

# Origins and Founder Effects on the Japanese Masked Palm Civet *Paguma larvata* (Viverridae, Carnivora), Revealed from a Comparison with its Molecular Phylogeography in Taiwan

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The source areas of the Japanese populations of the masked palm civet *Paguma larvata* (Viverridae, Carnivora), an alien species in Japan, have not been identified. In the present study, to reveal their origins and genetic features, we determined the full mitochondrial DNA cytochrome *b* sequences (1,140 base-pairs) of a total of 206 individuals of *P. larvata* from the Honshu and Shikoku islands of Japan (186 animals) and Taiwan (20 animals), and investigated their molecular phylogeography and the genetic relationships between populations in these countries. We found that each animal from Japan exhibited one of four haplotypes (JA1, JA2, JA4, and JA5), and that JA1 and JA4 were more frequent in eastern Honshu and Shikoku—central Honshu, respectively. By contrast, six haplotypes consisting of four new types (TW1, TW2, TW3, and TW4) and the previously reported two types (JA1 and JA4) were identified from 20 animals from native populations in Taiwan. Within Taiwan, one haplotype set (JA1, TW1, and TW2) was distributed in the western region, while a second (JA4, TW3, and TW4) was found in the eastern region; these regions are separated by high mountain ranges. Our comparison of haplotype distributions strongly demonstrated that the eastern Japanese populations originated from animals of western Taiwan, and that the western Japanese populations originated from those of eastern Taiwan. In addition, the lower genetic variability and particular distribution patterns of haplotypes in Japan showed founder effects, which may have resulted from multiple introductions of *P. larvata* to Japan from Taiwan.

**Key words:** founder effects, Japan, masked palm civet, mitochondrial DNA phylogeography, origin, *Paguma larvata*, Taiwan

## INTRODUCTION

Globalization of human activities increases the chances of artificial migration of organisms from their native habitats to new environments, and the number of such introduced and non-native organisms (called “alien species”) around

the world. When alien species are able to adapt themselves to their new environments, they have the potential to expand their habitat range widely, and to invade foreign ecosystems.

In Japan, the masked palm civet *Paguma larvata* (Viverridae, Carnivora, Mammalia) is considered an alien mammalian species (Torii 2009). This species is a medium-sized mammal, and is natively distributed in the region from Bangladesh, through Southeast Asia, to China, Taiwan, and Indonesia (Ewer, 1973; Lekagul and McNeely, 1988; Corbet

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and Hill, 1992; Wozencraft, 1993; Torii, 2009). On the four main islands of the Japanese archipelago, namely, Hokkaido, Honshu, Shikoku, and Kyushu, their distribution is sporadic, and they are not found in Kyushu, where many endemic mammals to Japan can be found. Furthermore, no fossils of *P. larvata* have been recorded from any geologic layer in Japan (Torii, 2009), and this species has also never been reported in any archaeological remains. Because *P. larvata* is omnivorous (Torii, 1986), it is thought to be very adaptive to environments in Japan. For these reasons, *P. larvata* can be regarded as an alien species.

Although the origin of this species in Japan is a long-standing mystery, recent studies have begun to shed light into its origins. Masuda et al. (2008) studied the molecular phylogeny of mitochondrial DNA (mtDNA) cytochrome *b* of this species from Japan and Southeast Asia, and reported the genetic differentiations between them as well as the variations within Japan; the origin of the Japanese populations, however, could not be identified. Patou et al. (2009) examined partial sequences of the mtDNA cytochrome *b* and control region of *P. larvata* in a wide area of Asia, and reported that sequences of three animals of Japan were very closely related to that of one animal of Vietnam and that of one animal of Taiwan, suggesting an Indo-Chinese origin for the Japanese *P. larvata*. But Patou et al. (2009) did not find any identical haplotypes between Japan and those countries, making it impossible to resolve the precise region of origin for Japan's *P. larvata*.

In the present study, we determined the full cytochrome *b* sequences (1,140 base-pairs, bp) for animals from native populations in Taiwan and non-native populations in Japan, and investigated the molecular phylogeography of *P. larvata* based on these genetic data. We further discuss the geographic isolation of Taiwanese populations by mountains,

and the precise origins and founder effects in the Japanese populations.

