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Chromosomal Polymorphism in the Gray Shrew *Crocidura attenuata* (Mammalia: Insectivora)

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ABSTRACT—Conventional and G-banded karyotypes of *Crocidura attenuata* Milne-Edwards, 1872 from Guangdong, southern China, are reported. The diploid chromosome number (2n) varied from 35 to 38 among specimens, while the fundamental arm number (FN) consistently was 54. Of the autosomes, 14 pairs including four meta- or submetacentric, three subtelocentric, and seven acrocentric pairs showed no variation in all specimens, whereas the remaining pairs showed Robertsonian polymorphism. The X and Y chromosomes were medium sized submetacentric and small acrocentric chromosomes, respectively. These karyotypes differ from that of *C. attenuata* from Taiwan, which has 40 chromosomes with 56 arms. Such differences are largely attributable to a non-Robertsonian rearrangement, where both 2n and FN values are different from each other. The largest metacentric pair observed in karyotypes from Guangdong may have resulted from the centromere-telomere translocation between an acrocentric and a subtelocentric pairs of karyotype homologous to that from Taiwan. Both morphometric difference and sequence divergence in mitochondrial cytochrome *b* gene between samples from Guangdong and Taiwan was relatively small. However, the non-Robertsonian rearrangement assumed between karyotypes of the shrews from Guangdong and Taiwan suggest that they are reproductively isolated from each other. The eastern continental and Taiwanese populations therefore may represent different species under the names, *C. attenuata* (sensu stricto), and *C. tanakae* Kuroda, 1938, respectively.

Key words: Robertsonian polymorphism, centromere-telomere translocation, taxonomy, cytochrome *b* gene, *Crocidura tanakae*

INTRODUCTION

The gray shrew *Crocidura attenuata* Milne-Edwards, 1872 is distributed in the East and Southeast Asia including the southern part of China, Indochina Peninsula and Assam in the Eurasian continent, and in Taiwan, Hainan Island and Batan Island of the Philippines (Hutterer, 1993; Heaney and Ruedi, 1994; Ruedi, 1995; Wolsan and Hutterer, 1998). Although this species was confused with *C. fuliginosa* by several previous authors (e.g., Lekagul and McNeely, 1977), the validity of these two species has been well demonstrated by recent morphological and karyological studies (Heaney and Timm, 1983; Jenkins, 1982; Ruedi *et al*., 1990; Ruedi, 1995; Jenkins and Smith, 1995). On the basis of these studies, Ruedi (1995) resurrected *C. malayana*, once synonymized to *C. fuliginosa*, as a valid species and Jenkins and Smith (1995) described *C. hilliana* as a new species similar to *C. attenuata* and *C. fuliginosa*. Thus, at least four species, *C. attenuata*, *C. fuliginosa*, *C. malayana*, and *C. hilliana* are currently recognized for the medium-sized shrews from the continental part of Southeast Asia (Ruedi, 1995; Jenkins and Smith, 1995).

Of these species, three have been examined karyologically on the basis of continental samples, yielding different combinations of diploid chromosome number (2n) and total number of chromosome arms (FN): in *C. fuliginosa* from the Malay Peninsula 2n and FN are 40 and 54–58 (Ruedi *et al*.,

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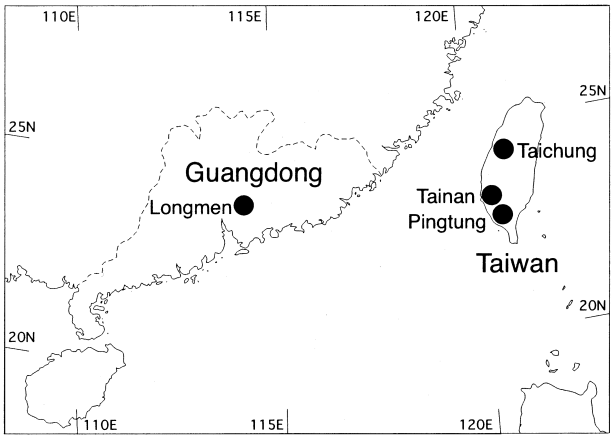

Fig. 1. Sampling localities of *Crocidura attenuata* in Guandong and Taiwan.

