of closely placed terminal branches and only one representative tree was shown (Fig. 5).

Shrews from Hokkaido and Sakhalin were clustered into one clade, (Hokkaido-Sakhalin Clade) and the shrews from Sakhalin were included in a clade of Hokkaido (Fig. 5). Monophyly of the individuals from Primorye was strongly supported (Primorye Clade). Then, Primorye Clade was clustered with Hokkaido-Sakhalin Clade with relatively high confidence (Maritime Northeast Asia Clade). Monophyly of *Sorex yukonicus* in Alaska was supported in the MP and BY analyses but less than 50% in the ML method (Fig. 5). Further, *S. yukonicus* made a clade with shrews from eastern Siberia to Kamchatka although the supporting value of the ML analysis was less than 50% (Eastern Siberia-Alaska Clade). Monophyly of Fennoscandian shrews was supported only by the BY analysis. Finnish shrews were divided into at least two groups (southeastern and northeastern parts of Finland). Some individuals from western and central Siberia (Nov, Tom, and Nad1) were located in Southeastern Finland Clade (Fig. 5). In addition, one individual (FiEn; location #66) in southeastern Finland was included in Central-Northern Finland-Northern Norway Clade.

The branching patterns of the MJ network based on *cytb* (Fig. 6) are basically similar to those of the ML tree (Fig. 5). Unlike the ML tree, however, *Sorex yukonicus* was not closely situated with *S. minutissimus* from eastern Siberia and Kamchatka. *Sorex yukonicus* was rather closed to *S. minutissimus* from western Eurasia in the MJ network (Fig. 6).

The combined data

The ML analysis of combined *cytb* and the control region data sets yielded a single most likely tree (-ln = 4753.15). Note that no samples from *S. yukonicus* in Alaska were included in this analysis because sequence data for the control region were not available (Appendix), and that the root was not fixed in this analysis (Fig. 7).

When *S. hosonoi* is an outgroup, monophyly of Western Eurasia Clade, which consists of all Fennoscandian shrews, 2 shrews from western Siberia (Nad2 and Nad3) and 1 from central Siberia (Tuv1), was strongly supported (Fig. 7). However, monophyly of Eastern Eur-

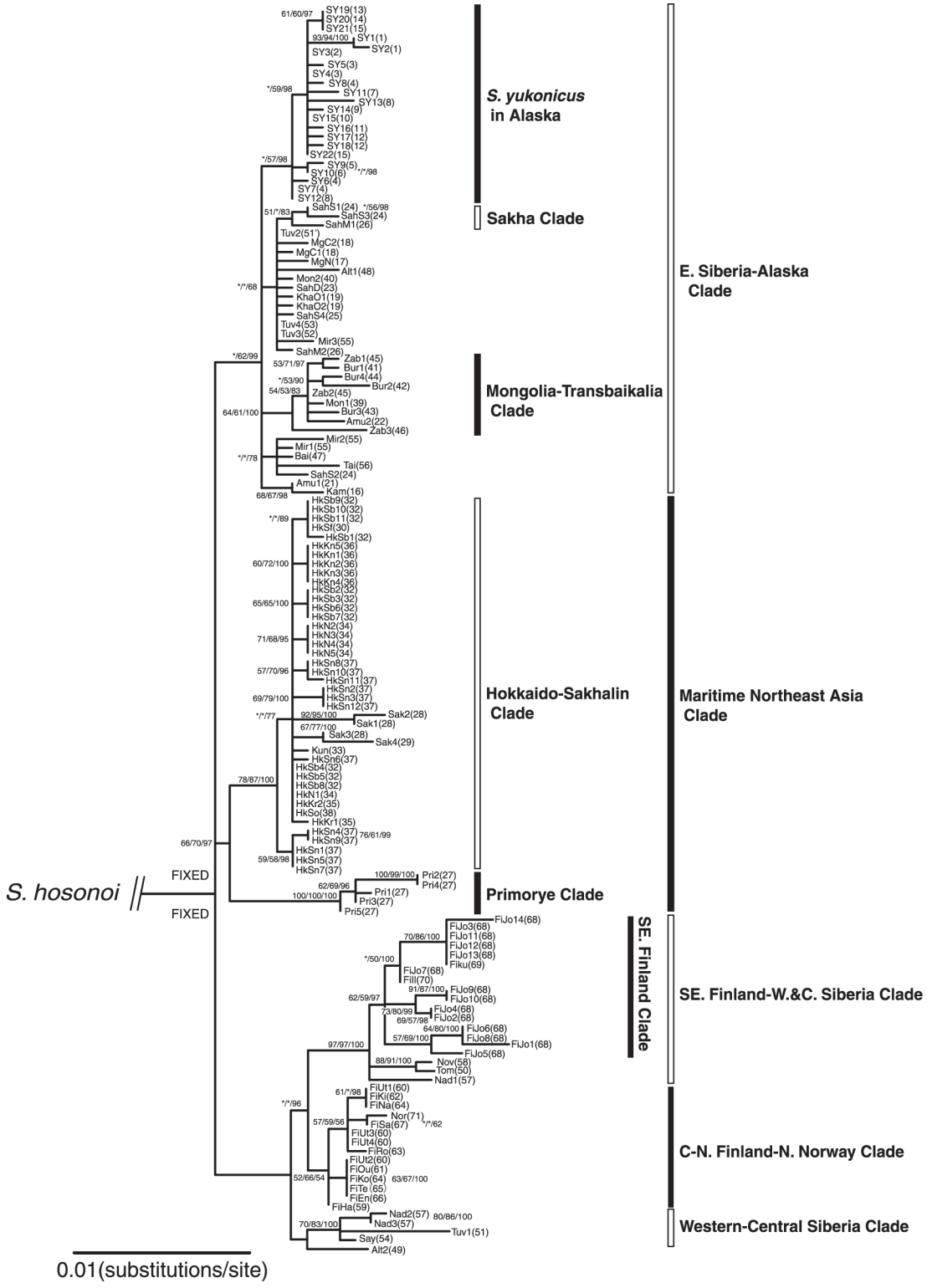

Fig. 5. One of the 34 ML (maximum likelihood) trees of *Sorex minutissimus* and *S. yukonicus* based on nucleotide sequences of the mitochondrial cytochrome *b* gene (960–1,140 bp). Numbers near nodes indicate bootstrap values (>50%) for the maximum likelihood analysis, the maximum parsimony analysis, and Baysian posterior probabilities, respectively. Bootstrap values were calculated by 100 replications. *Sorex hosonoi* is the outgroup and its root position was fixed expediently. See text for details for the fixation of rooting. There are only trivial differences in terminal branches among the 34 ML trees. *: nodes with confidence less than 50%.

