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Biogeographical studies on the least weasel (*Mustela nivalis*) and the feral Japanese marten (*Martes melampus*)

（イイズナと移入種ニホンテンの生物地理学的研究）

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Abstract

To reveal the distribution and the dispersal history of the species is a basic concern of the biogeographical science. Among the carnivorans, Mustelidae has adapted to various habitat such as sea, river, forest, and high mountains. However, it is still unknown how they evolved and acquired their distribution and ecological niches for each species. In the present study, the dispersal history of the least weasel *Mustela nivalis*, distributed in Holarctic, was elucidated with phylogeographical analyses on the continental scale. Furthermore, the individual movements of the feral Japanese marten *Mates melampus*, in the local population, were revealed by the molecular ecological analyses.

The least weasel is one of the most widely distributed mustelids. While previous studies have identified distinct western and eastern mtDNA lineages of the species in the western Palearctic, their broader distributions across the Palearctic have remained unknown. To address the broad-scale phylogeographical structure, the sampling locations were spread to populations in Eastern Europe, the Urals, and Japan, and analyzed the mtDNA control region and cytochrome *b*, the final intron of the zinc finger protein on Y chromosome (*ZFY*), and the autosomal agouti signaling protein gene (*ASIP*). The mtDNA data analysis exposed the previous western lineage (Clade I) but poorly supported assemblage extending across Palearctic, whereas the previous eastern lineage (Clade II) was reconfirmed and limited in the south western part of the Palearctic. The *ZFY* phylogeny showed a distinctive split that corresponding to the mtDNA lineage split, although less phylogeographical structure was seen in the *ASIP* variation. The present data concur with the previous inference of the Black Sea-Caspian Sea area having an ancestral character. The Urals region harbored high mitochondrial diversity, with an estimated coalescent time of around 100,000 years, suggesting this could have been a cryptic refugium for least weasels. Based on the coalescent-based demographic reconstructions, the expansion of Clade I across the Palearctic was remarkably rapid, while Clade II was relatively stable for a longer time. It seems that Clade II has maintained a constant population size in the temperate region, and the expansive Clade I represents adaptation to the cold regions.

The Japanese marten is an omnivorous mustelid endemic to the mainlands of Japan. For fur production, this species was introduced to Hokkaido, around the 1940s. After the fur production was closed, fugitive individuals spread to southwestern Hokkaido, but little information on their ecological status make the management challenging. In the present study, the genetic population structure of the feral Japanese marten was assessed with a fecal DNA analysis, focusing on some local populations in Sapporo, Hokkaido. Surveys were carried out in a valley area and mountainous areas from 2016 to 2018. In total, 252 out of 317 fecal samples were assigned to the Japanese marten with mitochondrial DNA D-loop sequences, separating to two haplotypes. From 102 fecal samples, 36 Japanese martens were identified by genotyping of nine microsatellite loci. The genetic population structure in the valley was different from that in the mountainous areas: four full sibling sets were detected in the valley, whereas there was only one sibling set and small genetic variation in the mountainous areas. Similar to the populations from the mainlands of Japan, the feral Japanese marten increased its population size in autumn and preferred riparian forests. It was newly discovered that Japanese marten preferred the Sakhalin fir *Abies sachalinensis*, leading to a hypothesis that coniferous forests can be an important resource for the feral Japanese marten in Hokkaido.

As a result, the least weasel had experienced rapid expansion to the northern Palearctic. This trend was also observed in the other species, such as stoat, red fox, Eurasian lynx, and brown bear. In addition, the present study showed a new northern cryptic refugia of least weasel, suggesting the adaptation to the cold regions. Furthermore, the multiple individuals' movements of the feral Japanese marten were revealed by the intensive surveys on the local population. This provoked their novel habitat preference to the coniferous forest and the occurrence of full sibling sets with male dispersal patterns. Thus, the present study showed the movement patterns of two mustelids on continental and local scales with phylogenetical and molecular ecological studies. These results will contribute to elucidate the complex Mustelidae evolutionary history.

General Introduction

Some species have narrow distributions within limited geographic regions, while others are distributed broadly across the globe. A basic concern of biogeographical science is to understand how species have evolved and acquired their worldwide distributions.

