Phylogenetic history of mustelid fauna in Taiwan inferred from mitochondrial genetic loci.

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Title: Phylogenetic history of mustelid fauna in Taiwan inferred from mitochondrial genetic loci.

Abstract: Phylogenetic relationships among species of the family Mustelidae were examined using the combined nucleotide sequences of the three mitochondrial genetic loci, cytochrome *b* (MT-CYB; 1140 bp), NADH dehydrogenase subunit 2 (MT-ND2; 1044 bp), and displacement loop (MT-DLOOP; 540 bp), with special emphasis on the phylogenetic history of four Taiwanese mustelid species: *Martes flavigula* (Boddaert, 1785), *Melogale moschata* (Gray, 1831), *Mustela nivalis* L., 1766, and *Mustela sibirica* Pallas, 1773. Maximum likelihood phylogenetic analysis of the combined sequences of the mitochondrial genetic loci produced a topology largely congruent with that of previous studies at species level. Analyses of intraspecific genetic variations revealed two *Melogale moschata* individuals from Taiwan and Vietnam that showed genetic distances comparable to interspecific variations within the mustelid lineages. Furthermore, *Mustela nivalis*, recently discovered in Taiwan, was not as genetically differentiated from other continental conspecific individuals as a previous morphological survey suggested. Divergence time estimations for the mustelid lineages of Taiwan and the Eurasian continent by the Bayesian relaxed-molecular clock approach suggested multiple colonization of Taiwan by mustelids from the continent during the Pleistocene, creating a hierarchical pattern of endemism based on the differential isolation history of the mustelid species in Taiwan.

Introduction

Islands are hotspots of biodiversity (Whittaker and Fernandez-Palacios 2007), and the eastern peripheral islands of the Eurasian continent have played an important role in creating novel evolutionary lineages and harboring historically diverse organisms (Suzuki 2009). The unique characteristics of island organisms are thought to be the result of repeated isolations from and connections to the continental mainland (Ota 1998; Otsuka and Takahashi 2000). Thus, the endemism of island biota is achieved through a process of iterative receptions of continental organisms and subsequent geographic isolations, culminating in global centers of endemic richness (Kier et al. 2009). Among these centers of richness are the East Asian marginal islands (Whittaker and Fernandez-Palacios 2007).

The islands of Taiwan are located at the southeastern edge of the Eurasian continent. The main island of Taiwan is thought to have been formed by the collision of the Philippine Sea and continental Eurasian plates (Page and Suppe 1981; Ho 1986; Teng 1990; Huang et al. 1997). Since the emergence of the island in the Pliocene (Teng 1990; Huang et al. 1997), intermittent connections to the Asian mainland are thought to have occurred (Kizaki and Oshiro 1980; Kimura 2000), possibly due to sea-level lowering in glacial periods (Gascoyne et al. 1979; Voris 2000; Wang 2004). Therefore, the island likely has received multiple invasions of organisms from the Asian continent, as suggested in the palaeontological record (e.g., Otsuka and Takahashi 2000; Takahashi et al. 2001). The complicated biota of Taiwan is an intermingling of various organisms with different histories. The zoogeographic (faunal) region of Taiwan is near the boundary between the northern Palaearctic subregion of the Holarctic region and the southern Oriental subregion of the Paleotropical region. Thus, various northern and southern elements of the Asian continent are found in the Taiwan islands (Kano 1940; Tzeng 1986; Lin 2000), and no chronologically and biogeographically congruent evolutionary scenario explains the current patterns of species distributions in Taiwan.

Recent phylogeographic studies based on molecular genetic approaches have sought to unravel the complex genealogies of Taiwanese fauna. Two major hypotheses have been proposed to explain genetic variations observed on the island. The first proposes *in situ* differentiation, in which variations have been generated due to interruption of within-island gene-flow by physical barriers, e.g., a central mountain range for Pallas's squirrel *Callosciurus erythraeus* (Pallas, 1779) (Oshida et al. 2006) and the Indian rice frog *Rana limnocharis* Gravenhorst, 1829 (Toda et al. 1997, 1998). Some refugia during the Pleistocene may also have functioned as a mechanism of *in situ* differentiation (Wang et al. 1999; Oshida et al. 2006; Yuan et al. 2006). The alternative hypothesis is immigration from the mainland, in which multiple colonization events occurred via land bridges formed sporadically during the Pleistocene at the current Taiwan Strait (Yu 1995; Yu et al., 1996; Tu et al. 2000; Creer et al. 2001, 2004; Lin et al. 2002; Jang-Liaw et al. 2008; Zhong et al. 2008). Determining which hypothesis better explains the genetic variations of specific organisms in Taiwan is difficult (Oshida et al. 2006; Watanabe et al. 2007), owing to limited information about the geology of Taiwan-continent interactions and the lack of comparisons between Taiwanese and continental organisms. Knowledge of the influence of island history on the modern fauna in Taiwan is thus fragmentary. A comparison of Taiwanese lineages of diverse organisms to corresponding continental counterparts with different origins and histories would help to disentangle the complex histories of the Taiwanese fauna. In particular, comparisons of the possible colonization ages of different organisms would provide insight into the importance of the landbridge hypothesis.

