Phylogeny of Eurasian Soricine Shrews (Insectivora, Mammalia) Inferred from the Mitochondrial Cytochrome b Gene Sequences

Authors: Ohdachi, Satoshi, Masuda, Ryuichi, Abe, Hisashi, Adachi, Jun, Dokuchaev, Nikolai E., et al.

Source: Zoological Science, 14(3) : 527-532

Published By: Zoological Society of Japan

URL: https://doi.org/10.2108/zsj.14.527

ZOOLOGICAL SCIENCE **14**: 527–532 (1997)

Phylogeny of Eurasian Soricine Shrews (Insectivora, Mammalia) Inferred from the Mitochondrial Cytochrome *b* Gene Sequences

Satoshi Ohdachi[1]*, Ryuichi Masuda[2], Hisashi Abe[3], Jun Adachi[4,7], Nikolai E. Dokuchaev[5], Voitto Haukisalmi[6] and Michihiro C. Yoshida[2]

[1]*Institute of Low Temperature Science, Hokkaido University, Sapporo 060, Japan*
[2]*Chromosome Research Unit, Faculty of Science, Hokkaido University, Sapporo 060, Japan*
[3]*Laboratory of Applied Zoology, Faculty of Agriculture, Hokkaido University, Sapporo 060, Japan*
[4]*Institute of Statistical Mathematics, Minato-ku, Tokyo 106, Japan*
[5]*Institute of Biological Problems of the North, Magadan, Russia*
[6]*Department of Ecology and Systematics, University of Helsinki, Helsinki, Finland*

ABSTRACT—Phylogenetic relationships among 31 operational taxonomic units of shrews (Soricidae, Mammalia), mainly from eastern Eurasia, were inferred from partial nucleotide sequences of the mitochondrial cytochrome *b* gene by maximum likelihood (ML) and neighbor joining (NJ) methods. Eleven monophyletic groups were recognized among the soricine shrews examined in the ML tree. However, branching orders of the groups were obscure judging from the local bootstrap values, and two out of the 11 groups were not monophyletic in the NJ tree. The phylogenetic relationships among *Sorex caecutiens*, *S. shinto*, and *S. sadonis* in the Japanese and Sakhalin islands, whose taxonomy was controversial, were clarified. *S. shinto* in the Honshu and Shikoku Islands is genetically differentiated enough to be considered a separate species from *S. caecutiens*, while *S. sadonis* could be treated as a subspecies of *S. shinto*. Some other taxonomic problems are also discussed.

INTRODUCTION

The modern Soricidae (Insectivora, Mammalia) is divided into two subfamilies, the Soricinae, red-toothed shrews, and the Crocidurinae, white-toothed shrews (Repenning, 1967). Some authors recognize a third subfamily, the Scutisoricinae (armored shrew), as being independent from the Crocidurinae (Simpson, 1945). Soricine shrews are distributed throughout the Holarctic region, consisting of approximately 110 species, while the crocidurines are diversified mainly in Africa and southern Asia (MacDonald, 1984; Abe, 1985; Corbet and Hill, 1986; Churchfield, 1990; Hutterer, 1993). In the northeastern Asiatic region, including eastern Siberia, up to 10 soricine species co-exist, with the highest species diversity in Eurasia (Dolgov, 1985). Therefore, northeastern Asia is thought to be a key region in which to investigate the evolution of soricine shrews.

It is quite difficult to reconstruct the speciation process of shrews, because their fossil record is poor owing to minute and fragile skeletons (but see, Repenning, 1967; Savage and Russell, 1983; Reumer, 1987) and because their morphology is similar to each other (Churchfield, 1990). The recent development of molecular phylogenetics has made it relatively easy to estimate phylogenetic relationships among modern stocks, even if they have very similar morphologies. Phylogenetic relationships of some Nearctic and Palaearctic soricine species have been investigated by biochemical techniques (George, 1988; George and Sarich, 1994) and mitochondrial DNA (Stewart and Baker, 1994; Taberlet *et al.*, 1994). However, phylogenetic relationships based on molecular data among most Eurasian species remain unresolved, although Dannelid (1991) proposed a hypothetical phylogeny of Eurasian *Sorex*, mainly based on karyotypes.