Phylogeography explores the evolutionary and dispersal histories of species using the genealogical information embedded in their DNA (Avise 2000). Many studies of phylogeography have tried to reconstruct the migration histories of mammalian species in the Palearctic and to identify their glacial refugia, which have frequently been located in the Balkan and Iberian peninsulas, in the Caucasus and the Russian Far East (e.g. Frantz et al. 2014; Hewitt 1999; Hope et al. 2010; Korsten et al. 2009). The location of these refugia evidently reflects the species' environmental tolerances (Schmitt and Varga 2012). Moreover, Řičánková et al. (2015) pointed out that Central Asia was important as a glacial refugium for the megafauna and that the "mammoth fauna" (these were the cold tolerant fauna, which developed and reached their peak during the late Pleistocene, see Vereshchagin and Baryshnikov, 1992) remained there with highest frequency. Recently, the Y chromosomal genes are also commonly used to reveal the history of paternal lineage (e.g. Hirata et al. 2017), which enable to approach the sex-biased dispersal history, combining with the maternal genetic markers such as the mitochondrial DNA (mtDNA).

On the other hands, some species have expanded their distribution without going through their original evolutionary history. It is the invasive non-native species. Most of them were introduced with human movements and activities, and now, they are one of the main threats for biodiversity in the world (Bellard et al. 2016). Recently, the application of genetic techniques is common and essential for the management of the invasive non-native species, because genetic techniques provide us the biological information such as their distribution, origins, population size, and the potential eradication of them (Browett et al. 2020). On studying the invasive non-native species, not only for the management, there

is also an evolutionary biological viewpoint as how they can survive in the non-native range of them (e.g. Barun et al. 2015).

Mustelidae is a prime candidate to investigate patterns of adaptive radiation (Sato et al. 2012; Law et al. 2018), because this family holds the largest number of species and various ecological niches among carnivorous mammals. Previously, the evolutionary history of Mustelidae has been discussed repeatedly (e.g. Koepfli et al. 2008; Sato et al. 2012; Law et al. 2018). Based on Sato et al. (2012), the Mustelidae arose approximately 16.1 million years ago (mya) within a period of global warmth (Mid-Miocene Climatic Optimum: one of Earth's most recent prolonged warming events). For extant Mustelidae, badgers and martens diverged from ancestral species at first, and then weasels, polecats, minks, and otters have diverged and adapted to other ecological niches. Common features of most Mustelidae are elongate body and sexual size dimorphism (SSD) with male-biased dimorphism (male's body become bigger). The elongated body is considered as a result of evolution to enter burrows and confined spaces to obtain prey, and they increased energy requirements with the greater surface area, which leads to that metabolism of cold stressed of Mustelidae is 50-100% greater than normal shape mammals (Brown and Lasiewski 1972). The male-biased SSD gives rise to the male sampling bias, because male's home ranges is larger than female's (Buskirk and Lindstedt 1989), and it might drove and maintained by both sexual selection and niche divergence (Law 2019).

In this way, the phylogenetic status and morphological characters were often investigated. However, the biogeographic history and ecological niches of each Mustelidae species are still insufficient, probably caused by their elusiveness. In fact, seven Mustelidae species inhabit in Japan, but their phylogeographic and ecological data were insufficient to discuss the mustelid faunal assemblage (Sato et al. 2013).

Mustelidae species have adapted to not only temperate regions but cool-temperate regions, although there are still few discussions on the cool-temperate adaptation of Mustelidae with their demographic history and adaptive traits. In addition, lately, some Mustelidae species were introduced to outside of their native range by human movements, and they also have become big threats to biodiversity.

Hence, to reveal phylogeographical history and ecological niches of Mustelidae will contribute to Evolutionary Biology.

In the present study, the biogeography of the least weasel *Mustela nivalis* and the Japanese marten *Mates melampus* were focused. The phylogeography of least weasel is still unclear for their worldwide distribution. The Japanese marten is an endemic species in the mainland of Japan, and it was introduced to Hokkaido and Sado islands in Japan, however, the biological data of the feral populations is still few. Based on the results, it is discussed how the least weasel expanded to their distribution and which factors affect the movements of the feral Japanese marten.

Chapter I

Phylogeography and population history of the least weasel (*Mustela nivalis*) in the Palearctic based on multilocus analysis

(The content of this chapter was published in *Journal of Zoological Systematics and Evolutionary Research* 58:408–426.)