We evaluated the genetic status of the lineages of the four Taiwanese species in the family Mustelidae (Carnivora, Mammalia): *Martes flavigula* (Yellow-throated marten), *Melogale moschata* (Chinese ferret badger), *Mustela nivalis* (Least weasel), and *Mustela sibirica* (Siberian weasel). The former two species mostly inhabit the southern Oriental subregion,

Mustela nivalis has expanded to the northern Holarctic (both Palaearctic and Nearctic) region, and *Mustela sibirica* has both Oriental and Palaearctic distributions (Wozencraft 2005). Thus, the mustelid fauna in Taiwan provides an excellent opportunity to test the role of different processes involved in the assembly of an island fauna. We examined nucleotide sequences of three mitochondrial genetic loci, cytochrome *b*, NADH dehydrogenase subunit 2, and displacement loop of mustelids, with special emphasis on Taiwanese and continental individuals of the four species present in Taiwan. *Lutra lutra* (L., 1758) and *Aonix cinerea* (Illiger, 1815), which inhabit Taiwan, were not examined in this study. On the basis of the genetic diversity of the Taiwanese mustelids, we considered their taxonomy and evolutionary history.

Materials and Methods

Sample collection and gene nomenclature

In total, we examined 24 species of the family Mustelidae (Carnivora, Mammalia). Four species (*Martes flavigula*, *Melogale moschata*, *Mustela nivalis*, and *Mustela sibirica*) included individuals from both Taiwan and the continental mainland. One to two representatives of the other species were analyzed. We determined the nucleotide sequences of one to three mitochondrial genetic loci for 45 individuals from 24 mustelid species and downloaded 32 sequences from the DDBJ/EMBL/GenBank international DNA database (Table 1). Gene locus symbols and names follow the nomenclature of the homologous human locus, as approved by the Human Genome Organisation (HUGO) Gene Nomenclature Committee, and are listed in the Human Gene Nomenclature Database (http://www.genenames.org/). Cytochrome *b* and NADH dehydrogenase subunit 2 were designated MT-CYB and MT-ND2, respectively. For the displacement loop region, which has been called the D loop or control region, sub-regions are named differently in the database.

For brevity, however, we designated it as the MT-DLOOP, following the naming system for MT-CYB and MT-ND2. Novel sequences were deposited in the international DNA databases as accession numbers AB564113–AB564168 and AB601552–AB601597 (Table 1).

PCR amplification and sequencing strategy

Total genomic DNA was extracted from tissues preserved in ethanol using the conventional phenol–chloroform method (Sambrook and Russell 2001). The DNA was amplified with nested polymerase chain reactions (PCRs) using an automated thermal cycler (model PC 808, Astec). For the PCR of MT-CYB and MT-ND2, we used Ex *Taq* Hot Start Version (Takara) according to the manufacturer's instructions. Each PCR mix was composed of Ex *Taq* Buffer containing 2.0 mM $MgCl_2$, 0.2 mM of each dNTP, 0.8 μM of each primer, 1.25 units of TAKARA Ex *Taq*™ HS polymerase, and 0.1–0.2 μg of template total genomic DNA in a total volume of 50 μl. The thermal cycling parameters for the first PCR consisted of 94°C for 3 min, 30 cycles of 94°C for 30 s, 50°C for 30 s, and 72°C for 1.5 min, and a final 72°C for 10 min. A 1-μl aliquot of each reaction mixture from the first PCR product was used as template for the second nested PCR in a 50-μl reaction mixture. The second nested PCR mixture included the same reagents as the first PCR, except for the primer pairs. The thermal cycling for the second nested PCR was performed using essentially the same conditions as the first PCR, except that the extension was decreased from 1.5 min to 40 s based on the expected length of the amplified fragments. For PCR of the MT-DLOOP, we used AmpliTaq Gold PCR Master Mix (Life Technologies) according to the manufacturer's instructions. Each PCR mix contained the appropriate amount of AmpliTaq DNA polymerase as well as the correct buffer, 2.5 mM $MgCl_2$, 0.2 mM of dNTP, 0.5 μM of each primer, and 0.1–0.2 μg of template total genomic DNA in a total volume of 20 μl. The thermal cycling parameters of the PCR were the same as in the second PCR for the MT-CYB and MT-ND2 analyses. The primers

used in this study are the same as those reported in Sato *et al*. (2009*b*). As a negative control, PCR without DNA template was performed, and no amplification was seen in the negative control. The final PCR products were sequenced according to the manufacturer's instructions using the BigDye Terminator Cycle Sequencing kit v3.1 (ABI), followed by automated sequencing on an ABI3130 genetic analyzer.

Multiple alignments and applied data matrix

The MT-CYB and MT-ND2 sequences generated in this study and collected from the DNA database were aligned manually, without using an alignment program, because the absence of sequence-length variation, which likely stemmed from functional constraints on these protein-coding regions, made alignment straightforward. Conversely, we used the program MUSCLE version 3.8 (Edgar 2004) for the multiple alignment of the MT-DLOOP due to the non-trivial nature of the alignment caused by sequence length polymorphisms. Sequence fragments that could not be aligned unambiguously were excluded from the analyses to avoid reconstruction of a phylogeny based on erroneous sequence homology. From the 574-bp alignment length of the MT-DLOOP sites after the MUSCLE analysis, we excluded 34 sites (sites 21–30 and 77–100) because of alignment ambiguities. Part of the MT-CYB sequence could not be determined for one *Martes flavigula* (HS844, the initial 593 bp were missing), two *Melogale moschata* (AK703 and HS2372, the initial 528 bp were missing), and one *Mustela erminea* L., 1758 (HS678, the final 6 bp were missing) because of unknowns in the PCR and sequencing processes. Despite some missing characters in our data, the large set of existing characters could lead to a correct phylogenetic reconstruction (Wolsan and Sato 2010). In our data, the sequences were determined for all taxa for many regions. Therefore, we treated the above-mentioned undetermined data as missing characters with the assumption that a small proportion of missing characters does not significantly affect the

phylogenetic reconstruction (*e.g.*, Wiens 2006). The assembled data matrix is available from TreeBASE (http://www.treebase.org/treebase-web/home.html) as study accession number S10586.

