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# Phylogenetic Relationships among East Asian Species of *Crocidura* (Mammalia, Insectivora) Inferred from Mitochondrial Cytochrome *b* Gene Sequences

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**ABSTRACT**—Phylogenetic relationships among the East Asian species of the genus *Crocidura* (Mammalia, Insectivora, Soricidae) were inferred from the partial sequences (402 base pairs) of the mitochondrial cytochrome *b* gene. Six species of *Crocidura* and one representative of another insectivoran genus *Suncus* were examined. In the neighbor joining tree, four distinct lineages were recognized: *C. lasiura* from northeastern China and Korea, and *C. dsinezumi* from the main-islands of Japan; *C. attenuata* from Taiwan; *C. watasei* from the Amami and Okinawa Groups, and *S. murinus* from the Miyako Group; and *C. suaveolens* from Tsushima and Taiwan, and *C. sibirica* from Central Asia in Russian territory. All these lineages excluding *C. lasiura* and *C. dsinezumi* were also recognized by the maximum parsimony analysis. Clustering of *C. watasei* with *S. murinus* suggested the non-monophyly of the genus *Crocidura* as of the present definition. Biogeographical implications of the present results were also discussed.

## INTRODUCTION

*Crocidura* is the largest mammalian genus consisting of no less than 164 species of small terrestrial shrews widely distributed in the Palaearctic, Ethiopian and Oriental regions (Hutterer, 1993; Wolsan and Hutterer, 1998). The taxonomic arrangement of this genus is still problematic, because several authors studying morphological and genetic properties of its African components casted a doubt to the monophyly of the genus as in relation to other closely related genera, *Suncus* and *Sylvisorex* (Butler *et al.*, 1989; Maddalena, 1990; McLellan, 1994). The non-monophyly as in relation to *Suncus* is also suspected for the Asian *Crocidura* species (Corbet and Hill, 1992), but without concrete evidence.

During the last decade, a number of systematic studies

for the Asian *Crocidura* have been performed based on morphological, karyological, and genetic data (Ruedi *et al.*, 1990, 1993, 1998; Heaney and Ruedi, 1994; Kitchener *et al.*, 1994; Ruedi, 1995, 1996; Ruedi and Vogel, 1995; Jenkins and Smith, 1995; Motokawa *et al.*, 1996, 1997; Fang *et al.*, 1997; Motokawa, 1998; Motokawa and Harada, 1998), leading to drastic taxonomic changes from the previous arrangement (Ellerman and Morrison-Scott, 1951; Jenkins, 1976; Lekagul and McNeely, 1977; Jameson and Jones, 1977). In those recent studies, however, the phylogenetic relationships have been assessed only for the Southeast Asian species (Ruedi, 1996; Ruedi *et al.*, 1998): no studies have been made for the comprehensive phylogeny of the East Asian species.

East Asia, consisting of a number of fairly old landbridge islands and the adjacent continent that range in both Palaearctic and Oriental faunal realms, is one of the most important regions to study mammal evolution. Such historical and biogeographical situations have let this region embrace a diverse, highly endemic mammal fauna (Abe *et al.*, 1994;

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Motokawa, 1999; Lin and Lin, 1983). It is generally considered that the ancestors of the East Asian mammal lineages were originally dispersed from areas of both north and south of this region (Abe *et al.*, 1994; Zhang *et al.*, 1997), but the geographical origin of each lineage remains controversial.

In East Asia, seven species are currently recognized for the genus *Crocridura*: *C. lasiura* in the northeastern part of the Asian continent including Korean Peninsula; *C. suaveolens* in eastern continent including Korean Peninsula, Tsushima, and Taiwan; *C. dsinezumi* in the main-islands of Japan; *C. orii* in the Amami Group of the central Ryukyus; *C. watasei* in the Amami Group and the Okinawa Group of the central Ryukyus; *C. attenuata* in southeastern continent and Taiwan; and *C. kurodai* in Taiwan (Corbet, 1978; Corbet and Hill, 1992; Abe *et al.*, 1994; Motokawa *et al.*, 1996, 1997; Fang *et al.*, 1997; Zhang *et al.*, 1997; Motokawa, 1998). Of these, *C. suaveolens* is distributed almost throughout the Palaearctic Eurasian continent, whereas *C. attenuata* occurs in the region from Taiwan to Malay Peninsula (Corbet, 1978; Corbet and Hill, 1992; Fang *et al.*, 1997; Zhang *et al.*, 1997). The remaining five species are endemic to East Asia.