## MATERIAL AND METHODS

### Samples and DNA extraction

A total of 186 animals from Japan and 20 animals from Taiwan were examined in the present study (Table 1 and Fig. 1). Tissue samples and sources were as follows: muscle tissues obtained from road kills or pest control (58 samples from Ibaraki Nature Museum; 16 from Tochigi Prefectural Museum; one from Dr. Yayoi Kaneko; 45 from Gunma Museum of Natural History; five from Nagano Environmental Conservation Research Institute; four from Iida City Museum; 48 from Shikoku Institute of Natural History; six from the National Museum of Natural Science of Taiwan; eight from the Endemic Species Research Institute of Taiwan); feces from zoo animals (two samples from Morioka Zoological Park); and blood collected from captured animals in ecological surveys (six samples from National Pingtung University of Science and Technology) were preserved in 70–99% ethanol at room temperature until use. Hairs were collected from seven captured animals from Nihondaira Zoo.

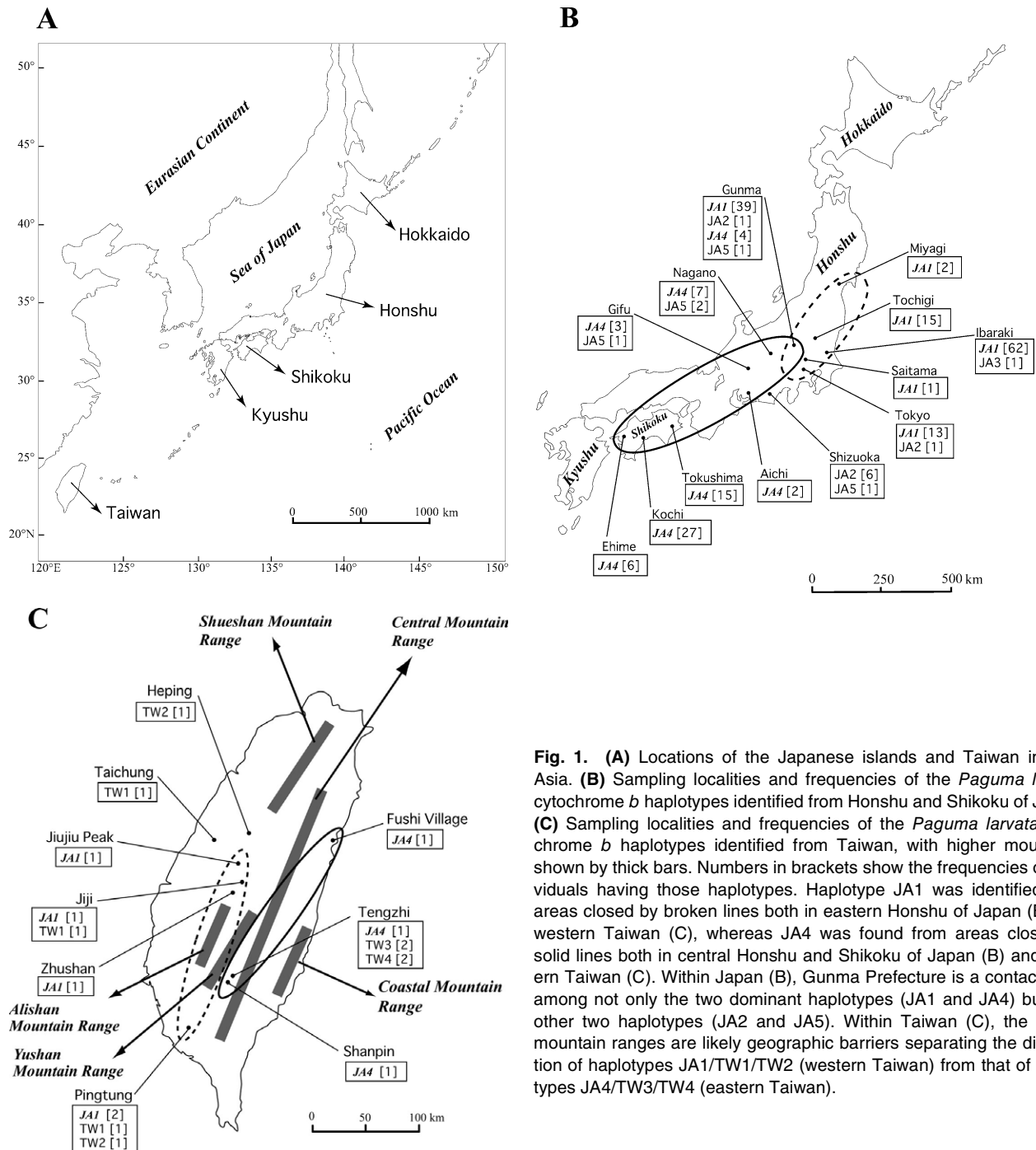
Total DNA was extracted from muscle and blood samples using the DNeasy Tissue & Blood Kit (Qiagen), feces using the QIAamp Stool Mini Kit (Qiagen), and hairs using the QIAamp DNA Micro Kit (Qiagen) following the manufacturer's instructions, and preserved in 100–200 µl of TE buffer. An aliquot (1–5 µl) of the DNA extract per animal was used as a template in the subsequent polymerase chain reaction (PCR) analysis, described below.