Phylogenetic analyses

Phylogenetic analyses were conducted on the combined mitochondrial genetic data consisting of 2,724 -bp nucleotide sequences of MT-CYB, MT-ND2, and MT-DLOOP using the maximum likelihood (ML; Felsenstein 1981) approach. Trees were rooted using species of the subfamily Guloninae (martens, wolverine, and allies), based on the phylogenetic hypothesis that the subfamilies Helictidinae (ferret badgers), Lutrinae (otters and allies), and Mustelinae (minks, polecats, weasels, and allies) are more closely related to each other, than to Guloninae (Sato et al. 2004, 2006, 2009*a*; Fulton and Strobeck 2006; Koepfli et al. 2008; Wolsan and Sato 2010). See Sato et al. (2009*a*) for the rationale for using the subfamily name Guloninae, instead of Martinae, as proposed by Fulton and Strobeck (2006) and followed by Koepfli et al. (2008).

The ML analysis was conducted using the program GARLI, version 0.96 (Zwickl 2006), and the nucleotide substitution model that best fit the data was applied. The model was determined using Akaike's information criterion (AIC) from the program Modeltest, version 3.7 (Posada and Crandall 1998; see also Posada and Buckley 2004). The GTR+I+Γ model was selected as the best model for the combined dataset. The ML tree was inferred from heuristic searches involving three runs of the genetic algorithm with 20,000 generations of a mutation-selection-reproduction cycle. The starting tree was generated from the ML stepwise-addition-sequence option. All the other parameters were set by default. Clade support was assessed using nonparametric bootstrap analyses (BP; 200 replicates; Felsenstein 1985).

Divergence-time estimations

To estimate the divergence times for the separation of the Taiwanese and Eurasian mustelids, we adopted the Bayesian relaxed molecular-clock dating approach as implemented in the program BEAST, version 1.6.1 (Drummond and Rambaut 2007). Before the BEAST analyses, we tested the global molecular clock by comparing the log likelihood of the unconstrained ML topology with the molecular-clock enforced ML topology in the program PAUP, version 4.0b10 (Swofford 2002). The likelihood-ratio test rejected the strict molecular clock ($-\ln L_{\text{unconstrained}} = 19645.29052$ and $-\ln L_{\text{molecular clock}} = 19690.24655$; $\chi^2 = 90$, df = 42, $P < 0.001$). Therefore, we used the approach that considers rate variation among different branches, in which the rates for each branch were drawn independently from a lognormal distribution and uncorrelated (Drummond et al. 2006). First, we created the input file for the BEAST using the program BEAUTi, version 1.6.1 (provided in the BEAST package). In BEAUTi, we set the substitution model for the sequence evolution, priors, and conditions of Markov Chain Monte Carlo (MCMC) for estimating posterior distributions of the time to the most recent common ancestor (MRCA) for the divergence between mustelids in Taiwan and those on the continent. We set the GTR+I+Γ for the substitution model as selected above, and the parameters in the model were unlinked among the three partitions (MT-CYB, MT-ND2, and MT-DLOOP). We selected the Yule speciation process as a prior on phylogeny. Two independent MCMC analyses were run for 10 million generations with trees sampled every 1,000 generations. Each log file was checked to confirm the convergence to the stationary posterior distribution and the sufficient effective sample size (ESS) of each parameter in the program Tracer, version 1.5 (Rambaut and Drummond 2009). We obtained sufficient ESS values exceeding 200 for most of the parameters, and over 2,000 for the divergence-time parameters of each node representing the MRCA of relevant Taiwanese and continental

individuals. The log files of both runs were then combined in LogCombiner version 1.6.1 (provided in the BEAST package), with the first 25% of the sampled parameters discarded as burn-in. We provided parameter estimates based on the combined samples from the two independent runs.

Multiple calibration points were set using the first appearance of fossils of some mustelids. The fossil information was adopted in the form of the lognormal prior distribution in line with the recommendation of Ho (2007) and Ho and Phillips (2009). The values of the time constraints based on fossil evidence were set to the zero-offset of the lognormal distribution as the minimum bound. The mean of the lognormal distribution was unavoidably set to somewhat subjective values (Ho 2007). The standard deviation was assigned so that upper limit of the 95% credible interval (CI) of the lognormal distribution took a value of times for divergences one step older in the phylogenetic topologies (the time for the first emergence of the stem lineage of the clade of interest) based on the estimations from Koepfli et al. (2008) and Sato et al. (2009a). When the age of the fossil record directly ancestral to the extant species could be used, the younger age for this ancestor (possible extinction age) was used for the upper limit of the 95% CI of the lognormal distribution. We used three time constraints for calibrations as minimum bounds. First, 3.4 Ma was adopted for the MRCA of *Mustela* and *Neovison* based on the earliest fossil findings of previously circumscribed *Mustela* (including *Neovison*) in the lower Pliocene strata (European Neogene Land Mammal Zone MN15), following Morlo and Kundrát (2001; also see Sato et al. 2003). The mean value was set to 2.0. The standard deviation was set so that the upper limit of the 95% CI took the value of 9.6, as the divergence time for *Mustela-Neovison* and the closest clade (consisting of the Lutrinae species) was estimated to be 8.7–9.0 Ma by Koepfli et al. (2008) and 9.6 Ma by Sato et al. (2009a). Second, 3.3 Ma was applied for the MRCA of the subgenus *Martes*, *M. americana* (Turton, 1806), *M. foina* (Erxleben, 1777), *M. martes* (L., 1758), *M. melampus*