In the present study, a part of the mitochondrial cytochrome *b* gene was sequenced for five of the East Asian *Crocridura* species, *C. sibirica* from Central Asia and *Suncus murinus* from East Asia, and resultant data were analysed phylogenetically. Our purposes are to hypothesize the phylogenetic relationships among them and to test the monophyly of the genus *Crocridura*.

## MATERIALS AND METHODS

We used 20 specimens of six *Crocridura* species and *Suncus murinus* (Table 1, Fig. 1, Appendix). Akademgorodok City in Russia, from which two specimens of *C. sibirica* were obtained (not plotted in Fig. 1), is located around 54°40' N in latitude and 83°05' E in longitude (Narita *et al.*, 1995). A specimen of *S. murinus* was from a closed colony, which had been originated from wild caught animals on Taramajima of the Miyako Group, the southern Ryukyus (Oda, 1985).

**Table 1.** Specimens of *Crocridura* and *Suncus* examined in the present study. Locality numbers given in brackets correspond to those in Fig. 1. Details of localities and voucher specimens are given in Appendix.

Species	Locality	Number of specimens examined
<i>C. dsinezumi</i>	Toyama [1]	1
	Gifu [2]	1
	Chiburijima [3]	1
	Yakushima [5]	2
<i>C. lasiura</i>	China [12]	1
	Korea [11]	1
<i>C. attenuata</i>	Taichung [9]	1
	Pingtung [10]	2
<i>C. watasei</i>	Amamioshima [6]	1
	Okinawajima [7]	2
<i>C. suaveolens</i>	Tsushima [4]	1
	Taichung [9]	3
<i>C. sibirica</i>	Russia	2
<i>S. murinus</i>	Taramajima [8]	1

Specific names follow Wolsan and Hutterer (1998). The voucher specimens are deposited in the Zoological Department of Kyoto University (KUZ), Osaka City University Medical School (OCUMS), and School of Dentistry of Aichi-Gakuin University (AGUD). The sequence data of *Soriculus caudatus*, a member of a subfamily (Soricinae) different from that for the genera *Crocridura* and *Suncus* (Crocridurinae: Hutterer, 1993), was taken from Ohdachi *et al.* (1997) and was incorporated into the phylogenetic analyses as outgroup.

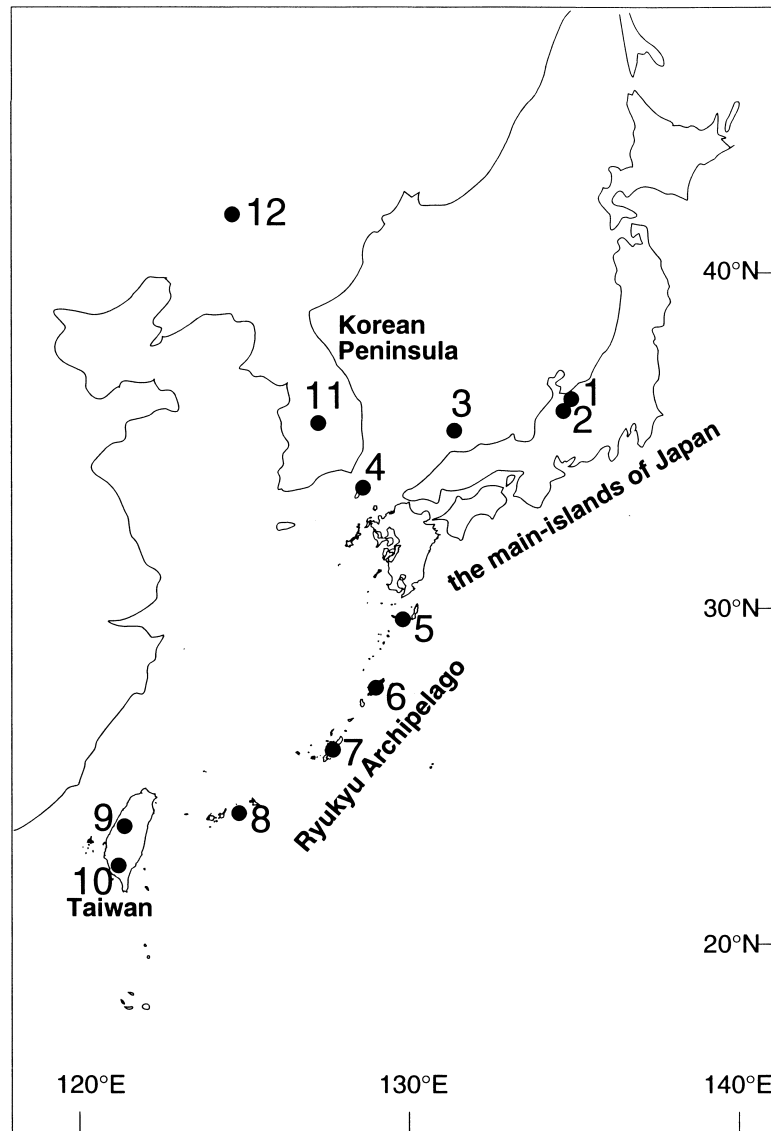
Liver samples were removed and stored at -80°C or in 95% ethanol until experiments. The DNA was extracted using the phenol-chloroform method described by Sambrook *et al.* (1989). A 402 base-pair fragment of the mitochondrial cytochrome *b* gene was analysed by using the semi-nested polymerase chain reactions (PCR) and direct sequencing method by Suzuki *et al.* (1997). The primers used for the first PCR (L14724 and H15915) and the second PCR (R-L14724 and U-H15155) are the same as those in Suzuki *et al.* (1997). Numbers refer to the positions of 3' base of the primers in the complete sequence of human mitochondrial DNA (Anderson *et al.*, 1981).