1990; Ruedi and Vogel, 1995), whereas those values in *C. malayana* from the Malay Peninsula and *C. hilliana* from Thailand are 2n=38–40 and FN=62–68 (Ruedi *et al*., 1990; Ruedi and Vogel, 1995), and 2n=50 and FN=66 (Motokawa and Harada, 1998), respectively.

Tsuchiya *et al*. (1979) described karyotype of one *C. attenuata* from Thailand as consisting of 2n=50 chromosomes with FN=64. This report, however, probably suffers erroneous identification of material examined (Motokawa *et al*., 1997), and this karyotype closely resembles that of *C. hilliana* as discussed by Motokawa and Harada (1998). The karyotype of *C. attenuata* from Taiwan, on the other hand, shows 2n=40 and FN=56, and consists of three meta- or submetacentric, four subtelocentric, and 12 acrocentric pairs in autosomes (Motokawa *et al*., 1997). It thus resembles the karyotype of *C. fuliginosa* (Motokawa *et al*., 1997; Fang *et al*., 1997). Because karyological examination of *C. attenuata* from the continental part of its range has not been reported, the karyological study of *C. attenuata* is strongly desired to verify Motokawa *et al*.'s (1997) assumption from Tsuchiya *et al*.'s (1979) report.

In this study, we examined the karyotype of *C. attenuata* from Guangdong, southern China (Fig. 1), and found considerable polymorphism, as well as its differentiation from the Taiwanese conspecific karyotype. These variations are attributable to both Robertsonian and non-Robertsonian rearrangement. We also made morphometirc and genetic analyses between the continental and the Taiwanese samples of *C. attenuata* to asess the morphological and genetic divergences between two samples, which are karyologically divergent. The partial sequence data of the mitochondrial cytochrome *b* gene was used for genetic analysis, because it is well studied for the East Asian congeners (Motokawa *et al*., 2000). Then, we discuss the phylogenetic relationships and systematics of the continental and Taiwanese samples of *C. attenuata*.

MATERIALS AND METHODS

Four animals (two males and two females) were captured on August, 2000, from Longmen, Guangdong, southern China (Fig. 1). They were identified as *Crocidura attenuata* on the basis of external and cranial features including overall sizes (Heaney and Timm, 1983; Jenkins and Smith, 1995). For comparisons, 11 specimens of *C. attenuata* from Taichung, Tainan and Pingtung, Taiwan, were also examined (Fig. 1). These specimens are deposited in the Guangzhou University, Guangzhou (GU), the Kyoto University Museum, Kyoto (KUZ), Osaka City University Medical School, Osaka (OCUMS), and Taiwan Endemic Species Research Institute, Chichi (TESRI) (Appendix).

The chromosomal preparations were made by tail or lung tissue cultures following Motokawa *et al*. (1997). The staining technique of G-banding was applied following Seabright (1971). Comparative karyological data for *C. attenuata* from Taiwan, used in this study, was taken from Motokawa *et al*. (1997).

The following standard external measurements were taken with a scale or a dial caliper to nearest 0.1 mm: total length, tail length, ear length, hind foot length without claw, and forefoot length without claw. Head and body length (subtracting tail length from total length) and ratio of tail length to the former were also calculated. Besides these, the following 14 cranial measurements were taken to nearest 0.01 mm using a digital caliper: condyloincisive length, braincase breadth, interorbital breadth, postpalatal depth, rostral breadth, postpalatal length, upper toothrow length, length from the upper fourth premolar to upper third molar, labial length between second upper molars, palatal width at upper third molars, mandibular length from the tip of first incisor to posterior end of condyle, mandibular height at coronoid process, lower toothrow length, and lower molar row length. Of these, the former 10 were measured following Heaney and Timm (1983). Univariate differences between samples from Guangdong and Taiwan were tested with t-test at 5% significant level. Only one measurement (labial length between second upper molars) was tested with Aspin-Welch's t-test because of significant heteroscedasticity between samples.