### PCR amplification, nucleotide sequencing, and data analysis

PCR amplification of the entire region (1,140 bp) of mtDNA cytochrome *b* gene and nucleotide sequencing was performed using the method same as in Masuda et al. (2008). For analysis, the nine haplotypes (JA1–JA5 and SE1–SE4: accession nos. AB303951–AB303959 in DDBJ/Genbank/EMBL databases) identified from 24 animals of Japan and five animals of Southeast Asia, all of which have been reported by Masuda et al. (2008), were added to the data analysis in the present study. A sequence align-

**Table 1.** Frequencies of masked palm civet cytochrome *b* haplotypes identified from Japan, Taiwan and Southeast Asia in the present and previous studies. Numerals in parentheses are the numbers of individuals cited from Masuda et al. (2008), and they are included in the left numbers.

Pref. in Japan or country	Regional group	Number examined	Cytochrome <i>b</i> haplotypes												
			JA1	JA2	JA3	JA4	JA5	TW1	TW2	TW3	TW4	SE1	SE2	SE3	SE4
Miyagi	Eastern Honshu	2	2	–	–	–	–	–	–	–	–	–	–	–	–
Ibaraki	Eastern Honshu	63 ( 4)	62 ( 3)	–	1 (1)	–	–	–	–	–	–	–	–	–	–
Tochigi	Eastern Honshu	15	15	–	–	–	–	–	–	–	–	–	–	–	–
Saitama	Eastern Honshu	1 ( 1)	1 ( 1)	–	–	–	–	–	–	–	–	–	–	–	–
Tokyo	Eastern Honshu	14 (13)	13 (12)	1 (1)	–	–	–	–	–	–	–	–	–	–	–
Gunma	Eastern Honshu	45	39	1	–	4	1	–	–	–	–	–	–	–	–
Shizuoka	Central Honshu	7	–	6	–	–	1	–	–	–	–	–	–	–	–
Nagano	Central Honshu	9	–	–	–	7	2	–	–	–	–	–	–	–	–
Gifu	Central Honshu	4 ( 4)	–	–	–	3 (3)	1 (1)	–	–	–	–	–	–	–	–
Aichi	Central Honshu	2 ( 2)	–	–	–	2 (2)	–	–	–	–	–	–	–	–	–
Kochi	Shikoku	27	–	–	–	27	–	–	–	–	–	–	–	–	–
Tokushima	Shikoku	15	–	–	–	15	–	–	–	–	–	–	–	–	–
Ehime	Shikoku	6	–	–	–	6	–	–	–	–	–	–	–	–	–
Taiwan	Western Taiwan	10	5	–	–	–	–	3	2	–	–	–	–	–	–
	Eastern Taiwan	7	–	–	–	3	–	–	–	2	2	–	–	–	–
	Somewhere in Taiwan	3	2	–	–	–	–	–	1	–	–	–	–	–	–
Thailand	Southeast Asia	4 ( 4)	–	–	–	–	–	–	–	–	–	1 (1)	1 (1)	1 (1)	1 (1)
Malaysia	Southeast Asia	1 ( 1)	–	–	–	–	–	–	–	–	–	1 (1)	–	–	–
Total		235 (29)	139 (16)	8 (1)	1 (1)	67 (5)	5 (1)	3	3	2	2	2 (2)	1 (1)	1 (1)	1 (1)