Phylogenetic relationships were inferred by two methods: the neighbor joining method (Saitou and Nei, 1987) using PHYLIP program version 3.5c (Felsenstein, 1993) on the basis of pairwise distances calculated following the two-parameter model of Kimura (1980); and the maximum parsimony method using the branch-and-bound search in PAUP (Phylogenetic Analysis Using Parsimony Version 3.1.1, Swofford, 1993) with all character state changes weighted equally. In both analyses, 1000 bootstrap replications (Felsenstein, 1985) were made to assess the degree of supports for internal branches.

## RESULTS

Nucleotide sequence data of 402 base-pairs of the cytochrome *b* gene are given in Fig. 2. Fourteen haplotypes were observed from the 20 specimens. Of the 402 base-pairs sequenced, 123 sites (30.6%) were variable among 15 haplotypes including the outgroup. Percentage similarities are shown in Table 2. Two haplotypes, differing from each other in one nucleotide site (99.8% similarity), were found in three specimens of *C. suaveolens* from Taichung. Four haplotypes showing a geographic pattern, were observed in *C. dsinezumi* (96.0–99.5%): two from the main-islands of Japan (Toyama and Gifu) and the remaining two from small adjoining islands (Chiburijima and Yakushima). Intraspecific geographical divergences were also observed between specimens of *C. lasiura* from China and Korea (99.0%); *C. attenuata* from Taichung and Pingtung (99.5%); and *C. suaveolens* from Tsushima and Taichung (98.8–99.0%). On the other hand, specimens of *C. watasei* from Amamioshima and Okinawajima showed no sequence differences. The pairwise sequence similarities between conspecifics were consistently larger (96.0–99.8%) than the pairwise values for different species of the genus *Crocridura* (85.3–94.3%). The similarities of *Suncus murinus* with *Crocridura* species examined were mostly smaller (83.8–86.8%) than those within *Crocridura*, but with a slight overlap.

The neighbor joining tree is shown in Fig. 3A. Monophyly was supported for each of the species having more than one haplotype (74.9–98.4% bootstrap values) and four major clusters were formed with more than 50% bootstrap values: *C. dsinezumi* and *C. lasiura*; *C. attenuata*; *C. watasei* and *S.*



**Fig. 1.** The map showing sampling localities of the species of *Crocidura* and *Suncus* examined in the present study. Locality numbers refer to those in Table 1.

*murinus*; and *C. sibirica* and *C. suaveolens*. Such relationships other than the monophyly of *C. dsinezumi* and *C. lasiura* were also recognized in the maximum parsimony tree (Fig. 3B).

## DISCUSSION

The genus *Suncus*, broadly occurring in Africa, and South and Southeast Asia, is considered to be closely related to the genus *Crocidura* (Corbet and Hill, 1992). Since the former is morphologically distinguished from the latter only by the retention of four upper unicuspid teeth, validity of the current taxonomic arrangement of the two genera has required verifications (Corbet and Hill, 1992). Our results showed that *C. watasei* is more closely related to *S. murinus* than to other East Asian *Crocidura*, and this supports the non-monophyly

of the genus in its current arrangement as was suspected by a few previous authors (Butler *et al.*, 1989; Maddalena, 1990; McLellan, 1994). Geographically, both the genus *Suncus* as a whole and *S. murinus* are considered to have been originated in South Asia (Butler, 1998; Yosida, 1982). It is thus likely that *S. murinus* and *C. watasei* had derived from a common ancestor in South Asia.