The initial 402 bp of the mitochondrial cytochrome *b* gene was sequenced for four Guangdong specimens following the procedure of Motokawa *et al*. (2000). The sequence data determined in this study are placed in the DDBJ nucleotide sequence database with the accession number, AB066261. Sequence data of *C. attenuata* from Taiwan and related taxa (genera *Crocidura* and *Suncus*: AB066247-AB066260) were taken from Motokawa *et al*. (2000). A neighbor-joining tree (Saitou and Nei, 1987) was constructed based on pairwise distances calculated with the two-parameter model of Kimura (1980). To assess the degree of supports for internal branches, 1000 bootstrap replicates (Felsenstein, 1985) were made by using PHYLIP package version 3.5c (Felsenstein, 1993). Sequence data for *Soriculus caudatus* reported by Ohdachi *et al*. (1997) were incorporated into the analysis as those of an outgroup.

RESULTS

The conventional karyotypes of *C. attenuata* from Guangdong are shown in Fig. 2 (left). Three different karyotypes were recognized from the four specimens, in which the diploid number (2n) varied from 35 to 38, but the fundamental number (FN) was constant at 54. One specimen (GU, collector's number 3380: 64 cells examined) had 2n=38 chromosomes consisting of eight meta- or submetacentric, six subtelocentric, and 22 acrocentric autosomal chromosomes (Fig. 2A). Two specimens (GU, 3384, 3385: 34 and 53 cells examined, respectively) had 2n=36 chromosomes consisting of ten meta- or submetacentric, six subtelocentric, and 18 acrocentric autosomal chromosomes (Fig. 2B). The last individual (GU, 3372: 55 cells examined) had 2n=35 chromosomes consisting of eleven meta- or submetacentric, six subtelocent-