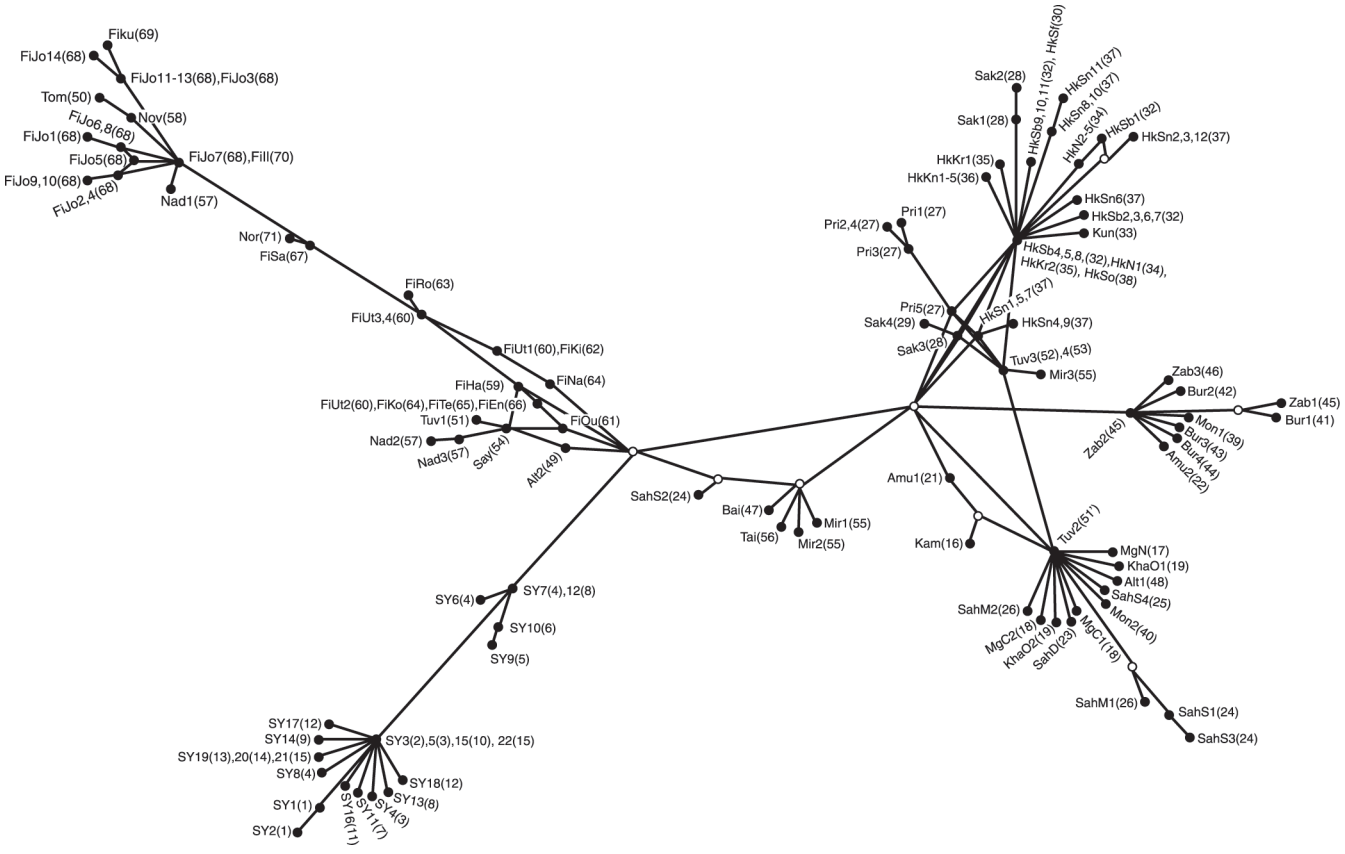

Fig. 6. Unrooted median joining network of *Sorex minutissimus* and *S. yukonicus* based on nucleotide sequences of the mitochondrial cytochrome *b* gene (960 bp). It was created by SpiltsTree 4. Open circles denote hypothetical taxonomic units and solid circles substantial operational taxonomic units. See Appendix for the codes of OTUs.

asia Clade was supported with low confidence (Fig. 7). Within Eastern Eurasia Clade, monophyly of Hokkaido-Sakhalin Clade and Primorye Clade was recognized without ambiguity. In addition, shrews from Sakhalin were paraphyletic (Fig. 7). Two individuals from Tuva Republic (location #51 and 51') were divided into Eastern Eurasia (Tuv2) and Western Eurasia (Tuv1) Clades although they captured from nearby sites (Fig. 1).

Genetic diversity

Nucleotide diversity (π) of *cytb* of the Alaskan population was rather low (Table 1). Nucleotide diversity of the shrews in Hokkaido (including Kunashiri and Kenbokki Islands) in all 3 data sets was lowest among the 8 regional groups. Shrews from Primorye also had small nucleotide diversity. Three regional groups (Kamchatka-Sakha, Sakhalin, and Mongolia-Transbaikalia) in eastern Eurasia tended to have medium nucleotide diversity (Table 1). In contrast, shrews from Cisbaikalia-western Siberia and Fennoscandia had high nucleotide diversity in all 3 data sets.