**Fig. 1.** (A) Locations of the Japanese islands and Taiwan in East Asia. (B) Sampling localities and frequencies of the *Paguma larvata* cytochrome *b* haplotypes identified from Honshu and Shikoku of Japan. (C) Sampling localities and frequencies of the *Paguma larvata* cytochrome *b* haplotypes identified from Taiwan, with higher mountains shown by thick bars. Numbers in brackets show the frequencies of individuals having those haplotypes. Haplotype JA1 was identified from areas closed by broken lines both in eastern Honshu of Japan (B) and western Taiwan (C), whereas JA4 was found from areas closed by solid lines both in central Honshu and Shikoku of Japan (B) and eastern Taiwan (C). Within Japan (B), Gunma Prefecture is a contact zone among not only the two dominant haplotypes (JA1 and JA4) but also other two haplotypes (JA2 and JA5). Within Taiwan (C), the higher mountain ranges are likely geographic barriers separating the distribution of haplotypes JA1/TW1/TW2 (western Taiwan) from that of haplotypes JA4/TW3/TW4 (eastern Taiwan).

ment was performed using GeneWorks (Intelligenetics). Polymorphic nucleotide sites, haplotype diversity and nucleotide diversity were calculated using ARLEQUIN software ver. 3.1.1 (Excoffier et al., 2005). A parsimony network of the cytochrome *b* haplotypes was constructed using the computer program TCS ver. 1.21 (Clement et al., 2000).

## RESULTS

### Haplotypes and phylogenetic relationships between *P. larvata* populations from Japan and Taiwan

Four cytochrome *b* haplotypes, all of which corre-

sponded with four haplotypes (JA1, JA2, JA4 and JA5) previously reported by Masuda et al. (2008), were identified at various frequencies from the 186 animals collected in Japan in the present study (Table 1). By contrast, four new haplotypes (TW1, TW2, TW3, and TW4) and two haplotypes matching JA1 and JA4 were found in 20 animals from Taiwan (Table 1). Of these 20, information on the sampling localities was not available for three, although they were certainly obtained from the field (Table 1). An alignment of the haplotypes identified in the present study and those of Southeast Asia (SE1, SE2, SE3, and SE4) reported in

**Table 2.** Alignment of cytochrome *b* haplotypes (1,140 bp) of the masked palm civet *Paguma larvata*. \*Cited from Masuda et al. (2008). Dots indicate identity with nucleotides of haplotype TW1 identified from Taiwan.

Haplotype	Nucleotide site numbers																Accession no.
	46	123	179	189	252	312	315	342	384	471	519	591	749	886	891	947	
TW1	G	C	T	C	T	T	T	C	C	A	C	G	T	T	G	T	AB511054
TW2	.	.	.	.	.	C	.	.	T	.	.	.	.	.	.	.	AB511055
TW3	.	.	.	T	.	C	.	.	.	.	.	.	.	.	.	.	AB511056
TW4	.	.	.	.	.	C	.	.	.	.	.	.	.	C	.	.	AB511057
JA1	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	AB303951*
JA2	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	AB303952*
JA3	.	.	.	.	C	C	.	.	.	.	.	.	.	.	.	.	AB303953*
JA4	A	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	AB303954*
JA5	.	.	.	.	.	C	.	.	.	.	.	A	.	.	.	.	AB303955*
SE1	.	T	.	.	.	C	.	.	.	G	T	.	.	.	.	.	AB303956*
SE2	.	T	.	.	.	C	.	.	.	G	T	.	.	.	.	C	AB303957*
SE3	.	T	C	.	.	C	.	T	.	G	T	.	C	.	.	.	AB303958*
SE4	.	T	.	.	.	C	.	.	.	.	T	.	.	.	A	.	AB303959*

Masuda et al. (2008), showed that 16 sites of the 1,140 bp were polymorphic, and that all nucleotide substitutions were transitions (Table 2).