(Wagner, 1840), and *M. zibellina* (L., 1758), based on the earliest fossil remains of the subgenus *Martes* represented by *Martes wenzensis* Stach, 1959 (see Sato et al. 2003 for more detail). The mean value was set to 1.0. The standard deviation was set so that the upper limit of the 95% CI took the value of 5.4, as the stem lineage of the subgenus *Martes* was estimated to have occurred at 4.7–5.1 Ma (Koepfli et al. 2008) and 5.4 Ma (Sato et al. 2009a). Third, 0.13 Ma was used for the MRCA of the two individuals of *Mustela erminea* based on the first fossil record of this species in the Illinoian age in North America (King 1983). The mean value was set to 0.5. The standard deviation was set so that the upper limit of the 95% CI took the value of 0.7, because *Mustela palerminea* L., 1758, a direct ancestor of *M. erminea*, was common until 0.7 Ma (King, 1983). Koepfli et al. (2008) and Sato et al. (2009a) dated the divergence of the *Gulo-Martes* and *Melogale*-Lutrinae-*Mustela-Neovison* lineages to be 11.6–11.9 Ma and 12.0 Ma, respectively. Therefore, we applied 12.0 Ma to the root height prior as a mean of the normal distribution with a standard deviation of 1.0.

Results

Phylogenetic relationships and genetic distances

The phylogenetic tree inferred from the combined sequences of MT-CYB, MT-ND2, and MT-DLOOP loci using the ML criterion supported the monophyly of the subfamilies Guloninae, Mustelinae, and Lutrinae, albeit with low support for the subfamily Lutrinae (Fig. 1). The interrelationships among these subfamilies were very weakly supported (BP = 44%). By contrast, basal intrasubfamilial relationships within the clade of Mustelinae comprising *Mustela* and *Neovison* were resolved with high support values. *Mustela kathiah* Hodgson, 1835 represented the monotypic lineage sister to the clade that included other musteline species except *M. nudipes* Desmarest, 1822, *M. strigidorsa* Gray, 1853, and *Neovison vison* (Schreber, 1777) (BP = 100%). *Mustela nudipes* and *M. strigidorsa* formed a well-supported

clade (BP = 100%), which was the second-oldest lineage within the clade of Mustelinae (BP = 100%). *Neovison vison* was the first offshoot of the musteline clade (BP = 97%).

Here, we described the relationships of the four mustelids that occur on Taiwan in more detail. The monophyly of *Martes flavigula*, *Melogale moschata*, *Mustela nivalis*, and *Mustela sibirica* was highly supported (BP = 100%). *Martes flavigula* was supported as a sister lineage to the clade that included species in the subgenus *Martes* (*M. americana*, *M. foina*, *M. martes*, *M. melampus*, and *M. zibellina*), albeit with low reliability (BP = 42%). Each *M. flavigula* individual from Kunming, Primorye, and Taiwan had different haplotypes. Additionally, an individual from Taiwan was closely related to one from Primorye, Russia, albeit with low reliability (BP = 53%). The average genetic distance (ML genetic distances inferred with parameters for the GTR+I+Γ model obtained in the Modeltest analysis) between the individuals from Taiwan and Primorye was 0.003. *Melogale moschata* was very weakly identified as the most basal lineage within the ingroup mustelids (BP = 44%). Each individual *M. moschata* from Taiwan and Vietnam had a different haplotype, with a genetic distance of 0.043. *Mustela nivalis* was the closest relative of *Mustela altaica* Pallas, 1811 (BP = 100%). *Mustela nivalis* individuals from Germany, Korea, and Taiwan formed distinct clades (BP > 95%). The clade composed of two Taiwanese individuals was closely related to that consisting of individuals from Germany, Korea, Russia (Primorye), and Japan (Hokkaido) with relatively high support (BP = 84%). The average genetic distance between individuals from Taiwan and the closest clade individuals was 0.009. *Mustela sibirica* was strongly supported as sister to the clade of *Mustela furo* L., 1758, *Mustela eversmanii* Lesson, 1827, *Mustela lutreola* (L., 1761), and *Mustela putorius* L., 1758 (BP = 100%). The Taiwanese lineage appeared in the basal position of the lineage of this species, albeit with low reliability (BP = 52%). The close relationship among individuals from Korea, Russia (Primorye), and

the Tsushima Islands was strongly supported (BP = 100%). The average genetic distance between the Taiwanese and other individuals was 0.016.

Among all of the individuals examined, the minimum and maximum genetic distances observed within species were 0 (two Taiwanese *Mustela nivalis* individuals and two Kunming *Martes flavigula* individuals) and 0.043 (Taiwanese and Vietnamese *Melogale moschata* individuals), respectively. Genetic distances less than the largest value of the intraspecific variation (0.043) were observed within the interspecific variation: 0.013 (*Mustela eversmanii* vs. *Mustela putorius*), 0.014 (*Mustela eversmanii* vs. *Mustela lutreola*), 0.014 (*Mustela lutreola* vs. *Mustela putorius*), 0.035 (*Martes martes* vs. *Martes zibellina*), and 0.041 (*Martes martes* vs. *Martes melampus*). The 0.048 value for *Martes melampus* vs. *Martes zibellina* was close to the maximum intraspecific difference observed within *Melogale moschata*.