Discussion

Phylogenetic and taxonomic status of S. yukonicus

The phylogenetic tree based on the mitochondrial cytochrome *b* (*cytb*) gene supported a monophyly of *S. yukonicus* in Alaska in MP and BY methods (Fig. 5) as in Hope et al. (2010). Hope et al. (2010), then, showed based on *cytb* that *S. yukonicus* in Alaska was clustered with *S. minutissimus* from Siberia, not with those from Maritime Northeast Asia (Hokkaido, Sakhalin, and Primorye), and with *S. minutissimus* both from Siberia and Maritime Northeast Asia based on nuclear apolipoprotein B (*ApoB*). In the present study (Fig. 5), *S. yukonicus* in Alaska were clustered with eastern Siberian *S. minutissimus*.

The shrews in Alaska had low nucleotide diversity, considering sampling area (Table 1). This is probably caused by a bottleneck event during the transcontinental immigration from Eurasia to North America at the Last Glacial Maximum, as Hope et al. (2010) suggested. The MJ network (Fig. 5) also implies a single ancestor (haplotype) of the shrews in Alaska.

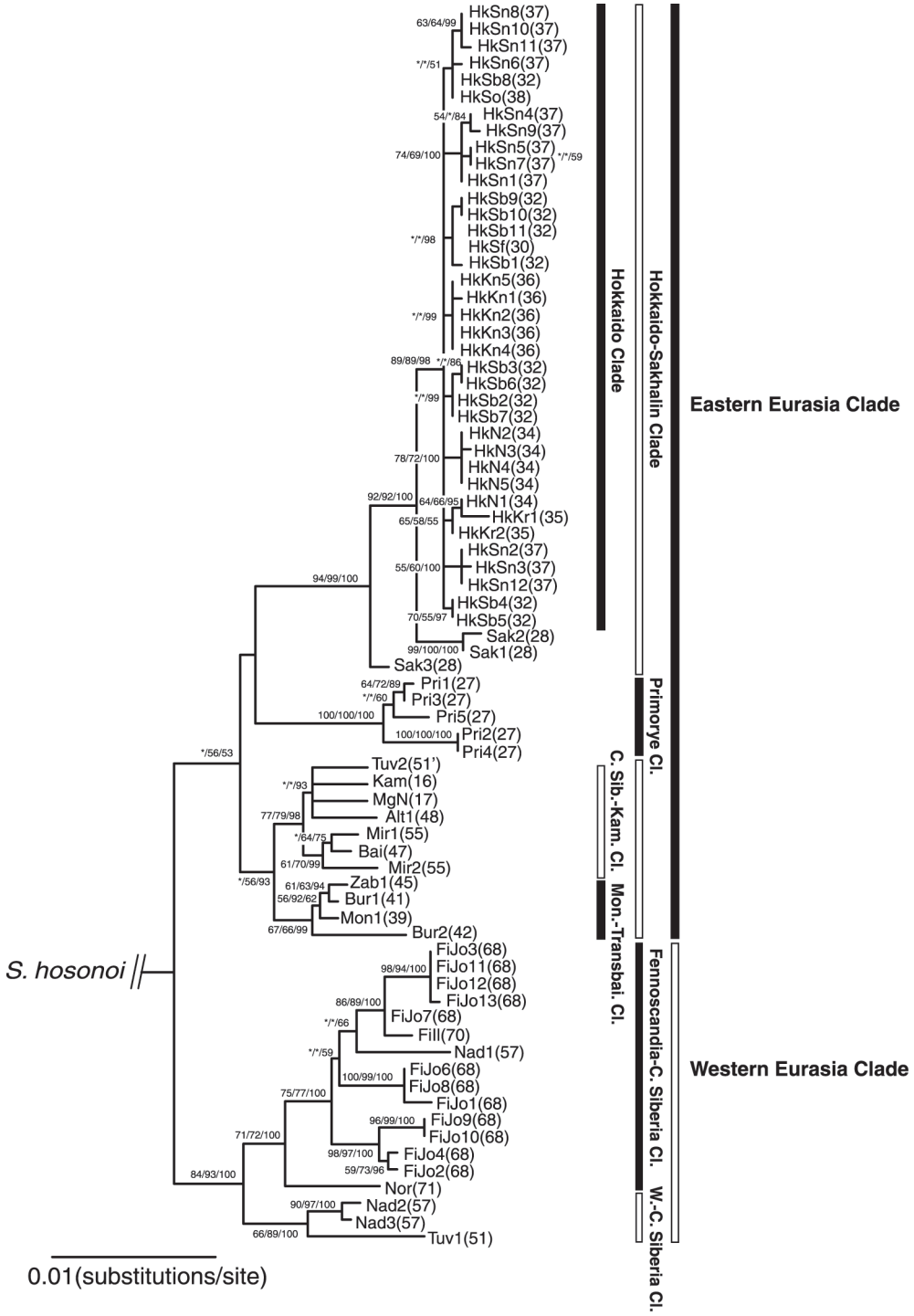

Fig. 7. Maximum likelihood tree of *Sorex minutissimus* based on combined nucleotide sequences (1,891 bp including gaps) of the mitochondrial cytochrome *b* and the control region genes. Numbers near nodes indicate bootstrap values (>50%) for the maximum likelihood analysis, the maximum parsimony analysis, and Baysian posterior probabilities, respectively. Bootstrap values were calculated by 100 replications. *Sorex hosonoi* is the outgroup (the position is not fixed). *: nodes with confidence less than 50%.