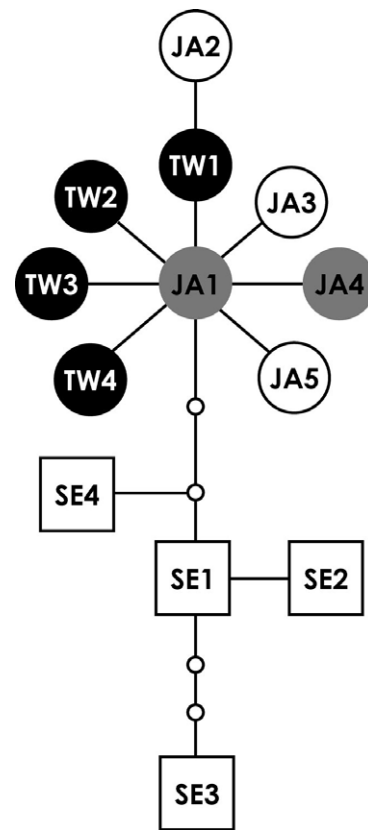
A parsimony network analysis (Fig. 2) indicated that haplotypes identified from Japan and Taiwan form a star-like tree with a central location of JA1 showing one-nucleotide substitution, whereas those from Southeast Asia are more remote from the others, showing more than three-nucleotide substitutions. Only JA2 had two-nucleotide substitutions with JA1. Haplotype TW1 was located between JA1 and JA2 in the parsimony network (Fig. 2).

The full set of sequences of the *P. larvata* cytochrome *b* gene determined in the present study were deposited to DDBJ/GenBank/EMBL nucleotide databases under the following accession numbers, AB511054–AB511057 (Table 1).

#### Frequencies and distribution patterns of the *P. larvata* haplotypes in Japan and Taiwan

Table 1 and Fig. 1B show that, of a total of 210 animals from Japan (186 animals of the present study in addition to 24 animals reported by Masuda et al. (2008)), the haplotype frequencies were as follows: 62.9% (132/210 animals) for JA1, 3.8% (8/210 animals) for JA2, 0.5% (1/210 animals) for JA3, 30.5% (64/210 animals) for JA4 and 2.4% (5/210 animals) for JA5. Haplotype JA1, which shows the highest frequency (62.9%), was found only in eastern Honshu, whereas JA4, with the secondly highest frequency (30.5%), occurred in Shikoku and central Honshu (Table 1 and Fig. 1B). Haplotypes JA2 and JA5 were identified from some localities in central Honshu (Fig. 1B). Only one animal from Ibaraki Prefecture in eastern Honshu exhibited haplotype JA3 (Fig. 1B). Thus, JA1 occupied a wide range in eastern Honshu, whereas JA4 was predominant in Shikoku and central Honshu. In particular, all 59 animals collected extensively within Ibaraki Prefecture of eastern Honshu shared only haplotypes JA1, and all 48 animals from Shikoku shared only JA4. Fig. 1B clearly shows that the area around Gunma Prefecture is a zone of contact among the distribution ranges of the four haplotypes (JA1, JA2, JA4 and JA5).

On the other hand, for the 20 Taiwanese animals (including three animals for which sampling localities were unavailable) examined in the present study, the frequencies



**Fig. 2.** A parsimony network among the *Paguma larvata* cytochrome *b* identified from Japan and Taiwan in the present study and those (JA1, JA2, JA3, JA4 and JA5) reported in the previous study (Masuda et al., 2008). Black circles (TW1, TW2, TW3 and TW4) show haplotypes newly identified only from Taiwan. White circles (JA2, JA3 and JA5) indicate haplotypes identified from only Japan, and gray circles (JA1 and JA4) show those identified both from Japan and Taiwan. Squares (SE1, SE2, SE3 and SE4) indicate haplotypes from Southeast Asian *P. larvata*, previously reported in Masuda et al. (2008). Small open circles without haplotype numbers mean missing haplotypes. A bar between haplotypes indicates one nucleotide substitution. Haplotypes (JA1 to JA5 and TW1 to TW4) found both from Japan and Taiwan form a star-like tree with a central location of JA1.