In the neighbor joining analysis, *C. dsinezumi* from the main-islands of Japan was exclusively clustered with *C. lasiura* from northeastern China and Korea. These two species have similar karyotypes ( $2n=40$ ), both consisting of three meta- or submetacentric, four subtelocentric, and twelve acrocentric pairs in autosomes ( $FN=56$ ) (Harada *et al.*, 1985; Zima *et al.*, 1998: see Maddalena and Ruedi [1994] for chromosome terminology). Such a chromosomal arrangement is also shared by two Oriental species: *C. attenuata* from Taiwan (Motokawa

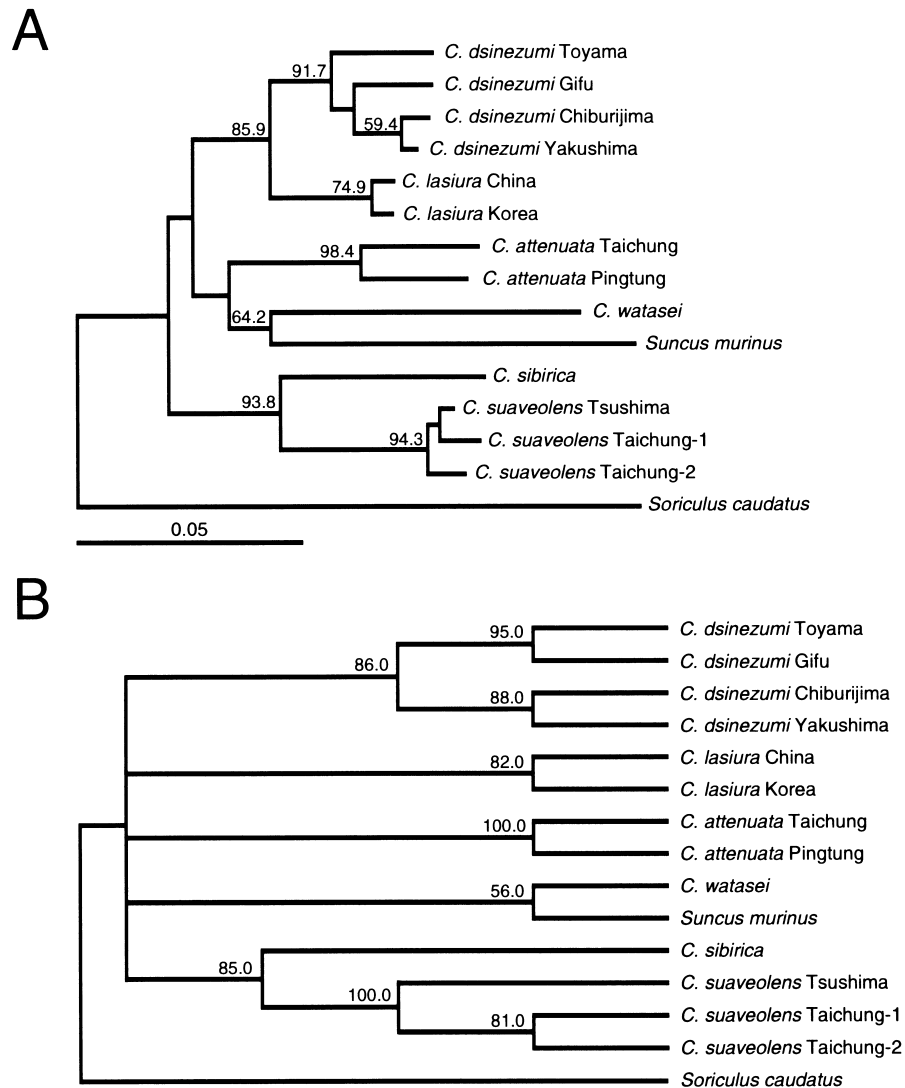
<i>Soriculus caudatus</i>	ATGACCAACATCCGAAAAACCCACCCACTAATAAAAAATCGTTAACAGTTTCATTATTGACCTCCGACCCCATCAACATTTCTTCATGATGAACCTCG
<i>S. murinus</i> Taramajima	.....T.....T.....T.....C..C..C.....A.....A..C.....T.....A.....T..T..T
<i>C. dsinezumi</i> Toyama	....AT..T.....C.....T.....C..T..C.....C.....A..T.....A.....T.....T..T..T
<i>C. dsinezumi</i> Gifu	....AT..T.....T.....T.....C..T..C.....C.....A..T.....A.....T.....T..T..T
<i>C. dsinezumi</i> Chiburijima	....AT.....T.....T..C.....C..T..C.....C.....A..T.....A.....T.....T..T..T
<i>C. dsinezumi</i> Yakushima	....A.....T.....T.....C.....C..T..C.....C.....A..T.....A.....T.....T..T..T
<i>C. lasiura</i> China	....A..T.....T.....T.....T.....C..T..C.....C.....A..T.....A.....T.....T..T..T
<i>C. lasiura</i> Korea	....A..T.....T.....T.....T.....C..T..C.....C.....A..T.....A.....T.....T..T..T
<i>C. attenuata</i> Taichung	....AT.....T..T.....T.....T.....C..T..C..C.....A..C.....A.....G.....T..T..T
<i>C. attenuata</i> Pingtung	....AT.....T..T.....T.....T.....C..T..C..C.....A..C.....A.....G.....T..T..T
<i>C. watasei</i> C.Ryukyus	....A.....T.....T..T..T.....T..A.....C..C..C.....T.....A..C.....T.....A.....T..T..T
<i>C. suaveolens</i> Tsushima	.....T..T.....T.....T.....C..C..C.....T..A.....A..T.....C..A.....T.....T..T..T
<i>C. suaveolens</i> Taichung-1	.....T..T.....T.....T.....C..C.....T..A.....A..T.....C..A.....T.....T..T..T
<i>C. suaveolens</i> Taichung-2	.....T..T.....T.....T.....C..C.....T..A.....A..T.....C..A.....T.....T..T..T
<i>C. sibirica</i> Russia	.....T.....A.....T.....T.....C..T.....T..A.....A..C.....C..A.....T.....T..T..T
<i>Soriculus caudatus</i>	GCTCCTTCTAGGTATCTGCTTAATCATTCAAATCCTAACTGGCCTTCTCTAGCAATACATTACAGTCCGACACCACAACCTGCCTTCTCCTCGCTCAC
<i>S. murinus</i> Taramajima	.T.....A..C..A.....TGCG.....T..C.....A.....T.....T..A.....A.....T..T.....A.....A.....