We support the taxonomic treatment for *S. yukonicus* by Hope et al. (2010); it should be a subspecies or a local population of *S. minutissimus* in Alaska. *Sorex yukonicus*, certainly, makes a monophyletic group and is phylogenetically closed to *S. minutissimus* in eastern Eurasia (Fig. 5; Hope et al. 2010). However, *S. minutissimus*

Table 1. Statics for genetic diversity of major regional groups of the *Sorex minutissimus-S. yukonicus* complex, based on sequences of the mitochondrial control region and cytochrome *b* gene

group (location numbers)	gene																	
	control region						*cytb*						combined data					
	n	*TS*	*S*	*h*	*Hd* ± *SD*	π ± *SD* (×10^{-3})	*n*	*TS*	*S*	*h*	*Hd* ± *SD*	π ± *SD* (×10^{-3})	*n*	*TS*	*S*	*h*	*Hd* ± *SD*	π ± *SD* (×10^{-3})
all individuals	74	717	77	40	0.96 ± 0.012	20.61 ± 1.18	140	676	75	68	0.970 ± 0.007	8.56 ± 0.38	74	1857	171	57	0.992 ± 0.004	14.27 ± 0.90
S. yukonicus in Alaska (1–15)	NA	NA	NA	NA	NA	NA	22	1140	20	16	0.957 ± 0.029	2.26 ± 0.34	NA	NA	NA	NA	NA	NA
S. minutissimus from Kamchatka to Sakha (16–26)	2	725	7	2	1.000 ± 0.500	9.66 ± 4.83	15	912	23	14	0.990 ± 0.028	4.20 ± 0.74	2	1865	12	2	1.000 ± 0.500	6.43 ± 3.22
S. minutissimus in Primorye (27)	5	731	10	3	0.800 ± 0.164	7.39 ± 1.67	5	1140	6	4	0.900 ± 0.161	2.81 ± 0.62	5	1871	16	4	0.900 ± 0.161	4.60 ± 1.01
S. minutissimus in Sakhalin (28–29)	3	729	7	2	0.667 ± 0.314	6.40 ± 3.02	4	1061	9	4	1.000 ± 0.177	5.03 ± 1.36	3	1869	15	3	1.000 ± 0.272	5.35 ± 2.21
S. minutissimus in Hokkaido (30–38)	37	727	9	11	0.853 ± 0.034	2.28 ± 0.32	38	1140	14	14	0.927 ± 0.017	1.87 ± 0.17	37	1867	22	25	0.976 ± 0.012	2.03 ± 0.13
S. minutissimus in Mongolia-Transbaikalia (39–46)	4	726	8	4	1.000 ± 0.177	5.97 ± 1.93	9	960	20	9	1.000 ± 0.052	5.15 ± 0.90	4	1866	16	4	1.000 ± 0.177	4.56 ± 1.46
S. minutissimus in Cisbaikalia and western Siberia (47–58)	9	718	39	9	1.000 ± 0.052	19.81 ± 2.06	17	960	38	14	0.971 ± 0.032	9.33 ± 1.11	9	1858	75	9	1.000 ± 0.052	14.32 ± 1.68
S. minustissimus in Fennoscandia (59–71)	14	724	25	9	0.923 ± 0.050	10.70 ± 1.12	30	725	16	11	0.906 ± 0.025	7.38 ± 0.32	14	1864	49	10	0.945 ± 0.045	7.72 ± 0.86

n = number of individuals; *TS* = total number of sites (excluding sites with gaps/missing data); *S* = segregating sites; *h* = number of haplotypes; *Hd* = haplotype diversity; π = nucleotide diversity. Location numbers in each group are indicated in parentheses; the numbers correspond with those in Fig. 1 and Appendix. *SD* = standar deviation. NA = not applicable. Note that the regional groups do not always corresponds with those of phylogenetic clusters.

in eastern Eurasia formed a monophyletic group different from western Eurasian group (Figs. 5 and 7; Hope et al. 2010). This means *S. minutissimus* is paraphyletic when *S. yukonicus* is regarded as an independent species. It is needed to make a final decision of the taxonomic status of *S. yukonicus* to compare genetic, karyological (Zima et al. 1998; Moribe 2011) and morphological (Dokuchaev 1997; Il'yashenko and Onishchenko 2003) features using more individuals from European part of Russia and Northwest China, and Chukotka (Andreev et al. 2006), the easternmost part of Eurasia, with the shrews from Alaska.

Shrews in Maritime Northeast Asia and Mongolia-Transbaikalia

In the present analysis of *cytb* (Fig. 5), monophyly of shrews from Maritime Northeast Asia (Hokkaido, Sakhalin, and Primorye) was supported by ML, MP, and BY methods, but not in the control region data set by ML and MP methods (Fig. 3) and the combined data set by the 3 methods (Fig. 7).

Shrews from Hokkaido and Sakhalin (= Karafuto) were included in a monophyletic group in the 3 data sets and shrews in Hokkaido and Sakhalin were genetically close to each other (Figs. 3, 5, and 7). In addition, the MJ networks (Figs. 4 and 6) demonstrated that individuals from Hokkaido and Sakhalin were clustered in vicinity. However, the phylogenetic positions of Sakhalin shrews are not straightforward; in the 3 data sets, Sakhalin shrews did not make a monophyletic group and are paraphyletic (Figs. 3, 5, and 7). Based on the data sets of the control region and the combined sequence (Figs. 3 and 7), it is the parsimonious interpretation that Hokkaido shrews were derived from Sakhalin population. However, additional samples from Sakhalin are needed to estimate the more exact evolutionary relationship between the populations in Hokkaido and Sakhalin.