Divergence-time estimation

The divergence of the mustelid lineages from Taiwan and from the continent was dated using the Bayesian relaxed-molecular clock approach with the program BEAST. The 95% highest posterior density (HPD) of the covariance of parent and child branch rates varied from -0.20 to 0.21, with a mean near zero, showing no strong evidence of autocorrelation of the rates among adjacent branches (see the BEAST manual). This validated our use of the uncorrelated lognormal distribution as a rate-variation model. The times to the most recent common ancestor for the Taiwanese individual and the closest clade or individual with upper and lower HPD intervals are indicated in Table 2. The mean divergence times obtained in the analyses (0.11, 1.46, 0.36, and 0.63 Ma for *Martes flavigula*, *Melogale moschata*, *Mustela nivalis*, and *Mustela sibirica*, respectively) gave four different ages for the divergence between the Taiwanese and closest lineages.

Discussion

Interspecific relationships within the mustelid phylogeny

Our combined phylogenetic analyses based on the ML criterion produced a topology largely congruent with previous hypotheses. As a whole, shallower interspecific relationships were well supported, with the opposite true for deeper nodes, a phenomenon consistent with the previous notion that the mitochondrial genetic loci suffer from the multiple-substitution problem in the phylogenetic reconstruction of the older past (*e.g*., Sato et al. 2003; Sato and Suzuki 2004). Nonetheless, the relationships we obtained among the four subfamilies studied (Guloninae, (Helictidinae, (Lutrinae, Mustelinae))) are consistent with the findings of recent molecular phylogenetic studies (Koepfli and Wayne 2003; Sato et al. 2004, 2006, 2009*a*; Fulton and Strobeck 2006; Koepfli et al. 2008). Interspecific relationships within the subfamilies Mustelinae and Guloninae mostly agreed with previous reports (e.g., Sato et al. 2003, 2004, 2006, 2009*a*; Koepfli et al. 2008; Wolsan and Sato 2010), although our data showed unprecedented strong support for the phylogenetic position of *Mustela kathiah*.

Intraspecific variations and two taxonomic implications

The intraspecific variation has implications for taxonomic issues concerning the Taiwanese mustelids. *Mustela nivalis* was first found in Taiwan in 1998 (Lin 2000; Lin et al. 2010). Its presence in Taiwan was unexpected, as this is a Holarctic species, and it is mainly distributed in the northern parts of Eurasia and North America (Wozencraft 2005). The considerable divergence of morphological characteristics of this newly discovered weasel from other described subspecies led Lin and Harada (1998) to initially regard it as a new species (but see Lin et al. 2010). However, our study demonstrated that Taiwanese individuals are not appreciably genetically differentiated from those of continental regions. This result is congruent with our previous study examining a 402-bp MT-CYB sequence of the Taiwanese

individual (Hosoda et al. 2000). Saarma and Tumanov (2006) used part of our MT-CYB sequence (312 bp) from the DNA database and compared it to the other *Mustela nivalis* subspecies. Their results agreed with ours and indicated that the genetic divergence of the Taiwanese individual was within intraspecific variation. Therefore, the newly discovered species was classified as *Mustela nivalis*, as noted in the most recent classification book (Wozencraft 2005). Previous observations of the morphological differentiation of *Mustela nivalis* in Taiwan are in accordance with the notion of the island effect as accelerating morphological evolution (Millien 2006).

Another taxonomic implication of this study is the evidence of significant differentiation between two *Melogale moschata* individuals from Taiwan and Vietnam, which showed genetic divergence comparable to that of interspecific genetic variation. To date, six subspecies of this species have been recognised (Storz and Wozencraft 1999; Wozencraft 2005). The Taiwanese and Vietnamese individuals that we examined belong to *M. moschata subaurantiaca* and *M. moschata taxilla*, respectively. The other subspecies are distributed from southeast China to northeast India, between the distributions of the two subspecies examined in this study. Therefore, the large genetic divergence we observed may not be due exclusively to the geographical separation of the Taiwan islands from the continent. Namely, the large genetic divergence might be established within the continent. Although it was impossible to specify the mechanism producing the large genetic diversity in this study by examining only two individuals, two possible explanations exist for the genetic divergence of the ferret badger in Taiwan. First, it might have anciently dispersed into Taiwan and have been isolated for a long time by the Taiwan–continent separation. Second, the genetic diversity might have arisen on the continent, and a diverged lineage might have recently dispersed into Taiwan. If the latter is correct, there should be a closely related lineage on the continent. Clearly, more samples, including those of other continental subspecies, are required

to understand the mechanism leading to such considerable divergence and to revise the taxonomy within this species. Regarding the other two mustelids on Taiwan (*Martes flavigula* and *Mustela sibirica*), no novel implications were gleaned from a taxonomical perspective.