Introduction

The least weasel *Mustela nivalis* Linnaeus 1766 is one of the most widespread carnivore taxa in the Northern Hemisphere with a range covering most of the Palearctic: Europe, North Africa, Northern Asia including the Japanese islands, and North America. Considering this fossil records, the least weasel is included in the "mammoth fauna" (Sheffield and King 1994; Youngman 1993, and references therein). This species is considered a mesopredator, and their diet consists mainly of rodents, lagomorphs with some insects (King and Powell 2007). The least weasel has significant variation in body and skull sizes and proportions throughout its huge distribution range (Abramov and Baryshnikov 2000). Along with an overall high variation of cranial characters, there is a tendency towards an increase of body size and relative tail length from the north to south and to some extent from the east to west (Abramov and Baryshnikov 2000). Abramov and Baryshnikov also suggested least weasel has unique geographical variations such as the distribution of the two pelage colorations (*nivalis* and *vulgaris*): the *nivalis* type is distributed in northern Palearctic and the *vulgaris* type in the Mediterranean region, and the ancestral cranial type, in turn, is distributed in Northern Africa, Spain, Caucasus, Middle East and Central Asia. It is also remarkable that the body sizes of least weasel do not follow the Bergmann rule (Bergmann 1847). King and Powell (2007) suggested that this phenomenon is affected by the unique evolutionary process of least weasel, which have decreased their body sizes so that they can prevent the heat loss and forage the small rodents in the cold environment. In fact, Marciszak and Socha (2014) reported a correlation between the temperature and the cranial size using the fossil materials from Polish caves: the size decreases during colder periods and increases in warmer intervals. In addition, Zub et al. (2011) suggested the winter survival rate is higher for smaller than for larger least weasel. It is plausible that several weasel characters including the ecological status, comprehensive distribution and great geographical variation have resulted from adaptation to the various environments, and it is interesting to further understand the evolutionary history of least weasel.

The chromosome number of the least weasel in Siberia and in Hokkaido, Japan, is 2n=42, whereas on the Honshu Island it is 2n=38 (Mandahl and Fredga 1980; Obara 1991). Distinct

mitochondrial DNA (mtDNA) control region (*CR*) sequences were observed in least weasel from Hokkaido and Honshu islands, as reported by Kurose et al. (1999). In a phylogeographic analysis of the mtDNA *CR* across Siberia, the Caucasus, Central Asia, and North America, the lineages around the Caucasus were the most variable probably due to the introgression or maintenance of polymorphic status of their ancestral population (Kurose et al. 2005). Meanwhile, Lebarbenchon et al. (2010) detected a subdivision of the Western Palearctic least weasel mitochondrial diversity into Western (Clade I) and Eastern (Clade II) lineages, using the mtDNA *CR* and cytochrome *b* (*Cytb*) sequences. Using only *Cytb* data, Mcdevitt et al. (2012) identified a presence of a suture zone between the lineages in Poland, and suggested that the Carpathians were one of the refugia for least weasel. Mcdevitt et al. (2012) also and confirmed the existence of the two main lineages, similar to the western Palearctic region reported by Lebarbenchon et al. (2010). Furthermore, Rodrigues et al. (2016) revealed the taxonomic status of the Egyptian least weasel that shared a haplotype with weasels in Turkey and Mediterranean islands. Additionally, least weasel could have been imported to some Mediterranean islands artificially, and the gene flow could have affected the genetic structure (Lebarbenchon et al. 2010; Rodrigues et al. 2017). However, the original dispersal and migration history of least weasel still remains unclear in Palearctic.

To further understand the molecular phylogenetic and biogeographical relationships among least weasels, mtDNA *CR* and *Cytb*, as maternally inherited genes were analysed in the present study, from a range of new localities across the Palearctic region. In addition, the final intron of the zinc finger protein locus on the Y chromosome (*ZFY*) as a paternally inherited gene and the agouti signaling protein locus (*ASIP*) as a biparentally inherited gene were also sequenced and analysed. Combining the present data with the previous results, the phylogeography and migration history of least weasel in Palearctic were discussed.