In the present study, shrews from Transbaikalia (including one shrew in northwestern Amur region) to Mongolia (except for one shrew from central Mongolia; location #40) made a monophyletic group except for ML method of the control region (Figs. 3, 5, and 7). Although this region borders on central Siberia and on eastern Siberia (Fig. 1), the phylogenetic positions of the shrews from the latter two regions did not always closed to the clade in Mongolia-Transbaikalia (Fig. 3, 5, and 7). In addition, the MJ network of *cytb* (Fig. 6) implies that shrews of Mongolia-Transbaikalia Clade might have recently derived from a single ancestor.

Refugia and movement of shrews in Fennoscandia and western-central Siberia

Monophyly of Fennoscandian shrews was basically supported in the 3 data sets (Figs. 3, 5, and 7). Hope et al. (2010) certified the monophyly of European shrews based on *cytb* and *ApoB* genes. Furthermore, the MJ networks based on the control region (Fig. 4) and *cytb* (Fig. 6) showed that Fennoscandian shrews were grouped together with some shrews from central and western Siberia.

In the present study, *S. minutissimus* from western Eurasia was monophyletic (Western Eurasia Clade) with high confidence in the combined data set (Fig. 7), and monophyly of *S. minutissimus* from eastern Eurasia was fundamentally supported by MP and BY methods (Eastern Eurasia Clade). However, 4 individuals (Nad1-3 and Tuv1) in Western Eurasia Clade were from western and central Siberia (Fig. 7).

Two shrews from Tuva Republic (Tuv1 and Tuv2, location #51 and 51') were located very distantly in the phylogenetic trees (Figs. 3, 5, and 7); one was included in Eastern Eurasia Clade whereas the other was in Western Eurasia Clade (Fig. 7). Likewise, one individual from Nadym (Nad1, location #57) in northwestern Siberia was included in a clade with shrews in southeastern Finland although the other two (Nad2 and Nad3) made a clade with those from western to central Siberia (Figs. 3, 5, and 7). Likewise, two shrews (Alt1 and Alt2) from Altai region (location #49) were phylogenetically distantly located based on *cytb* (Fig. 5). These facts imply that some shrews in western-central Siberia and Finland moved in a long-distance after ice sheets began to retreat. Most individuals from southeastern Finland were included in a clade (Southeastern Finland Clade) but one individual of southeastern Finland (FiEn; location #66) was grouped with those from central-northern Finland (Central-northern Finland and Northern Norway Clade), based on *cytb* gene (Fig. 5). Besides, the shrews from the west of Baikal Lake and Fennoscandia had higher genetic diversity than those from regions of the eastern Eurasia and Alaska (Table 1). The high diversity in the west of Baikal Lake is attributed to the co-existence of individuals from several genetically distant clades, while that in Fennoscandia is to high diversification among individuals there (Figs. 3, 5 and 7).

In the Last Glacial Maximum, northern Europe and northern North America (except northwestern Alaska) were covered with huge ice sheets whereas Northeast Asia and Alaska were not (CLIMAP Project Members

1976; Grosswald and Hughes 1995; Mangerud et al. 2004; Peltier 2004; Clark et al. 2009). Thus, many small land mammals in Europe and North America were once extinct and retreated into various refugia in the last glacial age and recolonised after warming (Blondel and Vigne 1993; Runck and Cook 2005; Schmitt 2007; Lomolino et al. 2010). Meanwhile, there existed a land bridge in Bering Sea, and terrestrial animals could immigrate between Eurasia and North America (Repenning 1967;Vangengeim 1967).

In the case of *S. minutissimus*, following biogeographic scenario can be considered. At the Last Glacial Maximum, shrews in Fennoscandia and northern Siberia might have retreated to the southern or eastern regions where ice sheets were not covered, and they were genetically diverged there during the retreat. After the Last Glacial Maximum, *S. minutissimus* reimmigrated from somewhere in southern areas into Fennoscandia and northwestern Siberia (e.g., Nadym, location #57). The refugia of the shrews in central-northern Finland and Norway seem to be different from those of southeastern Finland because shrews of these regions were divided into two clades (Fig. 5). Some shrews in western and central Siberia were sometimes phylogenetically distantly located even when they were collected from the same or nearby sites, as observed in Alt1 vs. Alt2, Tuv1 vs. Tuv2, and Nad1 vs. Nad2&3 (Figs. 3, 5, and 7). In addition, several individuals from western Siberia (Nov. Tom, and Nad1) were included in Southeastern Finland Clade (Fig. 5). Thus, shrews from central Siberia to Fennoscandia might have moved from several refugia in southern and/or eastern regions.

Hope et al. (2010) inferred that some refugia existed in Carpathian Black Sea or Caucasian region, based on the ecological niche modeling. Moreover, Ukkonen (2001) suggested that there might have been some patchy refugia for small mammals in Fennoscandia even at the Last Glacial Maximum, most part of which was covered with continental ice sheets. In addition, fossils of *S. minutissimus* in the Late Pleistocene were found from Austria, France, Germany, Poland, and Slovakia (Rzebik-Kowalska 1998). Thus, *S. minutissimus* probably stayed in many patchy refugia in Fennoscandia and the central part of Europe. Small body size of *S. minutissimus* could have maintained populations in such small patchy "poor" habitats (Sheftel and Hanski 2002; Sheftel 2005).

Genetic status of the shrews of European part of Russia and the isolated population of *S. minutissimus* in southern Norway (Mitchell-Jones et al. 1992) has to be analysed to infer the recolonisation route to Fennoscandia. Further, exact dating and morphological reexamination of fossils of *S. minutissimus* excaved from Central Europe (Rzebik-Kowalska 1998) and surveys of fossils in Black Sea-Caucasian region at the Last Glacial Maximum will help to estimate to specify the locations of refugia.

