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Phylogenetical Positions of *Sorex* sp. (Insectivora, Mammalia) from Cheju Island and *S. caecutiens* from the Korean Peninsula, Inferred from Mitochondrial Cytochrome *b* Gene Sequences

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ABSTRACT—Phylogenetical positions of *Sorex* specimens unassigned to species from Cheju Island, Korea, and *S. caecutiens* from southern Korean Peninsula were investigated based on full nucleotide sequences (1,140 bp) of the mitochondrial cytochrome *b* gene, comparing specimens of the *S. caecutiens*/*shinto* group from locations throughout its range. In the phylogenetic tree obtained, *S. caecutiens* were separated into two main groups: Hokkaido and Continent-Sakhalin-Cheju clusters. Shrews from Cheju and Korean Peninsula were included in the latter cluster. Thus, we suggest that the shrew on Cheju Island should be ranked as *S. caecutiens*, although taxonomic description of the shrew has to be conducted elsewhere. The Cheju shrews formed a single sub-cluster while the peninsular shrews were not included in a single sub-cluster. The clustering of individuals in Continent-Sakhalin-Cheju cluster did not always reflect the geographical proximity of their capture locations. We interpret these findings as indicating ancestral isolation of a Hokkaido population and recent rapid range expansion of the modern population in Eurasian Continent-Sakhalin-Cheju.

Key words: *Sorex caecutiens*/*shinto* group, cytochrome *b*, molecular phylogeny, Cheju Island, Korean Peninsula

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INTRODUCTION

Sorex caecutiens Laxmann and *S. shinto* Thomas (Soricinae, Soricidae, Insectivora) form a monophyletic *Sorex caecutiens*/*shinto* group (Ohdachi *et al*., 1997, 2001). *Sorex caecutiens* is widely distributed throughout the northern part of the Eurasian Continent and neighbouring islands, including the Korean Peninsula and the islands of Hokkaido and Sakhalin (Karafuto), while extant *S. shinto* is known only from the islands of Honshu, Sado, and Shikoku (Ohdachi *et al*., 1997, 2001; Fig. 1). Fossils of *S. shinto*, however, have been excavated from the middle Pleistocene layers in Honshu and the late Pleistocene in Kyushu (Kawamura and Sotsuka, 1984; Kawamura et al., 1989). Thus, the ranges of *S. caecutiens* populations of Hokkaido and the Korean Peninsula were adjacent to that of *S. shinto*. Ohdachi *et al*. (2001) showed the unique phylogenetic position of *S. caecutiens* in Hokkaido, which is obviously different from those from the continent, but no samples from the Korean Peninsula had then been analyzed. It is therefore necessary to examine the phylogenetic position of *S. caecutiens* from the Korean Peninsula and compare it with other *S. caecutiens* populations and with *S. shinto* to understand the evolutionary process of the *caecutiens*/*shinto* group.

A *Sorex* shrew was recently discovered from Cheju Island (H.-S. Oh, unpubl.), but has not been assigned to species yet. Thus, it is also necessary to determine the correct phylogenetic position of shrews from Cheju, which is located between the Korean Peninsula and the Japanese Islands and is thus a key region for the investigation of biogeographic history in East Asia. The Cheju shrew definitely belongs to the *caecutiens/shinto* group, judging from external and cranial morphology according to our preliminary investigation. However, it is difficult on morphological information alone to identify species or subspecies (or local populations) in the *caecutiens/shinto* group, because this group shows complex morphological variation between local pop-