Materials and methods

Specimens

Tissue samples were obtained from collaborative laboratories and museums in Bulgaria, Russia, Finland,

and Japan (76 samples consisting of 65 ethanol-preserved muscle tissue and 11 dried skins; Table I-1 and Figure I-1). Of these, 31 were also used in the previous mtDNA *CR* studies of Kurose et al. (1999, 2005). In addition, published data were employed from public databases, as specified in Table I-1 and 2.

DNA extraction, amplification, and sequencing

Total DNA was extracted from the samples using the DNeasy Tissue and Blood Kit (QIAGEN) or QIAamp DNA Investigator Kit (QIAGEN), following the manufacturer's protocols, including extraction blanks for the negative control. All experiments were done with filter tips and disposal tubes for preventing the contamination in the present study.

Two fragments of the mtDNA, *CR* (around 550 base-pairs, bp) and *Cytb* (1140 bp), and one from the Y-chromosomal *ZFY* (687 bp) and one from autosomal *ASIP* gene (480 bp; intron and exon), were amplified by PCR with the primers shown in Table I-3. For DNA degraded samples, small fragments (165–705 bp) were amplified to overlap each other. In the samples of dried skins, relatively shorter DNA fragments were amplified. Furthermore, the experiments were duplicated for the data confirmation on the degraded samples.

PCR was carried out in a volume of 10 µl including 2.0 µl of 5×Prime STAR GXL DNA Buffer (Takara), 0.8 µl of dNTP mixture (2.5mM each dNTP; Takara), 0.2 µl of Prime STAR GXL DNA Polymerase (1.25 U/µl, Takara), 0.4 µl for each of forward and reverse primers (10 pmol/µl), 0.4 µl of bovine serum albumin (0.4 µg/µl, Roche), 1.0–2.0 µl of the DNA extract, and the volume was adjusted to a total of 10 µl with distilled water. The amplification was performed with 30–45 cycles of 98 ^{o}C for 10 s, 54–61.4 ^{o}C for 15s and 68 ^{o}C for 1min using the Thermal Cycler TP400 (Takara). To check the amplicon, 3µl of the PCR product was electrophoresed on a 2% agarose gel, stained with ethidium bromide, and observed under an ultraviolet illumination. Then, the PCR products were purified with the QIAquick PCR Purification Kit (QIAGEN). In addition, it was confirmed no PCR amplification in the negative controls.

The DNA cycle sequencing was performed with the BigDye v3.1 or 1.1 Cycle Sequencing Kit (Applied Biosystems, ABI), using the PCR primers shown in Table I-3. Sequencing reaction was performed in a volume of 10 μl containing 1.75 μl of 5×BigDye Sequencing Buffer (ABI), 0.5 μl of Ready Reaction Premix (ABI), 0.5 μl for each of the primers, 2.0 μl of DNA template, and the volume was adjusted to 10 μl with distilled water. Cycle sequencing was performed with a preheating at 96 ºC for 1 min, and 25 cycles of 96 ºC for 10 s, 50 ºC or 55 ºC for 5 s, 60 ºC for 4 min. The cycle PCR products were precipitated with ethanol, and dissolved in 10 μl of formamide, and applied to an ABI 3730 DNA Analyzer for sequencing. The sequence alignment for all loci was performed using MUSCLE (Edgar 2004) in MEGA6 (Tamura et al. 2013).

Phylogenetic trees and networks

The nucleotide substitution models for analyses of all loci were selected using the Bayesian information criterion (BIC) with Partitionfinder v1.1.1 (Lanfear et al. 2012). The selected models were used to reconstruct phylogenetic trees (gene genealogies) with two approaches and programs: MrBayes v3.2.6 (Ronquist et al. 2012) for analyses under Bayesian inference (BI) and Garli v2.01 (Bazinet et al. 2014) for Heuristic Maximum likelihood analyses (ML). For analysis of the concatenated *CR* and *Cytb* sequence segments, separate substitution models were estimated for the *CR* segment and for each of three codon positions in the *Cytb* (1,2,3): these were K81uf+I+G, K80+G, HKY and TrN+G, but in the phylogenetic analysis HKY and GTR models were used instead of K81uf and TrN models respectively, because these models were not implemented in MrBayes (Hasegawa et al. 1985; Kimura 1980, 1981; Lanave et al. 1984; Tamura and Nei 1993). The list of the other nucleotide substitution models is shown in Table I-4.