Comparison of phylogeography with S. caecutiens and S. tundrensis

The *S. minutissimus*-*S. hosonoi* group showed a distributional pattern similar to the *S. caecutiens*-*shinto* group. *Sorex caecutiens* Laxmann, 1788 is a Palearctic species and ranges widely from Chukotka, via Kamchatka, Korea, Hokkaido, Sakhalin and Siberia, to Norway, and its sister species, *S. shinto* Thomas, 1905 occurs in mountainous regions in Hondo (Honshu, Shikoku, and Sado Islands) of Japan (Ohdachi et al. 2001, 2003, 2010). *Sorex minutissimus* ranges throughout northern Eurasia, from Chukotka to Norway (and in Alaska if *S. yukonicus* is included in *S. minutissimus*) whereas its sister species, *S. hosonoi*, Imaizumi 1954 is restricted to higher regions of central Honshu, Japan (Hope et al. 2010; Ohdachi et al. 2010), although *S. hosonoi* was distributed in wider area of Honshu in the Middle Pleistocene (Dokuchaev et al. 2010).

Sorex caecutiens has small genetic variation based on *cytb* sequence from Kamchatka, via Sakhalin, North Korea and Siberia, to Finland, and the population in Hokkaido was first branched in a deep position of the phylogenetic tree (Ohdachi et al. 2001, 2003; Ohdachi 2008). *Sorex caecutiens* from the Eurasian Continent (including Sakhalin) were included in a monophyletic group with ambiguous regional diversification, and there was a deep phylogenetic gap between shrews from Hokkaido and Sakhalin. In contrast, *S. minutissimus* (and *S. yukonicus*) showed several well-diverged phylogroups in Eurasia and Alaska (Figs. 3–7; Hope et al. 2010); especially, at least eastern and western populations are definitely separated. Further, *S. minutissimus* from Hokkaido and Sakhalin are genetically similar to each other (Figs. 3, 5, and 7).

Therefore, it could be presumed that ancestors of *S. minutissimus* fled to various refugia in the southern part of Europe (and maybe in patchy small refugia in Fennoscandia) and eastern Eurasia at the Last Glacial Maximum, whereas *S. caecutiens* retreated to the southern part of continental East Asia and Hokkaido (Ohdachi 2008). When ice sheets began to shrink after the Last

Glacial Maximum, *S. minutissimus* re-colonised into Fennoscandia-northern Siberia from the various refugia while *S. caecutiens* spread throughout the Eurasian Continent from East Asia in a short time. In addition, it is inferred that *S. minutissimus* (= *S. yukonicus*) immigrated from eastern Siberia to Alaska around the Last Glacial Maximum when the Bering Strait was closed.

Sorex tundrensis Merriam, 1900 also has a Holarctic distribution similar to that of the *S. minutissimus*-*S. yukonicus* complex, although *S. tundrensis* does not occur in western Russia and Fennoscandia (Bannikova et al. 2010; Hope et al. 2011). *Sorex tundrensis* in Alaska is monophyletic (Bannikova et al. 2010; Hope et al. 2011), as in *S. yukonicus* in Alaska (Hope et al. 2010; Fig. 5). However, unlike *S. yukonicus* in Alaska, which is clustered with *S. mimutissimus* in eastern Eurasia (Hope et al. 2010; MP and BY methods in Fig. 5), the close connection of *S. tundrensis* in Alaska with those in eastern Eurasia was not significantly supported (Bannikova et al. 2010; Hope et al. 2011). This suggests that divergence period between Eurasian and Alaskan populations should be different between the *S. minutissimus*-*S. yukonicus* complex and *S. tundrensis*, but further comparison using more genetic information is needed to reveal the difference in divergence time between the two shrews.

Shortly summarized, although the *S. minutissimus*-*S. hosonoi* group has a similar geographical range to the *S. caecutiens*-*S. shinto* group and *S. tundrensis*, biogeographic history seems to be quite different among them. For further understanding of the process of community formation of the soricine shrew in Eurasia, it is needed to compare phylogeography of other transcontinental species in Eurasia, such as *Sorex isodon* Turov, 1924, *S. daphaenodon* Thomas, 1907, and *Neomys fodiens* (Pennant, 1771).

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Appendix

Codes, locations of capture and DNA data base accession numbers of specimens examined. Numbers of location correspond with those of Fig. 1. Samples with "SY" code series is for *Sorex yukonicus*. Other specimens is of *Sorex minutissimus*, except for the outgroup (*S. hosonoi*). Sequences with asterisks (*) are those of cited from DNA data bases.