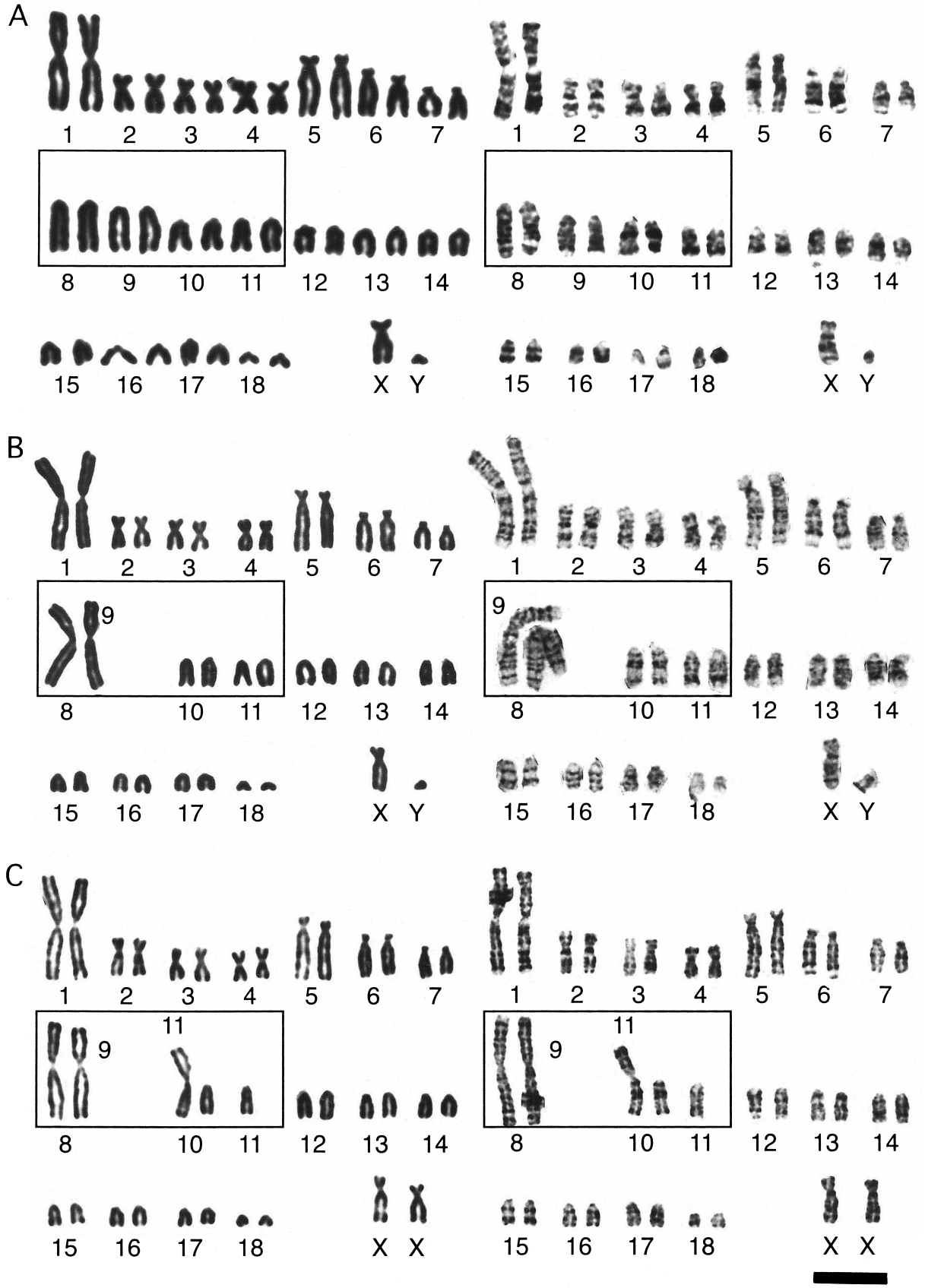

Fig. 2. The polymorphic conventional (left) and the G-banded (right) karyotypes of *Crocidura attenuata* from Guangdong. A: 2n=38 karyotype (GU, collector's number 3380), B: 2n=36 karyotype (GU, 3385), C: 2n=35 karyotype (GU, 3372). The polymorphic pairs are enclosed in rectangle frames. The bar represents 10 μm.

ric, and 16 acrocentric autosomal chromosomes (Fig. 2C). The X and Y chromosomes did not show significant individual variation and were medium-sized submetacentric chromosome and the smallest acrocentric chromosome, respectively (Fig. 2).

We tentatively considered 2n=38 karyotype (Fig. 2A) to be a standard format for *C. attenuata* from Guangdong and arranged chromosomes accordingly. The G-banded karyotypes (Fig. 2 right) indicated that intrapopulational polymorphisms are attributable to two Robertsonian variations. For nos. 8 and 9 arms, one specimen (3380) was homozygous for the unfused, twin acrocentric state (Fig. 2A). On the other hand, the remaining three (3384, 3385, and 3372) were homozygous for a metacentric state caused by Robertsonian translocations (Fig. 2B and 2C). For nos. 10 and 11 arms, three shrews (3380, 3384, 3385) were homozygous for the unfused, twin acrocentric state (Fig. 2A and 2B), whereas the remaining one (3372) was heterozygous with one metacentric and two acrocentric chromosomes (Fig. 2C).

Homologous G-bands corresponded well between the karyotypes from Guangdong and Taiwan (Fig. 3). In the karyotype from Taiwan, pairs 8, 9, 10 and 11, corresponding to the same numbers of pairs in the 2n=38 karyotype from Guangdong, respectively, were also acrocentric and showed no Robertsonian polymorphisms (Motokawa *et al.*, 1997).

The karyotypes from Guangdong differed from the karyotype from Taiwan in having a large metacentric chromosome pair (no. 1 in Fig. 2). This large metacentric pair showed homologous G-bands with one of the two largest subtelocentric pairs and the fifth largest acrocentric pair (nos. 5 and 12 in Motokawa *et al.* [1997], respectively) of the karyotype from Taiwan. This difference made the FN in the karyotypes from Guangdong (54) smaller than that from Taiwan (56). The remaining chromosome pairs from Guangdong (nos. 2–7, 12–18) were morphologically similar to the corresponding pairs from Taiwan (Fig. 3). The X chromosomes from Guangdong