<i>C. dsinezumi</i> Toyama	.....A.....A.....TGCA.....C..AT..A..TT.....T.....T..A..G..T.....T..A..T.....A.....
<i>C. dsinezumi</i> Gifu	.....A.....A.....TGCA.....C..AT..A..TT.....T.....T..A..G..T.....T..A..T.....A.....
<i>C. dsinezumi</i> Chiburijima	.....A.....A.....TGCA.....C..AT..A..TT.....T.....T..A..G..T.....T..A..T.....A.....
<i>C. dsinezumi</i> Yakushima	.....A.....A.....TGCA.....C..AT..A..TT.....T.....T..A..G..T.....T..A..T.....A.....
<i>C. lasiura</i> China	....AT.....A..T.....TGCA.....C..AT..A..TT.....T.....C.....A..A..T..T.....A..T.....
<i>C. lasiura</i> Korea	....AT.....A..T.....TGCA.....C..AT..A..TT.....T.....C.....A..A..T..T.....A..T.....
<i>C. attenuata</i> Taichung	.....T..AT.....C.....T.....TGCA.....C..A.....A.....T.....C.....A..A..T.....T.....A.....T.....
<i>C. attenuata</i> Pingtung	.....T..AT.....C.....T.....TGCA.....C..A.....A.....T.....C.....A..A..T.....T.....A.....T.....
<i>C. watasei</i> C.Ryukyus	.T.....T..A.....A.....T.....T.....T..A..TT.....C.....C.....A..A..T.....T.....A.....T.....
<i>C. suaveolens</i> Tsushima	.....T..A.....A..T.....TGCA.....T.....C..AT..A..TT.....T.....C..T..A..A..T.....T.....A.....T.....
<i>C. suaveolens</i> Taichung-1	.....T..A.....A..T.....GCA.....T.....C..AT..A..T.....T.....C.....A..A.....T.....A.....T.....
<i>C. suaveolens</i> Taichung-2	.....T..A.....A..T.....GCA.....T.....C..AT..A..T.....T.....C.....A..A.....T.....A.....T.....
<i>C. sibirica</i> Russia	.....T..A.....A..T.....A.....C.....A..A..TT.....T..G..C..T..A.....T.....T.....A.....T.....
<i>Soriculus caudatus</i>	CCACATTTGCCGAGATGTAATAATACGGTTGACTAATTCGGTACTTACATGCAAAATGGAGCATCCATATTCCTTATCTGTCTTCTCTTACAGTAGGACGA
<i>S. murinus</i> Taramajima	A.....C..T.....C.....T..C.....T.....T..T..G..C.....C.....T.....T.....T..C..T..T..C..T..G..C.....
<i>C. dsinezumi</i> Toyama	T..T.....C..T..C.....C..A..C..T..C..C..C.....C.....T..C..T..C..T.....C.....C.....
<i>C. dsinezumi</i> Gifu	T..T.....C..T..C.....C..A..C..T..C..C..C.....C.....T..C..T..C..T.....C.....C.....
<i>C. dsinezumi</i> Chiburijima	T.....C..T..C.....C..A..TC..T..C..C..C..G..C.....T..C..T..C..T.....T.....C.....
<i>C. dsinezumi</i> Yakushima	T.....C..T..C.....C..A..C..T..C..C..C..G..C.....T..C..T..C..T.....T.....C.....
<i>C. lasiura</i> China	T.....C..T.....C..A..TC..T..C..C..C.....C.....T..C..T.....C.....C.....
<i>C. lasiura</i> Korea	T.....C..T.....C..A..TC..T..C..C..C.....C.....T..C..T.....C.....C.....
<i>C. attenuata</i> Taichung	A..T..C..T.....T.....C.....C..A..TC..T..C.....C.....T..C..T..C..A.....T.....C.....