Code	Loc. #	Location	Acc. # for cytb	Acc. # for the control region from forward sequencing	Acc. # for the control region from reverse sequencing
SY1 (1)	1	Goodnews River, Alaska, USA	GU223640*	–	–
SY2 (1)	1	ditto	GU223642*	–	–
SY3 (2)	2	Serpentine Hot Springs, Alaska, USA	GU223647*	–	–
SY4 (3)	3	Devil Mountain Lakes, Alaska, USA	GU223644*	–	–
SY5 (3)	3	ditto	GU223645*	–	–
SY6 (4)	4	near Kathul Mountain, Alaska, USA	GU223655*	–	–
SY7 (4)	4	ditto	GU223652*	–	–
SY8 (4)	4	ditto	GU223653*	–	–
SY9 (5)	5	Carden Hills, Alaska, USA	GU223657*	–	–
SY10 (6)	6	Glenn Creek Cabin, Alaska, USA	GU223650*	–	–
SY11 (7)	7	Kandik Cabin, Alaska, USA	GU223651*	–	–
SY12 (8)	8	Yukon River from Glenn Creek Cabin, Alaska, USA	GU223648*	–	–
SY13 (8)	8	ditto	GU223649*	–	–
SY14 (9)	9	Chilchukabena Lake,Alaska, USA	GU223658*	–	–
SY15 (10)	10	near Red Devil, Alaska, USA	GU223643*	–	–
SY16 (11)	11	Fortress Mountain, Alaska, USA	GU223659*	–	–
SY17 (12)	12	Chulitna River and Turner Bay confluence, Alaska, USA	GU223660*	–	–
SY18 (12)	12	ditto	GU223661*	–	–
SY19 (13)	13	Baird Mountains, Salmon River, Alaska, USA	GU223685*	–	–
SY20 (14)	14	Baird Mountains, headwaters of Akillik River, Alaska, USA	GU223664*	–	–
SY21 (15)	15	Kakagrak Hills, Alaska, USA	HM002707*	–	–
SY22 (15)	15	ditto	GU223662*	–	–
Kam (16)	16	Elizovo, Kamchatka, Russia	AB028586*	AB668152	AB668229
MgN (17)	17	Nyuklya, Magadan, Russia	AB028585*	AB668153	AB668230
MgC1 (18)	18	Chelomdza River, Magadan, Russia	AB028584*	–	–
MgC2 (18)	18	ditto	AB028587*	–	–
KhaO1 (19)	19	Okhotsk, Khabarovskii Krai, Russia	GU223632*	–	–
KhaO2 (19)	19	ditto	GU223633*	–	–
Amu1 (21)	21	Svobodnyi, Amurskaya Oblast, Russia	AB668079	–	–
Amu2 (22)	22	Zeya, Amurskaya Oblast Russia	HM002679*	–	–
SahD (23)	23	Delyankir River, Sakha Republic, Russia	GU223631*	–	–
SahS1 (24)	24	near Sulgachi, Sakha Republic, Russia	HM002705*	–	–
SahS2 (24)	24	ditto	GU223634*	–	–
SahS3 (24)	24	ditto	GU223635*	–	–
SahS4 (25)	25	ditto	HM002694*	–	–
SahM1 (26)	26	near Mikhaylovka, Sakha Republic, Russia	GU223637*	–	–
SahM2 (26)	26	ditto	GU223636*	–	–
Pri1 (27)	27	Kedrovaya Pad' Reserve, Primorskii Krai, Russia	AB668080	AB668154	AB668231
Pri2 (27)	27	ditto	AB668081	AB668155	AB668232
Pri3 (27)	27	ditto	AB668082	AB668156	AB668233
Pri4 (27)	27	ditto	AB175129*	AB668157	AB668234
Pri5 (27)	27	ditto	AB175130*	AB668158	AB668235
Sak1 (28)	28	Sokol, Sakhalin, Russia	AB668083	AB668159	AB668236
Sak2 (28)	28	ditto	AB668084	AB668160	AB668237

Code	Loc. #	Location	Acc. # for cytb	Acc. # for the control region from forward sequencing	Acc. # for the control region from reverse sequencing
Sak3 (28)	28	ditto	AB668085	AB668161	AB668238
Sak4 (29)	29	Novoaleksandrovskaya, Sakhalin, Russia	HM002701*	–	–
HkSf (30)	30	Sarufutsu, Hokkaido, Japan	AB668086	AB668162	AB668239
HkSb1 (32)	32	Sarobetsu moor, Horonobe, Hokkaido, Japan	AB028591*	AB668163	AB668240
HkSb2 (32)	32	ditto	AB028590*	AB668164	AB668241
HkSb3 (32)	32	ditto	AB668088	AB668165	AB668242
HkSb4 (32)	32	ditto	AB668089	AB668166	AB668243
HkSb5 (32)	32	ditto	AB668090	AB668167	AB668244
HkSb6 (32)	32	ditto	AB668091	AB668168	AB668245
HkSb7 (32)	32	ditto	AB668092	AB668169	AB668246
HkSb8 (32)	32	ditto	AB668093	AB668170	AB668247
HkSb9 (32)	32	ditto	AB668094	AB668171	AB668248
HkSb10 (32)	32	ditto	AB668095	AB668172	AB668249
HkSb11 (32)	32	ditto	AB668096	AB668173	AB668250
Kun (33)	33	Kunashiri Is., the Chishima (Kuril) Islands	AB028589*	–	–
HkN1 (34)	34	Nemuro, Hokkaido, Japan	AB668097	AB668174	AB668251
HkN2 (34)	34	ditto	AB668098	AB668175	AB668252
HkN3 (34)	34	ditto	AB668099	AB668176	AB668253
HkN4 (34)	34	ditto	AB668100	AB668177	AB668254
HkN5 (34)	34	ditto	AB668101	AB668178	AB668255
HkKr1 (35)	35	Kiritapp moor, Hamanaka, Hokkaido, Japan	AB668102	AB668179	AB668256
HkKr2 (35)	35	ditto	AB668103	AB668180	AB668257
HkKn1 (36)	36	Kenbokki Is., Hokkaido, Japan	AB668104	AB668181	AB668258
HkKn2 (36)	36	ditto	AB668105	AB668182	AB668259
HkKn3 (36)	36	ditto	AB668106	AB668183	AB668260
HkKn4 (36)	36	ditto	AB668107	AB668184	AB668261
HkKn5 (36)	36	ditto	AB668108	AB668185	AB668262
HkSn1 (37)	37	Shiranuka, Hokkaido, Japan	AB668109	AB668186	AB668263
HkSn2 (37)	37	ditto	AB668110	AB668187	AB668264
HkSn3 (37)	37	ditto	AB668111	AB668188	AB668265
HkSn4 (37)	37	ditto	AB668112	AB668189	AB668266
HkSn5 (37)	37	ditto	AB668113	AB668190	AB668267
HkSn6 (37)	37	ditto	AB668114	AB668191	AB668268
HkSn7 (37)	37	ditto	AB668115	AB668192	AB668269
HkSn8 (37)	37	ditto	AB668116	AB668193	AB668270
HkSn9 (37)	37	ditto	AB668117	AB668194	AB668271
HkSn10 (37)	37	ditto	AB668118	AB668195	AB668272
HkSn11 (37)	37	ditto	AB668119	AB668196	AB668273
HkSn12 (37)	37	ditto	AB668120	AB668197	AB668274
HkSo (38)	38	Shikaoi, Hokkaido, Japan	AB668121	AB668198	AB668275
Mon1 (39)	39	Sangstai (upper region of Eroo river), Khentey Mts., Mongolia	AB668122	AB668199	AB668276
Mon2 (40)	40	Hangal district, Bulgan province, Mongolia	GU223629*	–	–
Bur1 (41)	41	Indola valley, Buryatia Republic, Russia	AB668123	AB668200	AB668277
Bur2 (42)	42	Shiringa, Buryatia Republic, Russia	AB668124	AB668201	AB668278
Bur3 (43)	43	Muhurshibir, Buryatia Republic, Russia	GU223626*	–	–
Bur4 (44)	44	NE of Baikal Lake, Buryatia Republic, Russia	HM002689*	–	–
Zab1 (45)	45	Chita, Zabaykalskii Krai Russia	AB668125	AB668202	AB668279
Zab2 (45)	45	ditto	AB668126	–	–
Zab3 (46)	46	Sochondinskii Reserve, Zabaykalskii Krai, Russia	HM002685*	–	–