Phylogenetic history of mustelid fauna in Taiwan

The divergence times of the Taiwanese and continental mustelids estimated from combined mitochondrial loci were 0.11, 1.46, 0.36, and 0.63 million years ago for *Martes flavigula*, *Melogale moschata*, *Mustela nivalis*, and *Mustela sibirica*, respectively (Table 2). These ages are all within the Pleistocene, suggesting that these divergences were influenced by the glacial–interglacial cycles (*e.g.*, Luthi et al. 2008). The continental fauna likely moved dynamically between northern and southern regions in response to these glacial cycles (Riddle 1996; Hewitt 2000; Sommer and Zachos 2009). Additionally, the sea between Taiwan and the continent was shallow enough during glacial periods to enable various organisms to cross the Taiwan Strait (Voris 2000). Consequently, Taiwan has probably received historically and geographically diverse lineages. We found no consistency in the migration scenarios of the four Taiwanese mustelids. *Martes flavigula* and *Melogale moschata*, from the southern Orient, are the youngest and oldest residents of Taiwan, respectively. Northern Holarctic *Mustela nivalis* and *Mustela sibirica*, which expanded both to the north and south, appear to have established themselves in Taiwan in periods between the migrations of the two Oriental species. The ability of four species of the family Mustelidae to coexist in Taiwan may be attributed to ecological niche differences, with arboreal martens (*Martes flavigula*), fossorial badgers (*Melogale moschata*), and open habitat weasels (*Mustela* spp.). These differences presumably permit coexistence with little competition. This idea, although implied from a smaller island system, is consistent with the suggestion from a study on a larger continental scale (Koepfli et al. 2008). They stressed that a phylogenetically and ecologically

heterogeneous set of species could coexist within the same mustelid community. The existence of two congeneric *Mustela* species, *M. nivalis* and *M. sibirica*, which might be expected to compete owing to similar ecological preferences, may be possible because of differences in foraging and breeding strategies, micro-habitats, and social dominance (King and Moors 1979; Powell and Zielinski 1983; Erlinge and Sandell 1988). Grant (1970) suggested a trend in island colonisation from ecological generalists to specialists, ultimately allowing closely related species to coexist on an island. In terms of diet, *M. nivalis*, which feeds almost entirely on small rodents, has characteristics of a specialist, whereas *M. sibirica* is a generalist that feeds more diversely (King and Moors 1979; Nowak 1991; Masuda 2009; Sasaki 2009). Therefore, the observation of Grant (1970) concurs with our estimated divergence times of *M. sibirica* (0.63 Ma) and *M. nivalis* (0.36 Ma) in Table 2. As an alternative explanation of the order of migration, *M. sibirica* may simply have had more opportunity, as its distribution in the continent is in closer proximity to the Taiwan islands than that of *M. nivalis*. Clearly, additional phylogenetic and ecological evidence, with larger samples, is necessary to elucidate the coexistence history of the Taiwanese mustelid fauna.

To conclude, our study found that the Taiwanese mustelids colonised the island at four different times during the Pleistocene, likely across land bridges that emerged owing to the lowering of sea levels during glacial periods. The results support the hypothesis of multiple episodes of immigration from the mainland, in agreement with other studies of the evolution of the Taiwanese fauna (Yu et al. 1996; Tu et al. 2000; Creer et al. 2001, 2004; Lin et al. 2002; Jang-Liaw et al. 2008; Zhong et al. 2008). The Taiwanese fauna (and flora) provide a good model for understanding the assembly and evolution of island communities that contain both old and new lineages.

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FIGURE CAPTIONS

Figure 1. Phylogenic relationships of the mustelids inferred from maximum likelihood analyses of the combined MT-CYB, MT-ND2, and MT-DLOOP sequence (2,724 bp). The negative log likelihood scores for optimal ML topology (-lnL) was -19649.848271. Numbers attached near nodes are bootstrap proportions calculated from the 200 pseudo-replicate topologies. The vertical thin lines indicated mustelid species including multiple individuals from different localities. Species names in bold faces indicate Taiwanese mustelid species. *Ma*, *Mu*, and *Me* indicate the genus names *Martes*, *Mustela*, and *Melogale*, respectively. The vertical thick lines indicate the subfamily names. For the rationale of those subfamily's names, see Sato et al. (2009*a*).