For the BI trees, Markov chain Monte Carlo (MCMC) analyses were run for 5×10^6 to 1×10^7 generations with trees sampled every 1000 generations, and the first 25% of the trees were discarded as burn-in. The convergence of MCMC analyses was confirmed by indicating the average standard deviation of split frequencies was below <0.01, and the parameter values sampled from MCMC runs

were checked in Tracer ver. 1.6 (http://tree.bio.ed.ac.uk/software/tracer/). The Bayesian posterior probabilities (PP) were also obtained from MrBayes. In the reconstruction of the ML trees, the effort to search for the best tree was repeated 20 times independently and terminated at 20000 generations with Garli. The bootstrap percentages (BP) were obtained from 1000 pseudoreplicates with Garli to assess the confidence values of internal branches.

Two confidence values (PP and BP) for the reconstructed branches were mapped on the trees using SumTrees 3.3.1 of the DendroPy package (Sukumaran and Holder 2010). Branches of the trees were regarded as well supported when their PP was ≥ 0.95 and BP was ≥ 70 %. Haplotypes from other three closely related species (Malayan weasel *Mustela nudipes* Desmarest 1822: AB601587 for *CR*, AB285332 for *Cytb*; yellow-belled weasel *Mu. kathiah* Hodgson 1835: AB601575, AB285331; stoat *Mu. erminea* Linnaeus 1758: AB006730, AB026101) were used as outgroups in the mtDNA tree. The stoat sequences of *ZFY* and *ASIP* (AB491595 and JX130732; respectively) were used as the references for these genes. The SINEs (short interspersed nuclear elements) region of the stoat's *ZFY* sequence was excluded in the analysis. These phylogenetic trees were visualized and edited with Figtree v1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/).

Median-joining networks (Bandelt et al. 1999) of the concatenated mtDNA *CR* and *Cytb*, of *ZFY*, and of *ASIP* separately were reconstructed with POPART 1.7 (Leigh and Bryant 2015). The program PHASE v2.1 (Stephens and Donnelly 2003; Stephens et al. 2001) implemented in DnaSP v5 (Librado and Rozas 2009) was used to estimate the haplotypes of *ASIP*. In all network analysis, the gap sites were treated as missing.

Divergence time and demographic history

Population divergence times were estimated from the concatenated mtDNA two-locus data set using BEAST v2.4.8 (Bouckaert et al. 2014) package, with the outgroups mentioned above. Three calibration points were set on the basis of the divergence times among outgroup species and least weasel. Following Sato et al. (2012) and Kinoshita et al. (2015), a normal distribution with a mean of following time and

SD were adopted to this analysis: 5.985 ± 0.315 million years ago (mya) between Malayan weasel and the others, 5.365 ± 0.195 mya between yellow-belled weasel and the others except Malayan weasel and 3.685 ± 0.285 mya between stoat and least weasel. In addition, the published estimate of least weasel mtDNA *Cytb* substitution rate of 2.1% Myr^{-1} from Dawson et al. (2014) and the strict clock model were also used. The tree prior was set as the coalescent constant population model. The HKY+I+G substitution model was used for all loci, which was the best model for the present data without partitions using BIC produced by Partitionfinder. The evolutionary rates of other loci were estimated based on these configurations. The MCMC analyses were run 1×10^8 generations with trees sampled every 1000 generations and the first 10% of the trees were discarded as burn-in. The effective sample sizes were confirmed by Tracer, with the requirements of convergence of MCMC chains and values for all parameters exceeding 200. The maximum clade credibility tree was selected using TreeAnnotator and visualized with Figtree.

Demographic changes (effective population size history) were simulated by the Extended Bayesian Skyline Plot (EBSP) approached in BEAST. The HKY+I+G model and the substitution rate of 2.1% Myr^{-1} for the mtDNA *Cytb* of least weasel were applied to each of the two identified main mitochondrial clades (Clade I and II; 106 and 56 individuals, respectively). The MCMC analyzes were run 1×10^8 generations with trees sampled every 1000 generations and the first 10% of the trees were discarded as burn-in. The effective sample sizes and the convergence of MCMC chains were confirmed by Tracer.

Genetic structure

Genetic diversity and demographic history were estimated from each data set separately (i.e. concatenated mtDNA *CR* and *Cytb*, *ZFY*, and *ASIP*) with Arlequin ver. 3.5.1.3 (Excoffier and Lischer 2010), calculating the haplotype diversity, the nucleotide diversity, the neutrality tests of Tajima's *D* (Tajima 1989), and Fu's F_s (Fu 1997).