* Corresponding author: Tel. +81-11-706-5499; FAX. +81-11-706-7142.
[7] Present address: Department of Zoology, University of Oxford, Oxford OX1 3PS, UK.

Furthermore, the taxonomy of some Japanese shrews (*Sorex caecutiens*, *S. shinto*, and *S. sadonis*) is controversial, because of the difficulty of interpreting morphological data (Imaizumi, 1960; Abe, 1967; Yoshiyuki and Imaizumi, 1986).

The major objective of this paper is to estimate the phylogenetical relationships and to clarify the taxonomic status among species or populations of Eurasian soricine shrews, especially, those from northeastern Asia, where there is high species diversity. We determined partial nucleotide sequences of the mitochondrial cytochrome *b* gene, which is thought to be an appropriate marker to estimate phylogenetic relations at species or subspecies level (Zhang and Ryder, 1993; Cao *et al.*, 1994; Masuda and Yoshida, 1994a; Sturmbauer *et al.*, 1994; but see William *et al.*, 1995). Then, phylogeny of the shrews was estimated by using the maximum likelihood and the neighbor-joining methods.

MATERIALS AND METHODS

OTUs examined

Thirty one operational taxonomic units (OTUs) of shrews (Soricidae), a mole (*Mogera wogura*), and the rat (*Rattus norvegicus*) were used for phylogenetic analysis (Table 1). These OTUs include all of the soricine species in northeastern Asia. We basically followed Hutterer's (1993) nomenclature; however, *Sorex caecutiens* in Hokkaido ('Ezo-togarinezumi' shrew) and *Mogera wogura* ('Kobe-mogura' mole) were used (Abe, 1967, 1995), instead of *S. shinto* and *M. robusta* by Hutterer (1993), respectively. Tribe names followed those used by Repenning (1967).

Sequencing of DNA

The first 402-base region of the mitochondrial cytochrome *b* gene was sequenced. Total DNAs were extracted by the phenol/proteinase K/SDS method (Sambrook *et al.*, 1989) or by the Chelex® 100 method (procedure of 'Chelex DNA extraction from hair' in Walsh *et al.*, 1991). The DNA extracts (ca. 500 μl) from dried tissues of old samples (Table 1) using the first method were concentrated to ca. 45 μl with TE buffer (10 mM Tris: 1 mM EDTA, pH 7.0-7.5) using Centricon™-30 microconcentrator columns (Amicon). The 402-base cytochrome *b* region was sequenced using the PCR (polymerase chain reaction) product/direct sequencing technique by Kocher *et al.* (1989) with a modification of Masuda and Yoshida (1994a, b). A slight modification of the present study was that 0.5-2 μl bovine serum albumin (20 μg/μl) was added to a 50-μl PCR reaction mixture to eliminate reaction inhibitors (Cooper, 1994), when DNA extracts were from dried tissues by the phenol/proteinase K/SDS method. For the PCR amplification, the forward primers on the light strand were designed as 5'-GACCAATGATATGAAAAACCATCG-3' (L14721), 5'-GATATGAAAAACCATC-