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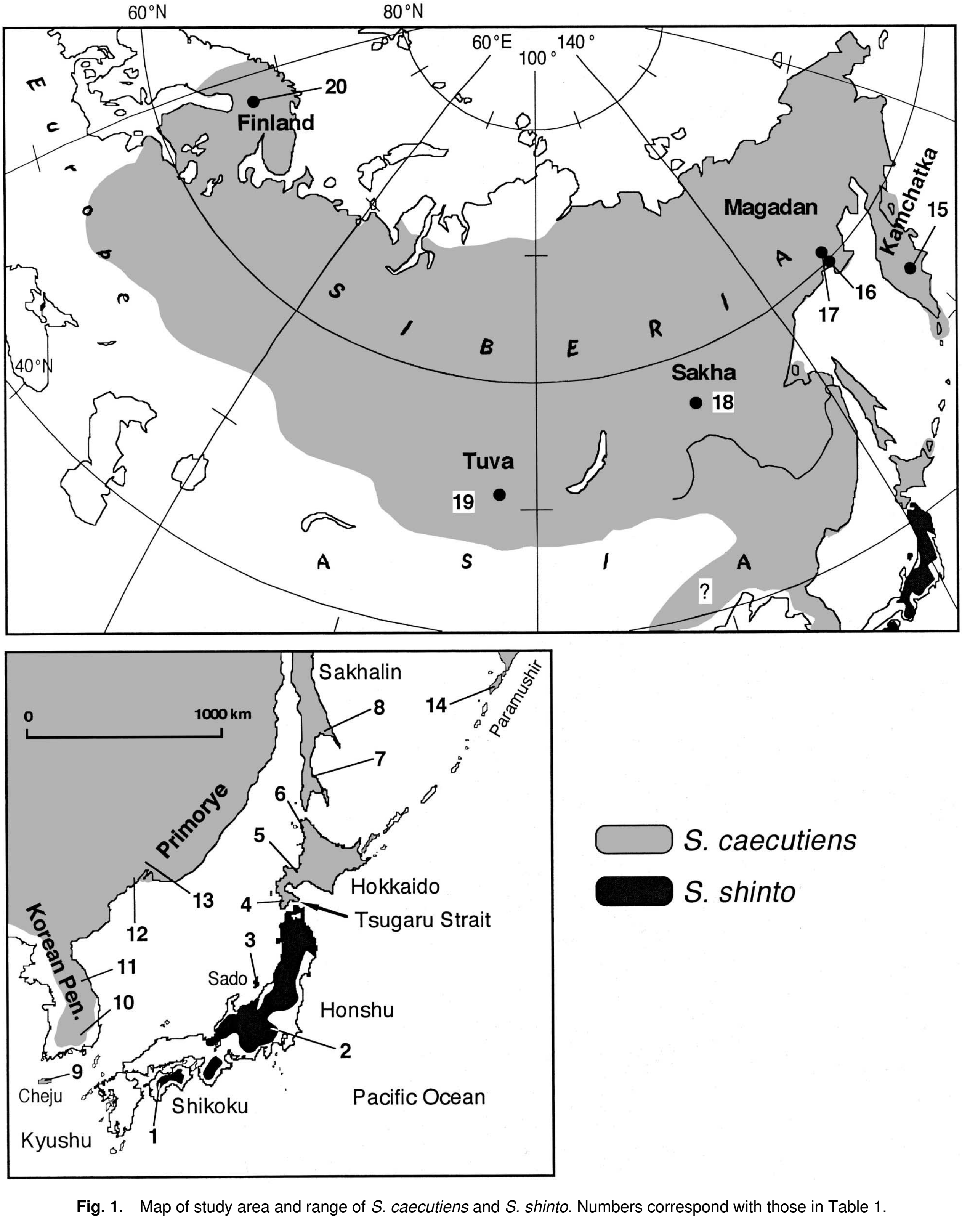

Fig. 1. Map of study area and range of *S. caecutiens* and *S. shinto*. Numbers correspond with those in Table 1.

ulations and sibling species (Dokuchaev *et al*., 1997). In cases of doubt, genetic information can provide clarification (e.g. Vogel and Sofianidou, 1996; Ohdachi *et al*., 1997; Iwasa *et al*., 2001). Accordingly, we recently captured five specimens of the genus *Sorex* at a higher location (ca. 1,000 m) of Mt. Halla on Cheju Island (33° 20’ 38”N, 126° 27’ 30”E) to investigate its phylogenetic status.

In this paper, we estimated phylogenetic relationships among different populations of the *caecutiens/shinto* group including those from Cheju Island and the Korean Peninsula by using full nucleotide sequences (1,140 bp) of the mitochondrial cytochrome *b* gene. We then propose a taxonomic status of the shrew from Cheju Island.

MATERIALS AND METHODS

Specimens and DNA analysis

Mitochondrial cytochrome *b* gene sequences (1,140 bp) were derived from 33 individuals of the *caecutiens/shinto* group from 20 locations throughout Eurasia (Table 1, Fig. 1). Of these, 17 shrews had been analyzed previously (Ohdachi *et al.*, 2001) and the remaining 16 were analyzed for the present study. In addition, one *S. isodon* Turov and one *S. mirabilis* Ognev from South Korea were used as outgroups in phylogenetic analysis. The biological information on the shrews (sex, age, collection date, etc.) as well as the sequence data are stored in DDBJ/EMBL/GenBank data bases (accession numbers are indicated in Table1). Sequencing method was given in Ohdachi *et al.* (2001).