**Table 3.** Genetic diversity of cytochrome *b* haplotypes in regional groups of the masked palm civet *Paguma larvata* from Japan and Taiwan. \*Referring to Table 2. \*\*Calculated from data of Table 1. Numerals in parentheses are the numbers of individuals cited from Masuda et al. (2008), and they are included in the left numbers.

Regional group*	Number examined**	Number of haplotypes	Number of polymorphic site	Haplotype diversity	Nucleotide diversity
All Honshu	162 (24)	5	5	0.3249	0.000387
– Eastern Honshu	140 (18)	5	5	0.1107	0.000124
– Central Honshu	22 ( 6)	3	4	0.6234	0.001458
Shikoku	48	1	0	0	0
All Taiwan	20	6	5	0.8316	0.001039
– Western Taiwan	10	3	2	0.6889	0.000721
– Eastern Taiwan	7	3	3	0.7619	0.001337
Southeast Asia	5 ( 5)	4	6	0.9000	0.002105
Total	235 (29)	13	16		

among the six haplotypes were more evenly distributed, as follows: 15.0% (3/20 animals) for TW1 (from Taichung, Jiji and Pingtung), 15.0% (3/20 animals) for TW2 (from Heping and Pingtung), 10.0% (2/20 animals) for TW3 (from Tengzhi), 10.0% (2/20 animals) for TW4 (from Tengzhi), 35.0% (7/20 animals) for JA1 (from Jiujiu Peak, Jiji, Zhushan and Pingtung), and 15.0% (3/20 animals) for JA4 (from Fushi Village, Tengzhi and Shanpin) (Table 1 and Fig. 1C). Of the three animals without information of sampling localities (included in the 20 animals of Taiwan), two exhibited haplotype JA1, and one exhibited TW2 (Table 1). Thus, the two haplotypes JA1 and JA4, both of which were predominant in Japan, were also found in animals from Taiwan. The other haplotypes (TW1 to TW4) were new and specific to Taiwan. In addition, haplotypes TW1, TW2, and JA1 were distributed in western Taiwan (North and South of the Alinshan and Yushan Mountain Ranges), whereas haplotypes JA4, TW3, and TW4 were located in eastern Taiwan (East of the Central Mountain Range and the area between the Central and Yushan Mountain Ranges) (Table 1 and Fig. 1C). This suggests that the *P. larvata* populations may have been geographically isolated by the higher mountain ranges in Taiwan.

The genetic diversity of the populations from Japan were much lower than those from Taiwan (Table 3). For example, the number of haplotypes identified was five from 162 animals of all Honshu and one from 48 animals of Shikoku, whereas there were six haplotypes from 20 animals of all Taiwan. Haplotype diversity was 0.3249 for all of Honshu and 0 for Shikoku, whereas the same figure was 0.8316 for all of Taiwan. Similarly, nucleotide diversity was  $3.87 \times 10^{-4}$  for all of Honshu and 0 for Shikoku, compared to  $10.39 \times 10^{-4}$  for all of Taiwan (Table 3). By contrast, the Southeast Asian animals showed the highest values for polymorphic site number (6), haplotype diversity (0.9000), and nucleotide diversity ( $21.05 \times 10^{-4}$ ), despite the fact that this sample size (5) was the smallest of all populations examined (Table 3).

## DISCUSSION

### Phylogeographical structures of the native *P. larvata* populations in Taiwan

The present study demonstrated the molecular phylo-

geographic features of the native *P. larvata* populations in Taiwan. The haplotype distribution patterns indicate that the *P. larvata* populations may have been geographically isolated between eastern and western Taiwan by mountain ranges (Fig. 1C). Of the six haplotypes identified from 20 animals, three haplotypes (TW1, TW2, and JA1) were found in western Taiwan, whereas the other three (TW3, TW4, and JA4) were found in eastern Taiwan (Table 1 and Fig. 1C). Information on the female home range would be useful in considering the expansion of maternally inherited mtDNA haplotypes, however, no such data on the home range of female *P. larvata* in Taiwan are available in the literature. Although a preliminary survey, constrained by the difficulties of long-term radio-tracking of this species, suggested that the home range size for adult female civets in Shizuoka, Japan was 45.6–68.0 ha ( $n = 4$ ) (Torii and Ohba 1996), the true extent of female home range remains unclear. Distribution patterns of maternally inherited mtDNA may serve as an indicator worthy of future study of the home ranges of female palm civets.