Table 1. Abbreviations and sources of the OTUs examined

Abbr. of OTU	Scientific name	Sources: locality; collection date; tissue; original no. (deposit)
S.cae-Fin	*Sorex caecutiens*	Lapland, Finland; Jul-94; liver in ethanol; 365 (VH)
S.cae-Sib	*Sorex caecutiens*	Magadan State, Russia; Jun-95; muscle in ethanol; N26/95 (SO)
S.cae-Sak	*Sorex caecutiens*	Sakhalin Is., Russia; Aug-95; muscle in ethanol; 94sak4 (SO)
S.cae-Hok	*Sorex caecutiens*	Hokkaido Is., Japan; Jun-94; dried hindfoot; 94sc1 (SO)
S.shin-Hon	*Sorex shinto*	Nagano Pref., Honshu Is., Japan; Oct-92; dried muscle; 5800 (HA)
S.shin-Shik	*Sorex shinto*	Shikoku Is., Japan; Apr-59; dried hindfoot; 1211 (HA)
S.sadonis	*Sorex sadonis*	Sado Is., Japan; Nov-94; muscle in ethanol; 5961 (HA)
S.minutiss.	*Sorex minutissimus*	Hokkaido Is., Japan; Sep-94; muscle in ethanol; 94/9/16-1 (SO)
S.hosonoi	*Sorex hosonoi*	Nagano Pref., Honshu Is., Japan; Jul-59; dried skin; 1218 (HA)
S.roboratus	*Sorex roboratus*	Magadan State, Russia; Sep-66; dried hindfoot; 2814 (HA)
S.minutus	*Sorex minutus*	Lapland, Finland; Jul-94; liver in ethanol; 363 (VH)
S.ung-Sak	*Sorex unguiculatus*	Sakhalin Is., Russia; Aug-94; muscle in ethanol; 94sak1 (SO)
S.ung-Hok	*Sorex unguiculatus*	Hokkaido Is., Japan; Jun-94; liver in ethanol; 94su1 (SO)
S.isodon	*Sorex isodon*	Magadan State, Russia; Jul-95; muscle in ethanol; N36/95 (SO)
S.grac-Hok1, 2	*Sorex gracillimus*	Hokkaido Is., Japan; Jun-94; muscle in ethanol; 94sg1, 94/9/14-6 (SO)
S.grac-Sak	*Sorex gracillimus*	Sakhalin Is., Russia; Aug-94; muscle in ethanol; 94sak2 (SO)
S.daph-Sib	*Sorex daphaenodon*	Primorskiy Territory, Russia; Sep-66; dried hindfoot; 696 (HA)
S.daph-Sak	*Sorex daphaenodon*	Sakhalin Is., Russia; Aug-95; muscle in ethanol; 95/8/19-1 (SO)
S.tundrensis	*Sorex tundrensis*	Tyumen State, Russia; Aug-65; dried hindfoot; 2962 (HA)
S.araneus	*Sorex araneus*	Lapland, Finland; Jul-94; liver in ethanol; 361 (VH)
S.raddei	*Sorex raddei*	Caucasian Region, Russia; May-67; dried forefoot; 74 (HA)
S.mirabilis	*Sorex mirabilis*	Primorskiy Territory, Russia; Jun-91; liver in ethanol; 96misc-1 (SO)
S.cinereus	*Sorex cinereus*	Alberta Province, Canada; Jul-72; dried forefoot; none (HA)
S.fumeus	*Sorex fumeus*	Pennsylvania State, U.S.A.; Sep-95; liver in ethanol; PA110 (VH)
Ne.fodiens	*Neomys fodiens*	southern Finland; Aug-88; liver in ethanol; 587 (VH)
Ch.platycephala	*Chimarrogale platycephala*	Shizuoka Pref., Honshu Is., Japan; Jun-82; dried hindfoot; 6095 (HA)
So.nigrescens	*Soriculus nigrescens*	central Nepal; Sep-75; dried hindfoot; 219 (HA)
So.caudatus	*Soriculus caudatus*	central Nepal; Sep-75; dried hindfoot; 134 (HA)
Su.murinus	*Suncus murinus*	Okinawa Is., Japan; Oct-92; frozen muscle; small (RM)
Cr.dsinezumi	*Crocidura dsinezumi*	Sado Is., Japan; Nov-94; muscle in ethanol; 5964 (HA)
Mo.wogura	*Mogera wogura*	Aichi Pref., Honshu Is., Japan; Jul-91; muscle in ethanol; 5727 (HA)
Rat	*Rattus norvegicus*	cited from Goertz and Feldmann (1982)

VH = V. Haukisalmi, SO = S. Ohdachi, HA = H. Abe, RM = R. Masuda.

GTTG-3' (L14724), and 5'-AAAAACCATCGTTGTTATTCAACT-3' (L14734), and the reverse primer on the heavy strand as 5'-CTCAGAATGATATTTGTCCTCA-3' (H15149) and 5'-GCCCCTCAG-AATGATATTTGTCCT-3' (H15151). Codes of primers in parentheses identify the light (L) or heavy (H) strand and the 3' end-position of the primer in the human mitochondrial DNA sequence (Anderson *et al.*, 1981). Internal primers were 5'-AGCAATACACTACACATCAGA-3' or 5'-CACATCTGCCGAGACGTAAA-3' for forward sequencing, and their complements for reverse sequencing. These internal primers were also used as forward or reverse primers for PCR when a full 402-base region could not be amplified. Choice of these primers was determined by 'trial and error' for each sample. For doubtful results, both forward and reverse sequencings were done. Sequence data are available from DNA Data Bank of Japan (http://www.ddbj.nig.ac.jp): accession numbers D85342-D85372 and D87025. Sequence of the rat was cited from Goetz and Feldman (1982).