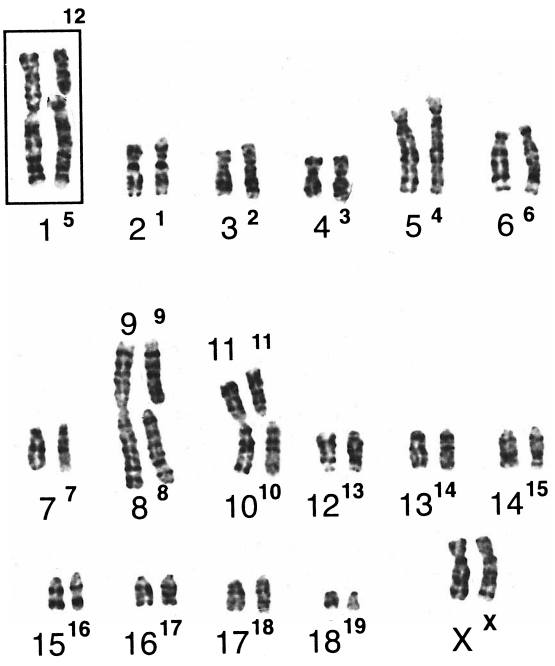

Fig. 3. Comparison of the G-banded karyotypes of *Crocidura attenuata* from Guangdong (2n=35; GU, collector's number 3372: left), and Taiwan (2n=40, OCUMS 6865; Motokawa *et al.*, 1997: right). Numbers in small font indicate chromosome pair numbers used in our previous report on the karyotype of the Taiwanese population (Motokawa *et al.*, 1997). A pair of chromosomes showing centromere-telomere translocation is enclosed in a rectangle frame.

Table 1. The external and cranial measurements of *Crocidura attenuata* from Guangdong and Taiwan. The means ± SD, and ranges (in the parentheses) are presented in milimeters.

Measurements	Guangdong (N=4)		Taiwan (N=11)		Difference* *P*-value
Head and body length	81.13±3.64	(77.5–86.0)	73.36±6.67	(64.5–84.5)	ns
Tail length	55.75±3.66	(50.5–59.0)	52.50±4.22	(45.0–60.5)	ns
Tail length ratio (%)	68.74±3.92	(63.5–72.9)	69.06±6.31	(57.0–79.4)	—
Ear length	9.08±0.74	(8.4–9.9)	9.05±0.91	(7.9–10.2)	ns
Hind foot length	12.85±0.68	(12.2–13.8)	13.05±0.54	(12.0–13.8)	ns
Forefoot length	8.55±0.30	(8.2–8.8)	8.85±0.29	(8.3–9.3)	ns
Condyloincisive length	20.42±0.77	(19.66–21.40)	20.85±0.41	(19.94–21.31)	ns
Braincase breadth	9.19±0.31	(8.85–9.58)	9.22±0.22	(8.87–9.50)	ns
Interorbital breadth	4.46±0.17	(4.23–4.60)	4.62±0.10	(4.47–4.74)	0.041
Postpalatal depth	3.90±0.25	(3.73–4.27)	3.89±0.13	(3.70–4.09)	ns
Rostral breadth	2.64±0.18	(2.42–2.85)	2.68±0.10	(2.52–2.86)	ns
Postpalatal length	9.18±0.38	(8.94–9.75)	9.18±0.30	(8.55–9.62)	ns
Upper toothrow length	8.93±0.23	(8.65–9.17)	9.33±0.19	(8.87–9.54)	0.005
Length from P^4 to M^3	5.15±0.15	(4.94–5.30)	5.36±0.16	(4.97–5.61)	0.044
Labial length between M^2s	6.31±0.32	(6.00–6.74)	6.41±0.13	(6.20–6.58)	ns
Palatal width at M^3	2.58±0.12	(2.41–2.71)	2.66±0.08	(2.53–2.83)	ns
Mandibular length	12.71±0.44	(12.23–13.25)	13.10±0.33	(12.31–13.52)	ns
Mandibular height	4.93±0.29	(4.65–5.33)	4.97±0.18	(4.57–5.19)	ns
Lower toothrow length	8.18±0.19	(7.93–8.40)	8.46±0.26	(7.93–8.72)	ns
Lower molar row length	4.48±0.21	(4.21–4.72)	4.36±0.10	(4.10–4.44)	ns

*ns: not significant

Table 2. Variable sites of the initial 402 bp of mitochondrial cytochrome *b* gene of *Crocidura attenuata* from Guangdong and Taiwan (Taichung and Pingtung).