Phylogenetic analysis

To infer phylogeny, maximum likelihood (ML) tree was estimated by the quartet-puzzling method (10,000 puzzling steps) using TREE-PUZZLE ver.5.0 (Strimmer and von Haeseler, 1996) based on 1,140-bp data set of mitochondrial cytochrome *b* sequences. According to the hierarchical likelihood ratio tests by MODELTEST ver. 3.06 (Posada and Crandall, 1998) with PAUP* ver. 4.0b10 (Swofford, 2000), the substitution model by Tamura and Nei (1993) with gamma distribution + invariable sites (TrN+G+I model) were chosen to construct an ML tree. Eight categories were used for gamma distribution (Yang 1996). Confidence of a node was assigned by a support value of quartet-puzzling (Strimmer and von Haeseler, 1996).

Table 1. List of samples used in analysis. Locality numbers correspond with those of Fig. 1.

Species	Code of OTUs	Locality (#)	Data source	Accession #
S. shinto	*S. shinto* (Shikoku)	Shikoku Is. (1)	1	AB028534
	S. shinto (Honshu)	Honshu Is. (2)	1	AB028540
	S. shinto (Sado)	Sado Is. (3)	1	AB028545
S. caecutiens	Kaminokuni (Hokkaido)	Kaminokuni, Hokkaido Is. (4)	1	AB028560
	Sapporo (Hokkaido)	Sapporo, Hokkaido Is. (5)	1	AB028562
	Sarobetsu (Hokkaido)	Sarobetsu, Hokkaido Is. (6)	1	AB028559
	Starodubskoe (Sakhalin)	Starodubskoe, Sakhalin Is. (7)	1	AB028556
	Trudovoe-1 (Sakhalin)	Trudovoe, Sakhalin Is. (8)	1	AB028549
	Trudovoe-2 (Sakhalin)	Trudovoe, Sakhalin Is. (8)	2	AB0062720
	Cheju Is.-1, 2, 3 and 4	Cheju Is. South Korea (9)	2	ABO062721-062724
	Mt. Gaya-1 and 2 (Korea)	Mt. Gaya, Korean Pen. (10)	2	AB0062725-062726
	Mt. Odae-1, 2 and 3 (Korea)	Mt. Odae, Korean Pen. (11)	2	AB0062727-062729
	Kedrovaya (Primorye)-1	Kedrovaya, Primorye (12)	1	AB028557
	Kedrovaya (Primorye)-2	Kedrovaya, Primorye (12)	2	AB0062730
	Ussuriiskii (Primorye)-1	Ussuriiskii, Primorye (13)	1	AB028554
	Ussuriiskii (Primorye)-2	Ussuriiskii, Primorye (13)	2	AB0062731
	Paramushir Is.	Paramushir Is., Northern Kuril (14)	1	AB028550
	Kamchatka-1	Milkovo, Kamchatka Pen. (15)	1	AB028555
	Kamchatka-2	Milkovo, Kamchatka Pen. (15)	2	AB0062732
	Magadan (Magadan)	Magadan City, Magadan state (16)	1	AB028551
	Elikchanskoye (Magadan)	Elikchanskoye, Magadan state (17)	1	AB028547
	Sakha-1	Nagornyi, Sakha republic (18)	1	AB028548
	Sakha-2	Nagornyi, Sakha republic (18)	2	AB0062733
	Tuva-1	southern part of Tuva republic (19)	1	AB028552
	Tuva-2	southern part of Tuva republic (19)	2	AB0062734
	Finland-1	Pallasjärvi, Finland (20)	1	AB028553
	Finland-2	Pallasjärvi, Finland (20)	2	AB0062735
S. isodon	*S. isodon*	Mt. Odae, Korean Pen. (11)	2	AB0062736
S. mirabilis	*S. mirabilis*	Mt. Odae, Korean Pen. (11)	2	AB0062737

[1] Ohdachi *et al.* (2001), [2] present study

RESULTS

The 1,140-bp region of the cytochrome *b* gene was successfully sequenced for the 18 new *Sorex* specimens (including one *S. isodon* and one *S. mirabilis*) used in the present study. None of the sequences contained any insertions or deletions.