Phylogenetic tree

A phylogenetic tree was inferred by the maximum likelihood (ML) method (Felsenstein, 1981), using the program NucML of MOLPHY Ver. 2.3 package (Adachi and Hasegawa, 1996). The third codon position of the cytochrome *b* gene (133 bases, excluding the first triplet) were included for analysis because few amino acid substitutions were found among the OTUs of the genus *Sorex*. A distance matrix was generated using a nucleotide substitution model by Hasegawa *et al.* (1985) and using the most likelihood value (25.5) of the rate of transition to transversion (α/β). The neighbor-joining tree (Saitou and Nei, 1987) was obtained using the distance matrix. Next, the tree which had the greatest log likelihood was searched for by using the local rearrangement search option of the NucML, starting from the neighbor-joining tree obtained (and also from some given topologies). To show the reliability of internal branches, local bootstrap values (Adachi and Hasegawa, 1996) of 1000 replications were calculated. The procedure using only the third codon position is not appropriate for the analysis among distantly related OTUs because nucleotide substitutions would be saturated. To complement the weakness of the ML method using only the third codon position, the neighbor-joining (NJ) method was also performed using the full 402-base data sets. The NJ tree was obtained by the computer program CLUSTAL W Ver. 1.5 (Thompson *et al.*, 1994) with the multiple substitution correction (Kimura, 1980). Bootstrap values of 1000 replications were calculated to assess the confidence of internal branches. Relative efficiencies of some algorithms for phylogeny reconstruction using molecular data were examined by Hasegawa and Fujiwara (1993), where the ML method tended to find a correct phylogeny.

RESULTS

A 402-base region of the cytochrome *b* was successfully sequenced for all OTUs. No insertions or deletions were observed.

In the ML tree (Fig. 1), monophyly among the OTUs from the Soricinae and the genus *Sorex* was strongly supported by the local bootstrap value (≥ 89%). We tentatively propose eleven groups within the soricine shrews examined, according to the bootstrap values of the ML tree (≥ 65%); (1) *Soriculus* group (*Soriculus nigrescens* and *So. caudatus*, Asiatic mountain shrews), (2) *Neomys*/*Chimarrogale* group (*Neomys fodiens* and *Chimarrogale platycephala*, water shrews), (3) *cinereus*/*fumeus* group (*Sorex cinereus* and *S. fumeus*, the subgenus *Otisorex* in North America), (4) *S. raddei*, (5) *unguiculatus*/*isodon*/*mirabilis* group (*S. unguiculatus*, *S. isodon*, and *S. mirabilis*), (6) *araneus*/*tundrensis*/*daphaenodon* group (*S. araneus*, *S. tundrensis*, and *S. daphaenodon*), (7)