Population structure was also assessed with SAMOVA (spatial analyses of molecular variance;

Dupanloup et al. 2002) implemented in SPADS 1.0 (Dellicour and Mardulyn 2014), using all 168 concatenated mtDNA *CR* and *Cytb* sequences. Specimens were grouped as populations by country, except for Japan where Hokkaido and Honshu were treated as different populations because there is a chromosomal difference between them. The number of a priori groups (*K*) was varied between 2 to 10, with 10000 iterations and 10 repetitions. Following Magri et al. (2006), the preferred *K* was selected by choosing the highest F_{CT}, and then configurations with single-population groups were excluded. In addition, the pairwise differences among populations were estimated using the fixation index Φ_{st} (Excoffier et al. 1992).

Results

DNA sequencing

A total of 210 novel sequences (45 for *CR*, 69 for *Cytb*, 29 for *ZFY* and 67 for *ASIP*) were obtained in the present study, and deposited to the DDBJ data base with accession numbers: *CR,* LC314601-LC314645; *Cytb*, LC324904-LC324972; *ZFY*, LC325040-LC325068; and *ASIP*, LC324973-LC325039 (Table I-1). The novel sequences were compared with the previously reported data (Table I-1 and 2), and then overlapped regions were used to classify the haplotypes. All haplotype numbers were shown in Table I-2 and 5.

Diversity and genealogy of mtDNA

In all, 92 distinct haplotypes were identified from the 215 sequences of the mtDNA *CR*. The length of *CR* was 514 bp including insert-deletion sites. The BI tree of *CR* was multifurcate and unstructured with no clear clusters except for the Hokkaido population (cluster Ie) (Figure I-2). Likewise, 116 haplotypes of *Cytb* were detected from 221 sequences of 1117 bp. The BI tree of *Cytb* was also poorly resolved, but some populations including the Hokkaido population and some European populations made up regional clusters (Figure I-3).

In the concatenated *CR* and *Cytb* data (1631 bp), 119 distinct haplotypes were recognized among 168 individuals (69 newly sequenced). This BI tree exposed two main clades (Figure I-4),

corresponding to the clades I and II of Lebarbenchon et al. (2010), however the support values were lower than the previous report. Clade I was in low monophyly with low supported values (0.77 PP and 9% BP), while Clade II had relatively high values (1.0 PP and 50% BP) (cf, 1.0 / 93% for Clade I and 0.94 / 76% for Clade II in Lebarbenchon et al. 2010) (Figure I-4). All samples were divided to clade I, II and the others, based on the topology of this BI tree (Figure I-4). The others consisted of the individuals of Turkmenistan (C25 and C28), Georgia (C30 and C31) and Ukraine (C32 and C33). These individuals were located at the most basal position. The ML tree in turn was multifurcated and did not have the main clades (Figure I-5).

The *CR* and *Cytb* concatenated BI tree also exhibited eight clusters with high support values (Figure I-4). Cluster Ia consisted of individuals from Spain to South Ural. Cluster Ib was shared by individuals from Finland. Cluster Ic distributed in Poland and South Ural. Cluster Ie consisted by only the Hokkaido population. Cluster If included only the North Ural individuals. Cluster IIb was shared by North African individuals and Sardinia. Cluster IId consisted of individuals from Greece, only. Cluster IIe was found from Greece to Poland. In addition, there were three clusters with relatively high support values: Cluster Id was consisted of South Korea, Cluster IIa was shared by Italian and its insular individuals, Cluster IIc was distributed in Serbia, Bulgaria, and Uzbekistan. Individuals of Honshu Island of Japan shared haplotype C66, which is more closely related to that C105 of a North Ural individual than the Hokkaido population. The locations of clades and clusters were plotted on a Palearctic map (Figure I-6).

The mtDNA haplotype network indicated two main clades clearly, compared to the BI tree (Figure I-7). Clade I was too complicated to read the phylogeographical relationships between haplotypes with many loops indicating the homoplasies. In contrast, Clade II had relatively clear internal structure and the mentioned clusters were supported. Haplotype C28 from Turkmenistan and the Ukrainian C32 and C33 were not placed in either of the two main clades but occupied an intermediate position of them.