A phylogenetic tree was obtained by the ML method (Fig. 2). The transition/transversion parameter was estimated from the data as 10.55±1.61 *SE* and Y/R transition parameter was 1.37±0.24 *SE*. Fraction of invariable site was 0.51±0.03 *SE*. Gamma distribution parameter, alpha, was estimated from the data set as 0.73±0.13 *SE*. Total rate heterogeneity was 0.79±0.08 *SE*. The percentage of unresolved quartets was 21.7% and -ln L was 3841.26 (without clock).

The ML tree showed that *S. caecutiens* and *S. shinto* were obviously separated, and shrews from Cheju Island were clearly included in *S. caecutiens* (Fig. 2). *Within S. caecutiens*, shrews from Hokkaido segregated unambiguously from those from the other locations (the islands of Cheju, Sakhalin, and Paramushir, and the Eurasian Continent); the former cluster is termed Hokkaido cluster and the latter Continent-Sakhalin-Cheju cluster (Fig. 2). Continent-Sakhalin-Cheju cluster in turn segregated into six subclusters: subclusters A-E and Cheju cluster. Sub-cluster A consisted of shrews from Sakhalin and locations throughout the Eurasian Continent (including Paramushir Island). Sub-cluster B consisted of shrews from southern Korean Peninsula, while one individual from Korea (Mt. Odae-3) was not included in this cluster. Sub-clusters C-E consisted mainly of shrews from northeastern continental Asia. All the shrews from Cheju Island were monophyletic (Cheju cluster), but phylogenetical relationships among those from Sakhalin-the Eurasian Continent did not always reflect the geographical proximity of their capture locations (Fig. 2).