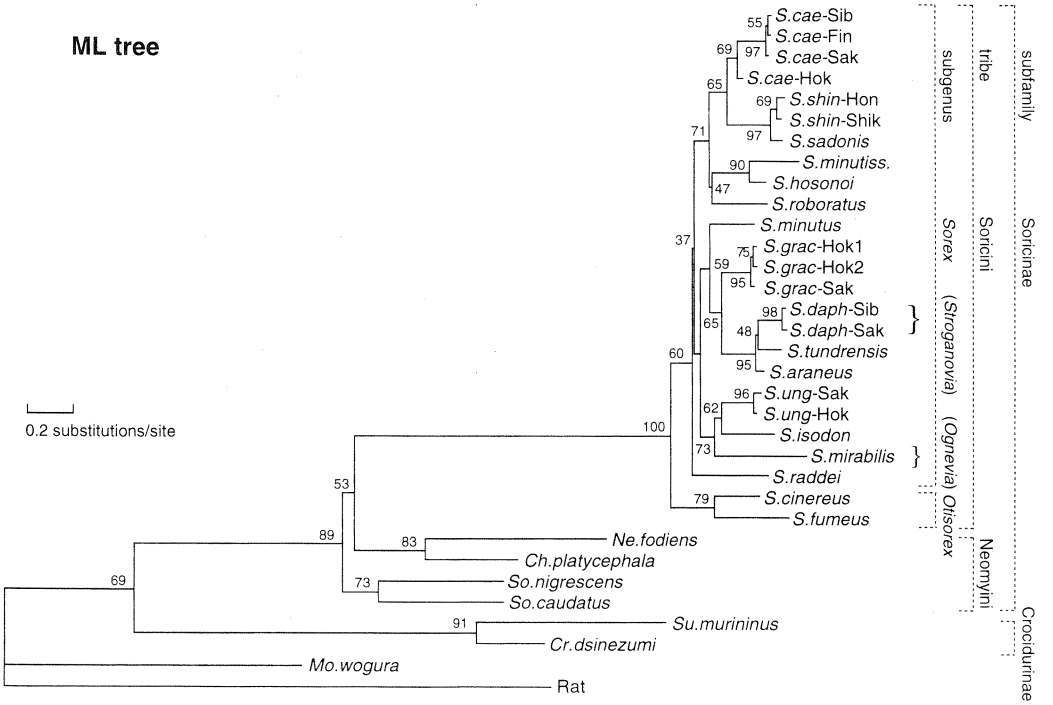

Fig. 1. The maximum likelihood (ML) tree by the nucleotide substitution model of Hasegawa *et al.* (1985), using third codon position (133 bases) of the mitochondrial cytochrome *b* gene sequences in insectivores. Branch length is proportional to the estimated number of nucleotide substitutions per site. Numbers near internal branches indicate the local bootstrap values (%) of 1000 replications.

S. gracillimus, (8) *S. minutus*, (9) *S. roboratus*, (10) *minutissimus/hosonoi* group (*S. minutissimus* and *S. hosonoi*), and (11) *caecutiens/shinto* group (*S. caecutiens*, *S. shinto*, and *S. sadonis*). Branching orders of these groups were obscure judging from the local bootstrap values.

Ignoring bootstrap values, nine out of the 11 groups proposed by the ML tree were also recognized in the NJ tree (Fig. 2), although branching orders of the groups were rather different from those of the ML tree. The *unguiculatus/isodon/mirabilis* group was non-monophyletic in the NJ analysis (Fig. 2). The *Soriculus* group was also non-monophyletic, but the four OTUs of the Neomyini were monophyletic in this tree (Fig. 2).

DISCUSSION

The hypothesis that all the OTUs from the Soricinae are monophyletic was strongly supported by the nucleotide sequences (Figs. 1 and 2). We did not examine any samples from the Blarini (short-tailed shrew in North America), the third tribe of the Soricinae, but a close relationship between the tribes Soricini and Blarini is accepted by morphological (Repenning, 1967) and biochemical (George, 1988; George and Sarich, 1994) data. Therefore, the Soricinae is presumably monophyletic.