Haplotype	Variable site				
	24	151	198	318	321
Guangdong (4 specimens)	C	T	C	C	T
Taichung (1 specimen, Motokawa *et al.*, 2000)	T	C	T	C	C
Pingtung (2 specimens, Motokawa *et al.*, 2000)	T	C	C	T	C

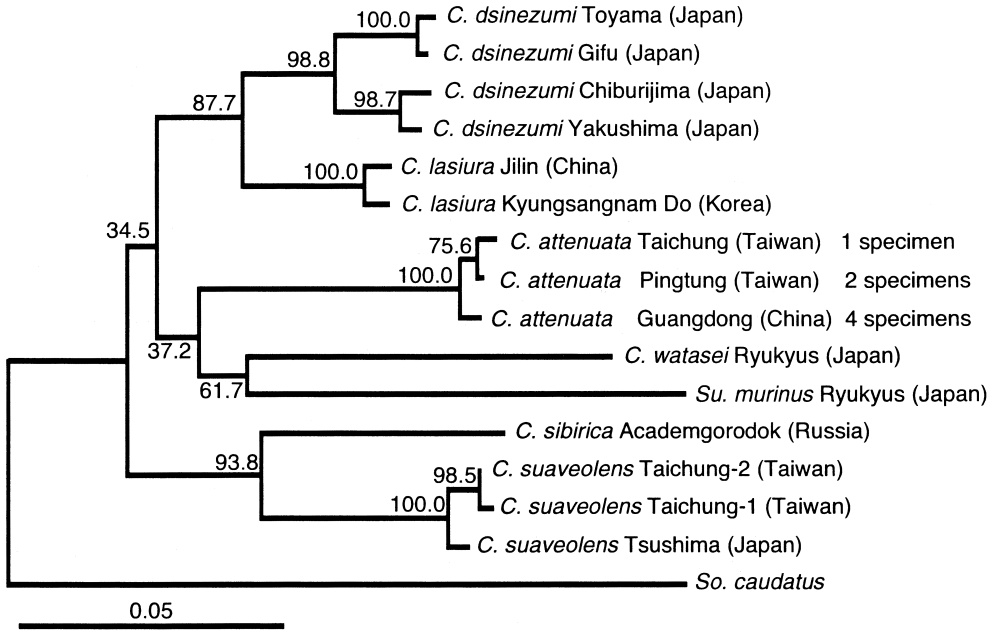

Fig. 4. Neighbor-joining tree for several East Asian *Crocidura* species, including *C. attenuata* from Guangdong, inferred from the initial 402 bp of the mitochondrial cytochrome *b* gene. Nodal values indicate percent support in 1000 bootstrap replicates.

and Taiwan were both submetacentric and similar to each other in size and G-band pattern (Figs. 3). The Y chromosome was a minute acrocentric in both karyotypes (Fig. 2 and Motokawa *et al.*, 1997).

Morphological measurements of *C. attenuata* from Guangdong and Taiwan were listed in Table 1. Measurements in specimens from Guangdong broadly overlapped those from Taiwan. The t-test revealed only three measurements as significantly different between the two samples (Table 1). These were interorbital breadth, upper toothrow length, and length from upper fourth premolar to upper third molar. In these measurements, the mean values from Guangdong sample were smaller than those from Taiwan.

The initial 402 bp of mitochondrial cytochrome *b* gene showed no individual variation among four specimens from Guangdong, and only slight variation between samples from Guangdong and Taiwan, or within samples from Taiwan (Table 2). For Taiwanese samples, those from Taichung and Pingtung differed at two sites (site numbers 198 and 318, 0.5% difference). Four sites (1.0%) differed between samples from Guangdong and Taichung (site numbers 24, 151, 198, and 321), or between the former and sample from Pingtung (site numbers 24, 151, 318, and 321). All mutations were transitions at silent positions. The neighbor-joining tree (Fig. 4) showed that the haplotype of Guangdong was joined with the cluster consisting of two haplotypes from Taiwan (Taichung and Pingtung) in all bootstrap replicates (100%). The tree topology among species of *Crocidura* was concordant with that provided in our previous report (Motokawa *et al.*, 2000).

DISCUSSION

Maddalena and Ruedi (1994) proposed the hypothetical ancestral karyotype of the genus *Crocidura* with 2n=38 and FN=54-58 on the basis of the G-banded karyotype comparison among several species from the Palaearctic, Oriental, and Afrotropical regions. In this work, they recognized two clades (the African clade and the Palaearctic-Oriental clade) showing differential evolutionary trends within the genus *Crocidura*. Of these, the African clade was characterized by increasing 2n and FN from the ancestral karyotype, while the Palaearctic-Oriental clade was assumed to have karyotypes with stable or decreasing 2n from the ancestral condition (Maddalena and Ruedi, 1994). The presumptive standard karyotype of *C. attenuata* from Guangdong, twin acrocentric state in two Robertsonian polymorphic pairs (Fig. 2A), 2n and FN values identical with those of the hypothetical ancestral karyotype of the genus (see above), and agree well with the evolutionary

scheme proposed by Maddalena and Ruedi (1994).