Fig. 1

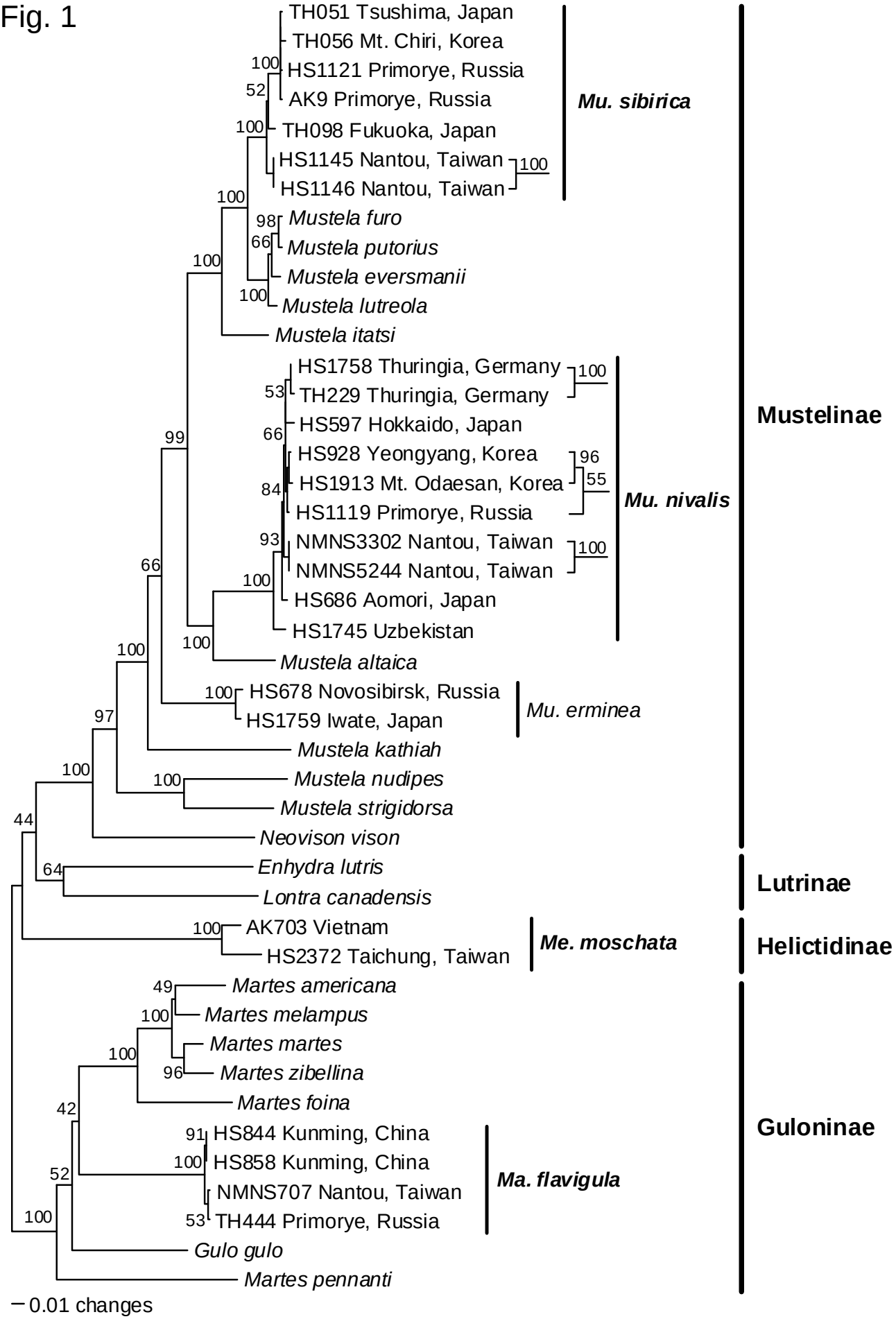
TH051 Tsushima, Japan
TH056 Mt. Chiri, Korea
HS1121 Primorye, Russia
AK9 Primorye, Russia
TH098 Fukuoka, Japan
HS1145 Nantou, Taiwan
HS1146 Nantou, Taiwan
Mu. sibirica
Mustela furo
Mustela putorius
Mustela eversmanii
Mustela lutreola
Mustela itatsi
HS1758 Thuringia, Germany
TH229 Thuringia, Germany
HS597 Hokkaido, Japan
HS928 Yeongyang, Korea
HS1913 Mt. Odaesan, Korea
HS1119 Primorye, Russia
NMNS3302 Nantou, Taiwan
NMNS5244 Nantou, Taiwan
HS686 Aomori, Japan
HS1745 Uzbekistan
Mu. nivalis
Mustela altaica
HS678 Novosibirsk, Russia
HS1759 Iwate, Japan
Mu. erminea
Mustela kathiah
Mustela nudipes
Mustela strigidorsa
Neovison vison
Enhydra lutris
Lontra canadensis
AK703 Vietnam
HS2372 Taichung, Taiwan
Me. moschata
Martes americana
Martes melampus
Martes martes
Martes zibellina
Martes foina
HS844 Kunming, China
HS858 Kunming, China
NMNS707 Nantou, Taiwan
TH444 Primorye, Russia
Ma. flavigula
Gulo gulo
Martes pennanti
0.01 changes
Mustelinae
Lutrinae
Helictidinae
Guloninae