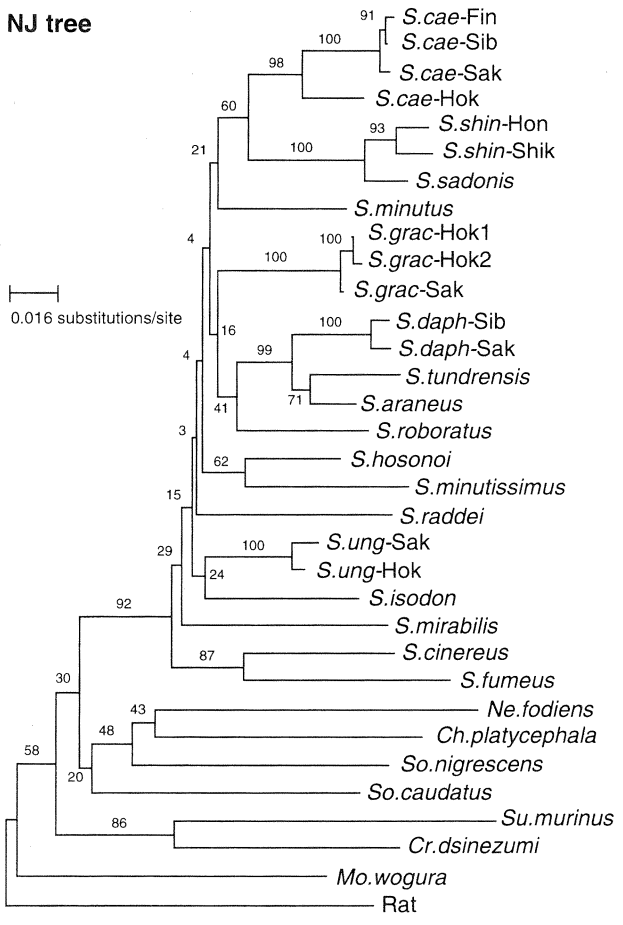

Fig. 2. The neighbor-joining (NJ) tree of the mitochondrial cytochrome *b* gene sequences (402 bases) in insectivores. Branch length is proportional to the estimated number of nucleotide substitutions per site by Kimura's two-parameter method. Numbers near internal branches indicate the bootstrap values (%) of 1000 replications.

The ML tree (Fig. 1) did not support a monophyletic relation between the two groups of the Neomyini, but the NJ tree (Fig. 2) demonstrated a monophyletic relation among the four species of the Neomyini. In the ML analysis, data included only the third codon position, and substitutions among the OTUs from the Neomyini, Crocidurinae, mole, and rat were almost saturated (Fig. 1). Therefore, non-monophyly of the Neomyini in the ML tree is probably an artifact of the analytical method used. In such a case, the results of the NJ analysis using the full 402-base data are supplementary to that of the ML analysis using only the third codon position. The NJ tree showed that the monophyly of the Neomyini although the *Soriculus* group was not monophyletic (Fig. 2). Non-monophyly of *Soriculus* in the NJ tree might be caused by insufficient data size, inferring from the low bootstrap values within the Neomyini. In addition, Reumer (1984) suggested that from the morphological and biogeographical view points the extant Neomyini should be divided into two different tribes, Notiosoricini and Soriculini. We did not analyze any of the samples from the Notiosoricini. Thus, the phylogenetic status of the Neomyini remains uncertain.

Hutterer (1993) listed some subgenera within Eurasian *Sorex*. However, the subgenera *Stroganovia* and *Ognevia* obviously should be included in the subgenus *Sorex* according to our result (Figs. 1 and 2), as Dannelid (1991) pointed out.

S. unguiculatus, *S. isodon*, and *S. mirabilis* are monophyletic in the ML tree (Fig. 1), but *S. mirabilis* was excluded from a cluster of the other two in the NJ tree (Fig. 2). Therefore, the phylogenetic status of *S. mirabilis* is not rigorously defined. Longer sequences and information of other gene sites are necessary to confirm its phylogenetic position. In contrast, a monophyletic relation of the *araneus/tundrensis/daphaenodon* group was strongly supported by both of the ML and NJ trees (Figs. 1 and 2). Their monophyletic relationships is also suggested by chromosome type (XY_1Y_2 sex- determination system; Fedyk and Ivanskaya, 1972; Reumer and Meylan, 1986; Dannelid, 1991).

Sorex gracillimus (Asiatic pygmy shrew) has long been regarded as *S. minutus* (European pygmy shrew), but is now accepted as a separate species (Hutterer, 1993). George (1988) also proposed from allozyme data that these two species should be separated, but showed that they still had a close relation. The present analysis based on mitochondrial DNA sequences, however, indicates that the two species are rather distantly related (Figs. 1 and 2), as proposed by Dannelid (1991).

There is taxonomic disagreement for the *caecutiens/shinto* group in Japan and Sakhalin. Abe (1967) did not recognize *Sorex shinto*. He regarded specimens from the Honshu, Shikoku, Hokkaido, and Sakhalin Islands all as being