The karyotypes of *C. attenuata* from Guangdong are different from the reported karyotypes of medium sized shrews on the Southeast Asia including *C. fuliginosa* (2n=40, FN=54–58: Ruedi *et al.*, 1990; Ruedi and Vogel, 1995), *C. malayana* (2n=38–40, FN=62–68: Ruedi *et al.*, 1990; Ruedi and Vogel, 1995), and *C. hilliana* (2n=50, FN=66; Motokawa and Harada, 1998). Thus, karyological information may be useful as one of keys for species identification among those species that have been often confused taxonomically.

The present results also demonstrate extensive differences between karyotypes of specimens from Guangdong and Taiwan, both identified as *C. attenuata*. The karyotype from Guangdong is characterized by having a distinctly enlarged metacentric pair (no. 1), which is homologous in G-bands to a subtelocentric (no. 5) and an acrocentric (no. 12) pairs in the karyotype from Taiwan. Because 2n=40 and FN=56 karyomorph occurs in both the *C. attenuata* lineage, which is supported by a 100% bootstrap value, and the *C. lasiura-C. dsinezumi* lineage with a 87.7% bootstrap value in the molecular phylogenetic tree (Fig. 4), the 2n=40 and FN=56 karyomorph is thought to represent the ancestral condition of these two lineages. Therefore, the largest no. 1 metacentric pair in karyotypes from Guangdong is thought to have been derived from 2n=40 and FN=56 karyomorph by the translocation with fusion between the telomere region of a subtelocentric chromosome and the centromere region of an acrocentric chromosome (Fig. 3). This non-Robertsonian chromosomal rearrangement induces changes in both 2n and FN numbers between the karyotypes from Guangdong and Taiwan.

Tandem translocations seem to be rare among closely related species of wild mammals. They have been reported only for limited groups, such as the cotton rats (Elder, 1980), muntjacs (Shi *et al.*, 1980), crocidurine and soricine shrews (Harada *et al.*, 1985; Zima *et al.*, 1998; Biltueva *et al.*, 2001), arvicoline voles (Modi, 1987), and phyllotine rodents (Walker and Spotorno, 1992). These chromosomal rearrangements are considered to be an important cue for speciation process in wild populations (e.g., King, 1993). The heterozygosity for telomere-centromere translocation is thought to give the most considerable impact on fertility among the structural chromosomal rearrangements (Walker and Spotorno, 1992; King, 1993), since the theory predicts that a heterozygote for only one telomere-centromere translocation will produce 50% unbalanced gametes (duplication or deficiency) (White, 1973; Walker and Spotorno, 1992; King, 1993). Such a high level of infertility may have an important reproductive isolation effect between the karyomorphs involved (White, 1973; King, 1993).

Therefore, we consider the populations from Guangdong and Taiwan, which differ by such centromere-telomere translocation, are probably reproductively isolated not only by their allopatric distribution but also by post-mating isolating mechanisms. Therefore, they most likely represent different species according to the biological species concept (Mayr and Ashlock, 1991). Considering the results of phylogenetic analysis of cytochrome *b* sequences (Fig. 4), as well as the fact that they showed less than 1% difference of cytochrome *b* gene sequences (Table 2), it is obvious that these two species are phylogenetically very close to each other. The low level of morphological divergence (Table 1) also supports a very recent common ancestory of these two species.