The occurrence of only one- or two-nucleotide substitution among the six haplotypes from Taiwanese animals suggests that the degree of genetic differentiation due to geographic isolation by the mountain ranges is not significant. This is in congruence with Patou et al. (2009), which indicated low genetic variability and suggested a lack of a phylogeographic structure across the distribution range of this species. Our study further indicated that the haplotype distribution patterns reveal the history of migration and habitat expansion of *P. larvata* in restricted areas such as Taiwan and Japan. In order to better understand the migration history of *P. larvata* in Taiwan, it is necessary to identify the contact zones between western and eastern groups, which are expected to be localized around the northern and southern limits of the Central Mountain Range. Oshida et al. (2006) studied mtDNA control region phylogeography of the Pallas's squirrel *Callosciurus erythraeus*, which is distributed widely in Taiwan, and reported that the Central Mountain Range had served as a geographic barrier, resulting in genetic differentiation between the western and eastern populations. In addition, using allozyme data, Toda et al. (1998) reported that the eastern population of the Indian rice frog *Rana limnocharis*, which is widely distributed in lowlands of Taiwan, had genetically diverged from other populations, again suggesting geographical isolation by the Central Mountain Range. Oshida et al. (2006) suggest that species distributed in low and middle elevations in Taiwan are strongly affected by the mountain ranges. Because the habitats of *P. larvata* in Taiwan are at altitudes of between 50 and 2,000 m (Cheng and Wang, 1993), the higher mountain ranges (heights of many of the mountains are more than 3,000 m high) may well have isolated them, resulting in genetic differentiation.

On the other hand, a previous study by Masuda et al. (2008), identified five haplotypes (JA1, JA2, JA3, JA4, and JA5) in 24 animals of Japan. Interestingly, no additional haplotypes were identified even when the sample size was expanded in the present study to include 186 animals newly collected from a wide range of locations in Japan. Among a total of 210 animals from the non-native Japanese populations, higher frequencies of haplotypes JA1 and JA4 were

identified in eastern Honshu and Shikoku/central Honshu, respectively, and lower frequencies of JA2 and JA5 were identified in central Honshu (Table 1 and Fig. 1B), indicating low genetic variability across Japanese populations. This is supported by the lower values of haplotype and nucleotide diversity in the Japanese population than the Taiwanese population (Table 3). These differences in population structure suggest a founder effect in the Japanese population, as described below.

### Origins and founder effects in non-native *P. larvata* populations in Japan

The present study of comparative phylogeography provides clear genetic evidence that some of the *P. larvata* populations found in Japan derive from native populations in Taiwan. The frequencies and distribution patterns of the two predominant haplotypes (JA1 and JA4) in Japan (Figs. 1b and 1c) suggest the possibility of artificial transportation between Taiwan and Japan; that is, founder animals may have been transported from western Taiwan to eastern Japan (eastern Honshu), and from eastern Taiwan to western Japan (central Honshu and Shikoku). As suggested by Wayne and Koepfli (1996), it is necessary in such cases for evolutionary biologists to frame their interpretations carefully in light of historical information and dispersal abilities, as well as differences in the mutation rate of genes. Although there are no detailed records on the history of transportation of *P. larvata* between the two countries, Miyashita (1977) suggested the introduction of *P. larvata* from Taiwan to Japan may have been for purposes of fur production during World War II. Nakamura (1994) also indicated that *P. larvata* were transported from Taiwan to Japan as food or pets. Imaizumi (1991) mentioned that the white-spot patterns on the face of *P. larvata* of Japan are similar to those of animals native to Taiwan. In addition, based on partial sequences of the mtDNA cytochrome *b* and a control region, Patou et al. (2009) reported that sequences of three animals from Japan (without information on sampling localities within Japan) were very closely related to those of one animal of Taiwan and of one animal of Vietnam, suggesting an Indo-Chinese origin for *P. larvata* of Japan, although they did not find any identical haplotypes among Japan, Taiwan, and Vietnam. Based on these findings and previous historical descriptions, it is reasonable to consider that at least some subsets of the Japanese populations of *P. larvata* have originated from Taiwanese founder animals.