S. caecutiens. On the other hand, Imaizumi (1960) treated *S. shinto* as a separate species from *S. caecutiens* and included "*S. caecutiens*" of Hokkaido and Sakhalin in *S. shinto* (names between double quotation marks are the usage in the present study). However, both of the ML and NJ trees produced two clusters in the *caecutiens*/*shinto* group (Figs. 1 and 2). One cluster consists of *S. caecutiens* in Finland, Siberia, Sakhalin, and Hokkaido, and the other contains *S. shinto* in Honshu and Shikoku and *S. sadonis*. The genetic distance between the two clusters is great enough to be regarded as a between species difference (Figs. 1 and 2). George (1988) also treated *S. shinto* as a separate species based on allozyme analysis. Yoshiyuki and Imaizumi (1986) described *S. sadonis* as a new species from the Sado Island, Japan. According to our usage of species names (Table 1), there would be no inconsistency between the nomenclature for *S. sadonis* and its phylogeny (Fig. 1). However, the genetic distance between *S. sadonis* and *S. shinto* appears to be small enough for these OTUs to be regarded as subspecies or populations, when compared with the distances among the other species examined (Figs. 1 and 2). Consequently, the usage of *S. shinto* or *S. shinto sadonis* might be appropriate for *S. sadonis*.

Branching orders among the groups suggested by the ML tree and among OTUs within the group were obscure within the genus *Sorex*, judging from the bootstrap values (Fig. 1). Further, many bootstrap values within *Sorex* were very low in the NJ tree (Fig. 2). Such uncertainty of the phylogenetical relationships of *Sorex* was probably caused by the insufficient data size. Data from longer sequences and other genes are needed to confirm the phylogenetical relationships of *Sorex*.

ACKNOWLEDGMENTS

We are grateful to V. Nesterenko, E. Pankakoski, H. Henttonen, and H. Shinyashiki for supplying some shrew samples. M. J. Toda and T. P. Craig critically commented on earlier versions of the manuscript. Without their cooperation, this work could not have been done. This study was supported in part by Grants-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture, Japan and by the cooperative research projects of the Institute of Low Temperature Science, Hokkaido University.

REFERENCES

Abe H (1967) Classification and biology of Japanese Insectivora (Mammalia) I. Studies on variation and classification. J Facul Agri, Hokkaido Univ 55: 191–269

Abe H (1985) Classification, distribution and ecology of the Soricidae, considered from the standpoint of adaptive radiation (in Japanese with English abstract). In "Suncus. Biology of Soricids as Experimental Animals" Ed by K Kondo, Gakkai Syuppan Center, Tokyo, pp 20–37

Abe H (1995) Revision of the Asian moles of genus *Mogera*. J Mamm Soc Japan 20: 51–68

Adachi J, Hasegawa M (1996) MOLPHY: Programs for Molecular Phylogenetics, ver 2.3. Institute of Statistical Mathematics, Tokyo

AndersonS, Bankier AT, Barrel BG, De Bruijn MHL, Coulson AR, Drouin J, Eperon IC, Nierlich DO, Roe BA, Sanger F, Schreier PH, Smith AJH, Staden R, Young IG (1981) Sequence and organization of the human mitochondrial genome. Nature 290: 457–465

Cao Y, Adachi J, Janke A, Pääbo S, Hasegawa M (1994) Phylogenetic relationships among eutherian orders estimated from inferred sequences of mitochondrial proteins: instability of a tree based on a single gene. J Mol Evol 39: 519–527

Churchfield S (1990) The Natural History of Shrews. A & C Black, London

Cooper A (1994) DNA from museum specimens. In "Ancient DNA" Ed by Herrmann B, Hummel S, Springer-Verlag, New York, pp 149–165

Corbet GB, Hill JF (1986) A World List of Mammalian Species. 2nd ed, British Museum (Natural History), London

Dannelid, E (1991) The genus *Sorex* (Mammalia, Soricidae) — distribution and evolutionary aspects of Eurasian species. Mamm Rev 21: 1–20

Dolgov VA (1985) Shrews in the North, South, East and West (in Russian). Moscow Univ Press, Moscow

Fedyk S, Ivanskaya EY (1972) Chromosomes of Siberian shrews. Acta Theriol 17: 475–492

Felsenstein J (1981) Evolutionary trees from DNA sequences: a maximum likelihood approach. J Mol Evol 17: 368–376

George, SB (1988) Systematics, historical biogeography, and evolution of the genus *Sorex*. J Mamm 69: 443–461

George SB, Sarich VM (1994) Albumin evolution in the Soricinae and its implications for the phylogenetic history of the Soricidae. In "Advances in the Biology of Shrews" Ed by Merritt JF, Kirkland Jr GL, Rose RK, Carnegie Museum of Natural History, Pittsburgh, pp 289–294