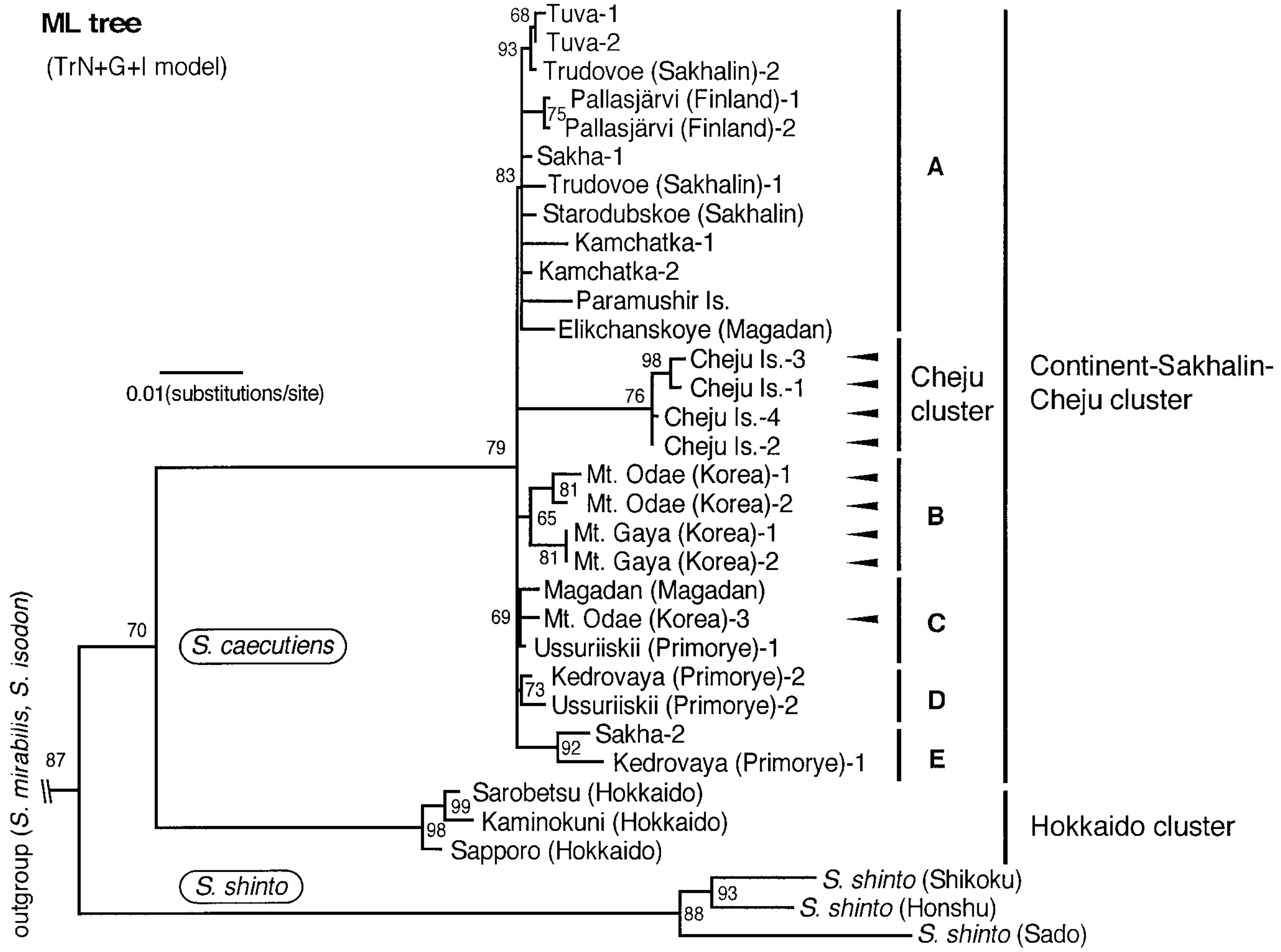

Fig. 2. Maximum likelihood tree by Tamura and Nei (1993) substitution model with 1 invariable and 8 gamma rates of site heterogeneity, based on nucleotide sequences (1,140 bp) of the mitochondrial cytochrome *b* gene in *Sorex caecutiens* and *S. shinto*. Numbers near internal branches indicate the support values of quartet-puzzling (%). Solid triangles denote shrews from peninsular Korea and Cheju Island.

DISCUSSION

Judging from the mitochondrial nucleotide sequences, the *Sorex* shrews on Cheju Island were phylogenetically allocated as *S. caecutiens* (Fig. 2), which is widely distributed throughout northern Eurasia (Fig. 1), and were not as *S. shinto*, which is endemic to the southern parts of the Japanese Islands. Thus, we suggest that the shrew on Cheju Island should be ranked as *S. caecutiens*, although traditional taxonomic description of the shrew has not been conducted yet since it was discovered (H.-S. Oh, unpubl.). Further, its morphological status among the *caecutiens/shinto* group should be investigated as the *Sorex* specimens from Cheju Island were distinctly larger and had relatively longer tails than those from the Korean Peninsula (unpubl.).

Shrews from peninsular Korea were clustered together with *S. caecutiens* from the continent and Sakhalin (Fig. 2). Thus, unlike the population of Hokkaido, the peninsular population did not have a unique phylogenetical position in *S. caecutiens.* Further, one sample from peninsular Korea (Mt. Odae-3) did not cluster into a single group with the other Korean shrews (Fig. 2). In contrast, four shrews from Cheju Island clustered together with a high support value (73%) in the ML tree (Fig. 2). This suggests that there is no clear demarcation between the peninsular and continental populations but that the Cheju population might have been isolated from the continental population for longer than the peninsular population.

Han *et al*. (in press) revealed based on mitochondrial phylogenetic investigation that there are two *Crocidura* species (Crocidurinae, Soricidae) (in Cheju) and proposed that one of them was introduced by humans and the other, which is a species common to the Korean Peninsula, was naturally distributed on Cheju Island. Hence, soricid fauna (*Sorex* and *Crocidura*) on Cheju Island is a part of that of the peninsula (= the continent) and was not directly influenced by the fauna of the Japanese Islands.

In Continent-Sakhalin-Cheju cluster of *S. caecutiens*, the ML tree showed brush-like diversification among six sub-clusters (Fig. 2), indicating the six clusters were founded almost simultaneously. Further, sub-cluster A was a main group in Continent-Sakhalin-Cheju cluster and consisted of shrews from different parts throughout Eurasia (Fig. 2). This suggests that the ancestral shrews of sub-cluster A spread throughout Eurasia in a short time that did not allow local differentiation of haplotypes, as suggested by Ohdachi *et al*. (2001). In contrast, the ancestor of the Hokkaido population of *S. caecutiens* seems to have been separated from that of Sakhalin and the continent considerably before the range expansion of these populations. The ancient separation of the Hokkaido population was also supported by Ohdachi *et al*. (2001) using a larger numbers of samples from Hokkaido.

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