The other Japanese haplotypes (JA2, JA3, and JA5) previously reported by Masuda et al. (2008) were not identified in any Taiwanese animals in the present study. It might be possible to find these three haplotypes from within Taiwan if more samples from wider areas of Taiwan are further analyzed. Alternatively, these three haplotypes might occur elsewhere on the Asian Continent; Patou et al. (2009) reported haplotypes showing sequence similarities with those of Japan in Vietnam.

It is notable that the area around Gunma Prefecture in Japan is a point of intersection among the distributions of not only the predominant JA1 and JA4, but also, at lower frequencies, JA2 and JA5 (Fig. 1B). Since 1943, when the first *P. larvata* reported in Japan was found in Shizuoka Prefecture (Nawa, 1965), this animal has been found spo-

radically, but across wide areas of Japan (Kuroda, 1955; Imaizumi, 1960; Obara, 1961), and is nowadays found in eastern Honshu and Shikoku (Torii, 1996; Abe et al., 2005; Torii, 2009). These records, and the genetic data obtained in the present study, suggest that there may have been at least three lineages of introduction and naturalization of *P. larvata* in Japan. In the first, *P. larvata* originating from eastern Taiwan may have entered central Honshu of Japan, although no haplotypes common between Japan and Taiwan have been found in Shizuoka Prefecture in central Honshu (Fig. 1B). In this scenario, introduced populations subsequently began dispersing to the east of Honshu, after which a subset of the central Honshu group carrying the JA4 haplotype reached Shikoku. Alternatively, animals with JA4 originating from eastern Taiwan may have been brought directly to Shikoku. In eastern Honshu, it appears that animals from western Taiwan carrying the JA1 haplotype were transported to eastern Honshu, and are now expanding their distribution to the west and north. We suggest that the two lineages (JA1 and JA4) may have begun to intersect at areas around Gunma Prefecture. To confirm the introduction routes of *P. larvata* to Japan and the after migration history, however, it will be necessary to obtain more genetic information and compare this with information on temporal changes in their distribution.

Previously, Oshida et al. (2007) investigated mtDNA control region phylogeny of the non-native Japanese populations and the native Taiwanese populations of the Pallas's squirrel (*Callosciurus erythraeus*), and reported genetic evidence that one of the Japanese populations originated from a population from northeastern Taiwan. The present study similarly suggests that human activities between Japan and Taiwan have affected not only the development of human culture and history, but also resulted in the transport of animals, leading to the introduction of alien species.

The present study also provides genetic evidence for founder effects in the Japanese populations of *P. larvata* following their introduction from Taiwan. The first evidence is the lower genetic diversity in the Japanese populations (Table 3), which may be the result of a very small founder population. The second is that the frequencies of JA1 and JA4 were relatively high both in Japan and Taiwan (Table 1), probably reflecting the haplotype frequencies in the founders introduced to Japan from Taiwan. Thirdly, as shown in Fig. 2, the Taiwanese haplotype TW1 described in the present study matched the missing haplotype (Masuda et al. 2008), situated between JA1 and JA2. This finding suggests that haplotype JA2 may also be native to Taiwan.

### ACKNOWLEDGMENTS

We thank Ryosuke Kishimoto (Nagano Environmental Conservation Research Institute), Ayao Kanesawa (Ibaraki Nature Museum), Terutake Hayashi (Tochigi Prefectural Museum), Akihiro Koizumi (Iida City Museum), Midori Ogura (Karasu-gawa Keikoku Ryokuchi of Nagano Prefecture), Megumi Matsushita (Nihondaira Zoo) and Tsunenori Tsujimoto (Morioka Zoological Park) for supplying specimens analyzed in the present study. We are also grateful to Yu-Cheng Chang (Tunghai University), Ako Yoshikawa (Hokkaido University) and Sachiko Ida (Hokkaido University) for their kind research assistance. This work was supported in part by a grant from the ESPEC Foundation for Global Environment Research and Technology (Charitable Trust) to R.M.

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(Received December 2, 2009 / Accepted January 20, 2010)