Diversity in the paternal and biparental genes

Only two *ZFY* haplotypes, D1 and D2, were found from among 30 individuals, of which 29 were newly sequenced in the present study. These two haplotypes were strongly diverged, with eight nucleotide differences and an 8-bp indel between them (Figure I-8). D1 was shared by 12 individuals of Japan (Hokkaido and Honshu), two of Russia and nine of Finland, whereas D2 was common to all six individuals of Bulgaria. The Bulgarian D2 featured the 8-bp insertion, which was also present in the *ZFY* sequence of the closely related stoat.

For *ASIP*, 12 haplotypes were detected from 67 individuals obtained in the present study (Figure I-8), which had 12 polymorphic sites. A central haplotype E1 was found in all regions except Honshu, where all individuals shared another haplotype E5. Hokkaido in turn had five haplotypes. However, no further phylogeographical structure was seen, and the differences between haplotypes were just one or two substitutions.

Phylogenetic trees from multilocus data

All sequence data on the four loci (mtDNA *CR*, mtDNA *Cytb*, *ZFY*, and *ASIP*) obtained in the present study were concatenated to reconstruct a BI tree (Figure I-9a). This tree consisted of 29 individuals of 2823 bp with stoat as an outgroup, the two main clades were supported with higher bootstrap values compared to the mtDNA tree: 92 % for Clade I and 98 % for Clade II. On the other hands, if *ZFY* was excluded from the data set, no strongly supported major clustering was seen, except for some regional clusters such as Finland, Hokkaido, and Bulgaria (Figure I-9).

Divergence time and population expansion history

The Bayesian phylogram with BEAST analyses, based on the two concatenated mtDNA loci, had a topology similar to that from MrBayes (Figure I-10). The time of most recent common ancestor (tMRCA) of least weasel would have been on 740 (560-940, 95%HPD) thousand years before present (kyBP), at the split of the Turkmenistan lineage (C25) from the others. The coalescence of the remaining lineages would have been 480 (370-600) kyBP. The divergence time of the two main clades could not

be traced from this tree, caused by the low PP support (< 0.95). The tMRCA of clade II was 200 (140-270) kyBP. The divergence times of the clusters were also obtained (Figure I-4). Based on the demographic fluctuation analysis, both clades I and II had experienced the population expansion from small populations. Clade II was relatively stable, whereas the expansion of Clade I was more rapid (Figure I-11).

Genetic structure

A hierarchical partition of genetic diversity in the concatenated mtDNA *CR* and *Cytb* data is shown by regions, clades, and intra-clade clusters in Table I-6. Overall, the nucleotide diversities were low (shallow genealogy) but haplotype diversities were high. Populations of the Black Sea-Caspian Sea region had the highest nucleotide diversities (0.028 for Turkmenistan; 0.025 for Ukraine and 0.026 for Georgia), and that of the Ural population (0.009) was also relatively high. Cluster If had the highest nucleotide diversity among clusters. The Honshu population in turn was monomorphic at all loci. Significant values of Tajima's D and Fu's F_s statistics are suggestive of the rapid expansion in the Palearctic population. Likewise, treating the mitochondrial lineages separately, Clade I and cluster Ia show signatures of rapid expansion. The Hokkaido population, Clade II, and cluster IIe also had significant values of Fu's F_s. The autosomal *ASIP* showed an overall low nucleotide diversity (0.003) and a high haplotype diversity (0.78). The Y-chromosomal *ZFY* showed relatively low nucleotide and haplotype diversities (0.008 and 0.331). The data of *ZFY* and *ASIP* did not indicate an expansion.

Least weasel had genetic differences to each other ($\Phi_{st} = 0.556$) in the Palearctic, but an attempt to divide the regional populations to subgroups by SAMOVA was not very successful. A search for the number of independent groups (K) that show the maximum inter-group diversity F_{CT} showed that this statistic increased with K and did not reach a plateau over $K = 10$ (Figure I-12). A configuration of $K = 3$, for which F_{CT} (0.368) was the highest among the others without single-population groups was very similar to the mtDNA haplotype network (Figure I-6). Group I represented populations distributed across the continent from Spain to Japan, which carry the mtDNA Clade I except for Georgia. Group II

in turn included populations of Romania, Serbia, Bulgaria, Greece, Turkey, Tunisia, and Morocco, which harbor mtDNA Clade II. Group III was composed of the populations from Turkmenistan and Ukraine (Table I-7).