<i>C. attenuata</i> Pingtung	A..T..C..T.....T.....C.....C..A..TC..T..C.....C.....T..C..T..C..A.....T.....C.....
<i>C. watasei</i> C.Ryukyus	A.....C..T.....C.....T..C..T.....C..A..TC..T..C..C.....C.....T.....T.....C..A..T..C.....G.....
<i>C. suaveolens</i> Tsushima	A..T.....C.....C..T..C.....C..A..C..C.....T..C..T..C..T.....T..C..T..CT..A.....C.....
<i>C. suaveolens</i> Taichung-1	A..T.....C.....C..T..C.....C..A..TC..C.....T..C..T..C..T.....T..C..T..CT..A.....C.....
<i>C. suaveolens</i> Taichung-2	A..T.....C.....C..T..C.....C..A..TC..C.....T..C..T..C..T.....T..C..T..CT..A.....C.....
<i>C. sibirica</i> Russia	A..T..C.....C.....T.....A..TC..C..C..T..C..T.....T.....T..C..A.....C.....C.....
<i>Soriculus caudatus</i>	GGCCTCTACTACGGCTCTTATATGTTTATAGAAACATGAAATATTGGCGTATTACTCTTATTCGCAGTGATAGCCACAGCCTTTATAGGGTACGTACTAC
<i>S. murinus</i> Taramajima	..AT..T..T..T.....C.....A..C..T.....C..T..C.....T.....A.....T..C..A..C.....A..T..T..C.....
<i>C. dsinezumi</i> Toyama	..G.....T.....A..A..C..T.....C.....T..CC.....G..C.....T..T.....C.....C.....
<i>C. dsinezumi</i> Gifu	..G.....T.....A..A..C..T.....C.....T..CC.....G..C.....T..T.....C.....C.....
<i>C. dsinezumi</i> Chiburijima	..G.....T..T.....C..A..C..T.....C.....T..CC.....G.....T.....T.....A..T..T..C.....
<i>C. dsinezumi</i> Yakushima	..G.....T..T.....C..A..C..T.....C.....T..CC.....G.....T.....T.....A..T..T..C.....
<i>C. lasiura</i> China	..A.....T..T.....C..A..A..C..T.....C.....T..CC.....T.....C.....T..T.....T..C.....
<i>C. lasiura</i> Korea	..A.....T..T.....C..A..A..C..T.....C.....T..CC.....T.....C.....T..T.....T..C.....
<i>C. attenuata</i> Taichung	..A..T..T..T..T..C..C..A..A..C..T.....C.....T..CC.....T.....T.....T..C.....T.....T..C.....
<i>C. attenuata</i> Pingtung	..A..T..T..T..T..T.....C..A..A..C..T.....C.....T..TC.....T.....T.....T.....T.....T.....T..C.....
<i>C. watasei</i> C.Ryukyus	..A..T..T..T.....C.....ACC..T.....C.....CC.....T.....T.....C.....T.....C.....A.....C.....C.....
<i>C. suaveolens</i> Tsushima	.....T..T.....C..A..ACC..T.....C.....T..CC.....C.....A.....A.....T.....T..T..C.....C.....
<i>C. suaveolens</i> Taichung-1	.....T..T.....C..A..ACC..T.....G..C.....T..CC.....A.....A.....T.....T..T..C.....C.....
<i>C. suaveolens</i> Taichung-2	.....T..T.....C..A..ACC..T.....C.....T..CC.....A.....A.....T.....T..T..C.....C.....
<i>C. sibirica</i> Russia	..A..T..T..T.....C..A..A..C..T.....C.....T..CC.....G.....A.....A.....T.....T..T..C.....