Table 1. Species and DNA sequences used in this study

Taxon	Voucher number[a]	Locality[a]	DDBJ/EMBL/GenBank accession numbers		
			MT-CYB	MT-ND2	MT-DLOOP
Enhydra lutris , sea otter	TH257	Alaska, USA	AF057120[b]	AY750618[c]	AB601556[g]
Gulo gulo , wolverine	HS1603	Sakhalin, Russia	AB051245[d]	AY377284[e]	AB601557[g]
Lontra canadensis , North American river ott	TH332	Unknown, Canada	AF057121[b]	AY598557[f]	AB601558[g]
Martes americana , American marten	TH330	Manitoba, Canada	AB051234[d]	AY598546[f]	AB601559[g]
Martes flavigula , yellow-throated marten	HS844	Kunming, China	AB564113[g]	AB601552[g]	AB601560[g]
	HS858	Kunming, China	AB564114[g]	AB564137[g]	AB601561[g]
	NMNS707	Nantou county, Taiwan	AB564115[g]	AB564138[g]	AB601562[g]
	TH444	Primorye, Russia	AB564116[g]	AB564139[g]	AB601563[g]
Martes foina , beech marten	HS1753	Gera, Thuringia, Germany	AB051236[d]	AB564140[g]	AB601564[g]
Martes martes , European pine marten	AK702	Moscow, Russia	AB051237[d]	AB564141[g]	AB601565[g]
Martes melampus , Japanese marten			AB455595[h]	AB455695[h]	AB455645[h]
Martes pennanti , fisher	TH331	Manitoba, Canada	AF057131[i]	AY750624[c]	AB601566[g]
Martes zibellina , sable			AB455641[h]	AB455741[h]	AB455691[h]
Melogale moschata , Chinese ferret-badger	AK703	Unknown, Vietnam	AB564117[g]	AB564142[g]	AB601567[g]
	HS2372	Taichung County, Taiwan	AB564118[g]	AB564143[g]	AB601568[g]
Mustela altaica , mountain weasel	AK805	Altai region, Russia	AB051239[d]	AB564144[g]	AB601569[g]
Mustela erminea , ermine	HS678	Novosibirsk, Russia	AB564119[g]	AB564145[g]	AB601570[g]
	HS1759	Iwate, Japan	AB564120[g]	AB564146[g]	AB601571[g]
Mustela eversmanii , steppe polecat	HS2169	Chita region, Russia	AB026102[i]	AB564148[g]	AB601572[g]
Mustela furo , ferret	TH27	Experimental Animal (The Jikei University)	AB026103[i]	AB564149[g]	AB601573[g]
Mustela itatsi , Japanese weasel	TH50	Ishikari, Hokkaido, Japan	-	AB564150[g]	AB601574[g]
	TH89	Aomori, Japan	AB564121[g]	-	-
Mustela kathiah , yellow-bellied weasel	TH321	Kunming, China	AB285331[k]	AY882063[l]	AB601575[g]
Mustela lutreola , European weasel	AK13	Novosibirsk, Russia	AB026105[i]	AY750628[c]	AB601576[g]
Mustela nivalis , lease weasel	HS597	Eniwa, Hokkaido, Japan	AB564122[g]	AB564152[g]	AB601577[g]
	HS686	Aomori, Japan	AB564123[g]	AB564153[g]	AB601578[g]
	HS928	Yeongyang, South Korea	AB564124[g]	AB601553[g]	AB601579[g]
	HS1119	Kedrovaya, Primorye, Russia	AB564125[g]	AB564154[g]	AB601580[g]
	HS1745	Chizchik River, Uzbekistan	AB564126[g]	AB564155[g]	AB601581[g]
	HS1758	Thuringia, Germany	AB564127[g]	AB564156[g]	AB601582[g]
	HS1913	Mt. Odaesan, South Korea	AB564128[g]	AB564157[g]	AB601583[g]

	NMNS3302	Mt. Hohuanshan, Nantou County, Taiwan	AB046612[g]	AB564158[g]	AB601584[g]
	NMNS5244	Mt. Hohuanshan, Nantou County, Taiwan	AB564129[g]	AB601554[g]	AB601585[g]
	TH229	Thuringia, Germany	AB564130[g]	AB564160[g]	AB601586[g]
Mustela nudipes , Malayan weasel	BM2002-227	Tasek Merimbun Heritage Park, Tutong, Brunei Darussalam	AB285332[k]	AB564161[g]	AB601587[g]
Mustela putorius , European polecat	AK710	Moscow, Russia	AB026107[i]	AY750630[c]	AB601588[g]
Mustela sibirica , Siberian weasel	AK#9	Primorye, Russia	AB564131[g]	AB564162[g]	AB601589[g]
	HS1121	Primorye, Russia	AB564132[g]	AB564163[g]	AB601590[g]
	HS1145	Mt. Hohuanshan, Nantou County, Taiwan	AB564133[g]	AB564164[g]	AB601591[g]
	HS1146	Mt. Hohuanshan, Nantou County, Taiwan	AB051243[d]	AB601555[g]	AB601592[g]
	TH51	Tsushima, Japan	AB564134[g]	AB564165[g]	AB601593[g]
	TH56	Mt. Chiri, South Korea	AB564135[g]	AB564166[g]	AB601594[g]
	TH98	Chikushi, Fukuoka, Japan	AB564136[g]	AB564167[g]	AB601595[g]
Mustela strigidorsa , back-striped weasel	ANWC M32057	Oudomsouk, Nakai, Khammouan, Laos	AB305635[k]	AB564168[g]	AB601596[g]
Neovison vison , American mink	TH49	Nayoro, Hokkaido, Japan (Introduced)	AF057129[b]	AY377285[e]	AB601597[g]

[a] Voucher number and Locality refer only to sequences generated in this study.

[b] Koepfli and Wayne (1998); [c] Flynn et al. (2005); [d] Hosoda et al. (2000); [e] Davis et al. (2004); [f] Delistle and Strobeck (2005); [g] Sequences generated in this study; [h] Sato et al. (2009b); [i] Kurose et al. (2000); [j] Koepfli and Wayne (2003); [k] Sato et al. (unpublished); [l] Yu and Zhang (2006).

Footnote

Sato et al. (unpublished): Sato, J.J., Wolsan, M., Prevosti, F.J., D'Elía, G., Begg, C., Begg, K., Hosoda, T, Campbell, K.L., and Suzuki, H. Evolutionary and biogeographical history of weasel-like carnivorans (Musteloidea)

Table 2. Divergence times of mustelid lieages from Taiwan and the continent estimated with the Bayesian relaxed molecular-clock approach.

	Posterior mean (Ma)	95% HPD*
Martes flavigula	0.11	0.04 - 0.19
Melogale moschata	1.46	1.00 - 1.97
Mustela nivalis	0.36	0.26 - 0.48
Mustela sibirica	0.63	0.43 - 0.82

*: HPD, Highest Posterior Densities