Code	Loc. #	Location	Acc. # for cytb	Acc. # for the control region from forward sequencing	Acc. # for the control region from reverse sequencing
Bai (47)	47	Olkhon Is. on Baikal Lake, Irkutsk Oblast, Russia	AB668127	AB668203	AB668280
Alt1 (48)	48	Artybash, Teletskoe Lake, Altai Republic, Russia	AB668128	AB668204	AB668281
Alt2 (49)	49	Cherga, Altai Republic, Russia	HM002699*	–	–
Tom (50)	50	Kozhevnikovaya, Tomskaya Oblast, Russia	HM002684*	–	–
Tuv1 (51)	51	Hindiktig-Khol' Lake, Tuva Republic, Russia	AB668129	AB668205	AB668282
Tuv2 (51')	51'	Talaity River, Tuva Republic, Russia	AB668130	AB668206	AB668283
Tuv3 (52)	52	Shurmakaya, Totginskii district, Tuva Republic, Russia	HM002683*	–	–
Tuv4 (53)	53	Azas Reserve, Totginskii district, Tuva Republic, Russia	HM002686*	–	–
Say (54)	54	Abaza, Krasnoyarskii Krai, Russia	HM002700*	–	–
Mir1 (55)	55	Mirnoye station, Turukhansk district, Krasnoyarskii Krai, Russia	AB668131	AB668207	AB668284
Mir2 (55)	55	ditto	AB668132	AB668208	AB668285
Mir3 (55)	55	ditto	HM002687*	–	–
Tai (56)	56	Taimyr, Krasnoyarskii Krai, Russia	GU223638*	–	–
Nad1 (57)	57	Nadym, Yamalo-Nenets Autonomous Okrug, Russia	AB668133	AB668209	AB668286
Nad2 (57)	57	ditto	AB668134	AB668210	AB668287
Nad3 (57)	57	ditto	AB668135	AB668211	AB668288
Nov (58)	58	Karasukskii, Novosibirskaya Oblast, Russia	GU223630*	–	–
FiHa (59)	59	Mieluskylä, Haapavesi, Finland	HM002675*	–	–
FiUt1 (60)	60	Pälli, Utajärvi, Finland	HM002664*	–	–
FiUt2 (60)	60	Pälli, Utajärvi, Finland	HM002665*	–	–
FiUt3 (60)	60	Pälli, Utajärvi, Finland	HM002666*	–	–
FiUt4 (60)	60	Pälli, Utajärvi, Finland	HM002667*	–	–
FiOu (61)	61	Kaukovainio, Oulu, Finland	HM002668*	–	–
FiKi (62)	62	Kiiminki, Finland	HM002669*	–	–
FiRo (63)	63	Kaihua, Rovaniemi, Finland	HM002670*	–	–
FiKo (64)	64	Korkiakangas, Kuivaniemi, Finland	HM002671*	–	–
FiNa (64)	64	Näsiö, Kuivaniemi, Finland	HM002672*	–	–
FiTe (65)	65	Karsikkokangas,Tervola, Finland	HM002674*	–	–
FiEn (66)	66	Enonkoski, Finland	HM002676*	–	–
FiSa (67)	67	Salla, Finland	GU223627*	–	–
FiJo1 (68)	68	Lake Sysmä, Joroinen, Finland	AB668136	AB668212	AB668289
FiJo2 (68)	68	ditto	AB668137	AB668213	AB668290
FiJo3 (68)	68	ditto	AB668139	AB668215	AB668292
FiJo4 (68)	68	ditto	AB668140	AB668216	AB668293
FiJo5 (68)	68	ditto	AB668141	AB668217	AB668294
FiJo6 (68)	68	ditto	AB668142	AB668218	AB668295
FiJo7 (68)	68	ditto	AB668143	AB668219	AB668296
FiJo8 (68)	68	ditto	AB668144	AB668220	AB668297
FiJo9 (68)	68	ditto	AB668145	AB668221	AB668298
FiJo10 (68)	68	ditto	AB668146	AB668222	AB668299
FiJo11 (68)	68	ditto	AB668147	AB668223	AB668300
FiJo12 (68)	68	ditto	AB668148	AB668224	AB668301
FiJo13 (68)	68	ditto	AB668149	AB668225	AB668302
FiJo14 (68)	68	ditto	AB668138	AB668214	AB668291
FiKu (69)	69	Kuhmo, Finland	HM002693*	–	–
FiIl (70)	70	Koitere lake, Ilomantsi, Finland	AB668150	AB668226	AB668303
Nor (71)	71	Dividalen, Troms county, Norway	AB668151	AB668227	AB668304
S. hosonoi (outgroup)		Shiojidaira, Nagano Pref., Japan	AB028592*	AB668228	AB668305

Location numbers 20 and 31 are missing after arranging data.