The species from Guangdong can be identified as *C. attenuata*, as this taxon was originally described by Milne-Edwards (1872) from Moupin, central Sichuan, continental China (Corbet and Hill, 1992). On the other hand, the species from Taiwan should be referred to *C. tanakae*, which was originally described by Kuroda (1938) from Taiwan and subsequently considered as a subspecies (Ellerman and Morrison-Scott, 1951; Jameson and Jones, 1977; Fang *et al.*, 1997) or a junior synonym (Corbet and Hill, 1992; Hutterer, 1993) of *C. attenuata*. The holotype of *C. tanakae* was presumably destroyed by the fire during the World War II in Tokyo (Imaizumi, 1962), but it is possible to examine the remaining eight specimens used in the original description of *C. tanakae*, currently deposited in the Yamashina Institute for Ornithology in Abiko (YIO 701-708). Morphological reexamination of these two species (*C. attenuata* and *C. tanakae*) is strongly desired to produce identification keys between them. This study has failed to identify good discriminating characters between these two species (Table 1).

The telomere-centromere translocation, forming a large metacentric pair from a subtelocentric and an acrocentric pairs of 2n=40 and FN=56 karyomorph in the genus *Crocidura*, was also reported in two pairs of chromosomes in *C. watasei* from the Ryukyu Archipelago, Japan, by Harada *et al.* (1985). However, observations by G-banding show that two pairs of large metacentric chromosomes in *C. watasei* (nos. 2 and 3 in Harada *et al.*, 1985) were homologous to another combination of chromosomes of *C. tanakae*, i.e., no. 5 subtelocentric and no. 10 acrocentric pairs, and no. 4 subtelocentric and no. 12 acrocentric pairs of the latter (Harada *et al.*, 1985; Motokawa *et al.*, 1997). Therefore, the formation of large metacentric autosomal pairs by the centromere-telomere translocation may have occurred independently in *C. attenuata* and *C. watasei*. This view is not discordant with the molecular phylogenetic relationships (Fig. 4) and the chromosome phylogeny (Biltueva *et al.*, 2001) that indicate that these two species (*C. attenuata* and *C. watasei*) are not monophyletic.

The intrapopulational karyological variation seems to be very rare within the subfamily Crocidurinae to which the genus *Crocidura* belongs (Zima *et al.*, 1998). Robertsonian polymorphisms have been reported only in *C. hutanis* and *C. lepidura* from Sumatra (Ruedi and Vogel, 1995), in *C. russula* from a Mediterranean island (Vogel *et al.*, 1992), and in *C. suaveolens* from Japan (Tsuchiya, 1987). In this study, extensive Robertsonian polymorphisms were observed among specimens from Guangdong (Fig. 2). These polymorphic pairs of chromosomes had long arms corresponding to the largest four acrocentric pairs of *C. tanakae*. Intrapopulational Robertsonian polymorphism is generally thought produced by chromosomal mutation or by hybridization between two or more geographical chromosomal races (Searle and

Wójcik, 1998). Because the karyotype of *C. attenuata* is thought to have been derived from the karyotype of *C. tanakae* with 2n=40 and FN=56 as discussed above, the twin acrocentric states may be the ancestral condition of both two pairs showing the Robertsonian polymorphism. Since no variation was detected in the cytochrome *b* gene sequence among individuals from Guangdong, it is more likely that these Robertsonian variations are transmitted within the same random mating unit. Further intensive karyological study of *C. attenuata* in the continent should be carried out to clarify the current status of the chromosomal polymorphism and to discuss the evolutionary history of the chromosomal changes in this species.

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APPENDIX

Specimens examined in this study.
They are deposited under the numbers given in parentheses. See text for abbreviations of acronyms.
Pingling, Longmen Prefecture, Guangdong, 23.40N, 114.20E (GU, 2 uncataloged specimens with collector's numbers 3372 female, 3380 male); Longmen, Longmen Prefecture, Guangdong, 23.43N, 114.13E (GU, 2 uncataloged specimens with collector's numbers 3384 female, 3385 male); Tunghai University, Taichung City, Taiwan, 24.11N, 120.35E (KUZ-M 967 male, 977 female, 978 male, 1126 male); Takeng, Taichung City, Taiwan, 24.11N, 120.45E (KUZ-M 1121 female); Taidusan, Lungchin, Taichung Prefecture, Taiwan, 24.12N, 120.35E (TESRI, 1 uncataloged specimen, female); Neipu, Pingtung Prefecture, Taiwan, 22.37N, 120.35E (OCUMS 6865 female, 6866 male); Kueijen, Tainan Prefecture, Taiwan, 22.58N, 120.17E (KUZ-M 755 female, 884 female, 887 male).