*et al.*, 1997; Fang *et al.*, 1997), and *C. fuliginosa* from Malay Peninsula (2n=40, FN=54–58 with intraspecific variation; Ruedi *et al.*, 1990; Ruedi and Vogel, 1995). Dobson (1994) assumed on the basis of fossil evidence that the ancestral form of *C. dsinezumi* had migrated to Japan from eastern China or Korean Peninsula by the middle Pleistocene. In these possible “original regions” of *C. dsinezumi*, two congeneric species, *C. suaveolens* and *C. lasiura*, are currently distributed (Corbet, 1978), of which *C. suaveolens* was considered to be closer to *C. dsinezumi* by several authors (Kuroda, 1934:

The geographic origin of the common ancestor of *C. dsinezumi* and *C. lasiura* also remains uncertain. Some authors (Corbet, 1978; Corbet and Hill, 1992; Hutterer, 1993) considered that the Shanghai form (*C. campuslincolnensis*) in the south of Yangtze River, a border of the Palaearctic and

**Table 2.** Percentage similarities of cytochrome *b* sequences of *Crocidura*, *Suncus*, and *Soriculus caudatus* (outgroup taxon).

Sample	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1. <i>Soriculus caudatus</i>	—														
2. <i>S. murinus</i> Taramajima	79.9	—													
3. <i>C. dsinezumi</i> Toyama	82.8	85.1	—												
4. <i>C. dsinezumi</i> Gifu	82.8	85.1	99.5	—											
5. <i>C. dsinezumi</i> Chiburijima	81.6	86.6	96.0	96.5	—										
6. <i>C. dsinezumi</i> Yakushima	82.6	86.1	96.5	97.0	99.0	—									
7. <i>C. lasiura</i> China	82.8	84.8	93.8	94.3	93.3	93.8	—								
8. <i>C. lasiura</i> Korea	82.3	85.3	93.8	94.3	93.8	94.3	99.0	—							
9. <i>C. attenuata</i> Taichung	80.1	86.8	89.3	89.1	89.3	88.8	91.5	91.0	—						
10. <i>C. attenuata</i> Pingtung	80.6	86.6	89.6	89.6	89.8	89.3	92.0	91.5	99.5	—					
11. <i>C. watasei</i> C. Ryukyus	79.9	86.3	86.6	86.3	86.3	86.1	88.3	87.8	88.1	87.6	—				
12. <i>C. suaveolens</i> Tsushima	80.6	84.1	89.6	89.8	90.3	90.8	88.6	89.6	87.1	87.1	85.6	—			
13. <i>C. suaveolens</i> Taichung-1	80.8	83.8	88.3	88.6	89.6	89.6	88.3	88.8	87.3	87.3	85.3	98.8	—		
14. <i>C. suaveolens</i> Taichung-2	81.1	84.1	88.6	88.8	89.8	89.8	88.6	89.1	87.6	87.6	85.6	99.0	99.8	—	
15. <i>C. sibirica</i> Russia	81.8	85.1	87.6	87.8	88.3	88.3	88.3	88.3	88.3	88.3	85.3	91.8	91.5	91.8	—

**Fig. 3.** The neighbor joining (A) and maximum parsimony (B) trees for *Crocidura* and *Suncus* inferred from the cytochrome *b* gene sequences. *Soriculus caudatus* was used as outgroup. Nodal values indicate percent supports (>50%) for branches in 1000 bootstrap replications.

Oriental mammal faunas in eastern China (Huang, 1985; Zhang *et al.*, 1997), is conspecific with *C. lasiura*. Although the present results do not give any information regarding the relationship of this lineage (*C. lasiura* and *C. dsinezumi*), the high karyological similarity with the Oriental *C. attenuata* and *C. fuliginosa* (see Zima *et al.*, 1998) and the distribution of the species of *C. lasiura* crossing Yangtze River suggest the southern origin of *C. lasiura* and *C. dsinezumi*.

*Crociodura watasei* is an endemic species to the central Ryukyus, which ancestor may have colonized there during the late Miocene (Motokawa, 1999) when the most recent landbridge connected the central Ryukyus to the continent (Ota, 1998). This species is characterized by its unique karyotype with the reduced chromosome number ( $2n=26$ ,  $FN=52$ ) within the genus *Crociodura* (Maddalena and Ruedi, 1994). Nevertheless, the G-banding pattern of *C. watasei* is highly homologous to the typical  $2n=40$ ,  $FN=56$  format (e.g., *C. dsinezumi*), and their differences are thus explained by assuming the occurrences of several Robertsonian fusions and translocations in the process of emergence of the former (Harada *et al.*, 1985). Two geographic groups (i.e., northern and southern groups) are recognized in *C. watasei* on the basis of the morphometric variation (Motokawa *et al.*, 1996), but in the present study no sequence differences were detected between one specimen from Amamioshima in the northern group and two from Okinawajima in the southern group.