Goertz G, Feldmann H (1982) Nucleotide sequence of the cytochrome b gene and adjacent regions from rat liver mitochondrial DNA. Current Genetics 5: 221–225

Hasegawa M, Fujiwara M (1993) Relative efficiencies of the maximum likelihood, maximum parsimony, and neighbor-joining methods for estimating protein phylogeny. Mol Phyl Evol 2: 1–5

Hasegawa M, Kishino H, Yano T (1985) Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. J Mol Evol 22: 160–174

Hutterer R (1993) Order Insectivora. In "A Taxonomic and Geographic Reference. Mammal Species of the World 2nd ed" Ed by Wilson DE, Reeder DM, Smithsonian Institution Press, Washington DC, pp 69–130

Imaizumi Y (1960) Coloured Illustrations of the Mammals of Japan (in Japanese). Hoikusha Publishing Osaka

Kimura M (1980) A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. J Mol Evol 16: 111–120

Kocher TD, Thomas WK, Meyer A, Edwards SV, Pääbo S, Villablanca FX, Wilson AC (1989) Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. Proc Natl Acad Sci USA 86: 6196–6200

MacDonald D (1984) The Encyclopedia of Mammals. Facts on File, New York

Masuda R, Yoshida MC (1994a) A molecular phylogeny of the family mustelidae (Mammalia, Carnivora), based on comparison of mitochondrial cytochrome b nucleotide sequences. Zool Sci 11: 605–612

Masuda R, Yoshida MC (1994b) Nucleotide sequence variation of cytochrome b genes in three species of weasels *Mustera itati*, *Mustela sibirica*, and *Mustela nivalis*, detected by improved PCR product-direct sequencing technique. J Mamm Soc Japan 19: 33–43

Repenning CA (1967) Subfamilies and genera of the Soricidae. Geol Surv Prof Paper 565: 1–69

Reumer JWF (1984) Ruscinian and early Pleistocene Soricidae (Insectivora, Mammalia) from Tegelen (The Netherlands) and Hungary. Scripta Geologica 73: 1–173

Reumer JWF (1987) Redefinition of the Soricidae and the

Heterosoricidae (Insectivora, Mammalia), with the description of the Crocidosoricinae, a new subfamily of Soricidae. Revue de Paléobiologie 6: 189–192

Reumer JWF, Meylan A (1986) New developments in vertebrate cytotaxonomy IX. Chromosome numbers in the order Insectivora (Mammalia). Genetica 70: 119–151

Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic tees. Mol Biol Evol 4: 406–425

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular Cloning: A Laboratory Manual. 2nd ed, Cold Spring Harbor Lab, New York

Savage DE, Russell DE (1983) Mammalian Paleofauna of the World. Addison-Wesley Publishing, London

Simpson GG (1945) The Principle of Classification and a Classification of Mammals. Bull Amer Mus Nat Hist 85, New York

Stewart DT, Baker AJ (1994) Pattern of sequence variation in the mitochondrial D-loop region of shrews. Mol Biol Evol 11: 9–21

Sturmbauer C, Verheyen E, Meyer A (1994) Mitochondrial phylogeny of the Lamprogini, the major substrate spawning lineage of cichlid fishes from lake Tanganyika in eastern Africa. Mol Biol Evol 11: 691–703

Taberlet P, Fumagalli L, Hausser J (1994) Chromosomal versus mitochondrial DNA evolution: tracking the evolutionary history of the southwestern European populations of the *Sorex araneus* group (Mammalia, Insectivora). Evolution 48: 623–636

Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. Nucleic Acids Res 22: 4673–4680

Walsh PS, Metzger DA, Higuchi R (1991) Chelex® 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. BioTechniques 10: 506–513

William J, Ballard O, Kreitman M (1995) Is mitochondrial DNA a strictly neutral marker? Trends Ecol Evol 10: 485–488

Yoshiyuki M, Imaizumi Y (1986) A new species of *Sorex* (Insectivora, Soricidae) from Sado Island, Japan. Bull Natl Sci Mus, Tokyo, Ser A 12: 185–193

Zhang YP, Ryder O (1993) Mitochondrial DNA sequence evolution in the Arctoidea. Proc Natl Acad Sci USA 90: 9557–9561

(Received January 9, 1997 / Accepted February 20, 1997)