The close relationship between *C. sibirica* from Central Asia and *C. suaveolens* from East Asia is well demonstrated in the present study. The standard karyotypes of these two species are same ( $2n=40$ ,  $FN=50$ ), comprising of two meta- or submetacentric, two subtelocentric and 15 acrocentric pairs in autosomes (Grafodatsky *et al.*, 1988; Tsuchiya, 1987; Fang *et al.*, 1997). This karyotypic format is also shared by the Central Asian and European *C. suaveolens* and *C. gueldenstaedtii* (Catzefflis *et al.*, 1985; Zima *et al.*, 1998). Geographic ranges of the *Crociodura* species having the  $2n=40$ ,  $FN=50$  format, almost exclusively spread over the Palaearctic region. But in East Asia, the range of *C. suaveolens* slightly extends to the southern side of Yangtze River in the continent (Zhang *et al.*, 1997) and to Taiwan in the insular region (Fang *et al.*, 1997). Fang *et al.* (1997) discussed that the Taiwanese *C. suaveolens* might have migrated from continent China in no earlier than the Pleistocene. Our results also suggest the recent colonization of *C. suaveolens* to Taiwan from the north, because the genetic divergence among populations of the species from Taiwan and Tsushima is smaller than that observed between *C. dsinezumi* populations.

Butler (1998) supposed from fossil evidence that the common ancestor of the genus *Crociodura* had evolved in Africa during the late Miocene and subsequently migrated to the Eurasian region. Such a view is also supported by the protein and chromosomal data (Maddalena, 1990; Maddalena and Ruedi, 1994). According to the hypothetical scenario of Maddalena and Ruedi (1994), this ancestral stock had the karyotype of  $2n=38$  and  $FN=54$  to 58, characterized by the

occurrence of four metacentric pairs. The  $2n=40$ ,  $FN=56$  karyomorph restricted to the southeastern continent and East Asia is much similar to the hypothetical ancestral format in general arrangement including the presence of four meta- or submetacentric pairs in autosomes. In addition, *C. attenuata* is distantly related to the other species with the  $2n=40$ ,  $FN=56$  karyotypes (*C. dsinezumi* and *C. lasiura*) (Fig. 3), and this suggests the plesiomorphic state of the  $2n=40$ ,  $FN=56$  format in the East Asian *Crociodura*. The common ancestor of the species with the  $2n=40$ ,  $FN=50$  karyomorph (e.g., *C. suaveolens*, *C. sibirica*) might have emerged somewhere in the Palaearctic region from the  $2n=40$ ,  $FN=56$  lineage.

The present molecular and the previous karyological data strongly suggest that the East Asian *Crociodura* is a mixture of southern and northern elements that are characterized by different fundamental numbers in karyotypes ( $FN=56$  and 50, respectively). Future studies based on more species from the Palaearctic and Oriental parts of the continent should be performed to reconstruct the phylogenetic relationships of the genus *Crociodura*.

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## APPENDIX.

Specimens examined in this study.

They are deposited with the numbers given in parentheses. See text for abbreviations of acronyms.

*Crociodura dsinezumi*: Tateyama-machi, Toyama, Japan (OCUMS 3703); Shirakawa-mura, Gifu, Japan (OCUMS 4346); Chiburijima Island, Oki Group, Japan (KUZ-M 951); Yakushima Island, Osumi Group, Japan (KUZ-M 938, 941). *C. lasiura*: Changchun, Jilin Province, China (AGUD 900722–55); Hapcheon Gun, Kyungsangnam Do, Korea (OCUMS 5272). *C. attenuata*: Taichung, Taiwan, R.O.C. (KUZ-M 1121); Pingtung Hsien, Taiwan, R.O.C. (OCUMS 6865, 6866). *C. watasei*: Amamioshima Island, Amami Group, Japan (OCUMS 2470); Okinawajima Island, Okinawa Group, Japan (KUZ-M 419, 440). *C. suaveolens*: Tsushima Island, Japan (KUZ-M 1432); Taichung, Taiwan, R.O.C. (KUZ-M 966, 1127 [Taichung-1], KUZ-M 1128 [Taichung-2]). *C. sibirica*: Academgorodok City, Russia (OCUMS 6410, 6412). *Suncus murinus*: Taramajima Island, Miyako Group, Japan (OCUMS 6882).