Discussion

Phylogeography of the least weasel in Palearctic

The present data of the concatenated mtDNA *CR* and *Cytb* sequences from a geographically expanded data set demonstrate a confused phylogeographic structure of least weasel in Palearctic. The tree topologies agree with the two main lineages (clades I and II) of Lebarbenchon et al. (2010), whereas the support values could not provide full confidence on clade I's monophyly (Figure I-4). Clade I has a broad distribution across northern Palearctic, from Spain in the west to Japan in the east, while clade II is limited to the South western part of the Palearctic, between North Africa and East Europe. At a lower level, the least weasels were tentatively classified into eleven mtDNA clusters. The Turkmenistan lineage of Central Asia plausibly represents the most ancestral split among all Palearctic populations, as in the data of Kurose et al. (2005).

The present data expanded the coverage of European sampling, e.g. Bulgaria and the Urals, supplementing the data of Lebarbenchon et al. (2010) and Rodrigues et al. (2016, 2017). The Bulgarian population was separated to two clusters (IIc and IIe), and the Northern Urals population consisted of one distinctive cluster (If) by itself. Individuals from around the Black and Caspian Seas had the new *Cytb* haplotypes that seemed to represent the most ancestral lineages branches of the genealogy (Figure I-4). Furthermore, the Turkmenistan and Ukraine haplotypes appeared at an intermediate position between clades I and II in the mtDNA haplotype network (Figure I-7). This configuration probably brings about the lower confidence values of the main clades, relative to those reported by Lebarbenchon et al. (2010) and McDevitt et al. (2012).

The SAMOVA analysis also suggests the ambiguity for the phylogeographic structure,

although the interpopulation component of variation is large (Φ_{st} = 0.556). The data point to a history of demographic and geographic expansions that have taken place across large areas while more ancestral diversity has been retained in certain segments of the range. The nucleotide diversity through the Palearctic population is quite low (0.012), while the haplotype diversity is very high (0.992) (Table I-6). This suggests that least weasel experienced a rapid demographic expansion from a small population, as also indicated by the Bayesian simulation, Tajima's *D*, and Fu's F_s statistics (Figure I-11 and Table I-6). Remarkably, similar results were also reported in the closely related species stoat (Dawson et al. 2014). The data showed low genetic differences and suggested a rapid expansion of stoats from Spain across the continent and through Beringia to North America. Such continent-wide expansions have also been inferred from other carnivorans, such as Red fox (*Vulpes vulpes* Linnaeus 1758) and Brown bear (*Ursus arctos* Linnaeus 1758) (Korsten et al. 2009; Statham et al. 2014).

The phylogeographic structure also shows some phenotypic correlates. It seems that Clade II has survived and maintained a relatively constant population size and high genetic diversity in the temperate region in the south western part of the Palearctic, whereas the expansive Clade I represents adaptation to the cold regions. On the other hand, the SAMOVA grouping (K = 2) coincided with the distribution of the two coloration types recognized by Abramov and Baryshnikov (2000). Group I correlates with the *nivalis*-type coat color and Group II with the *vulgaris*-type. Atmeh et al. (2018) demonstrated a clear relationship between the predation pressure and the camouflage of pelage coloration in a field experiment. One could speculate that this also affected the demographic and evolutionary histories. Clade I (Group I) expanded rapidly across Palearctic including the cold regions.

The present study also for the first time addressed the signals of population history in variation of a biparentally inherited gene *ASIP* and the paternally inherited *ZFY*. The *ASIP* haplotype network however did not have any phylogeographic variation (Figure I-8). Even the Turkmenistan individual, which had a distinct mtDNA lineage, shared haplotype E2 widespread across the range, in Bulgaria, Urals, Russia, Finland, and Hokkaido. The *ZFY* haplotype network in turn had a distinct subdivision of two main lineages that corresponded with the mtDNA Clade I-II split (Figure I-8). Haplotype D1 was

distributed from Finland to Japan. This distribution pattern is indeed similar to that in the gray red-backed vole (*Craseomys rufocanus* Sundevall 1846) which exhibits a monomorphism from western to far eastern Russia, within the partial sequence of the Y chromosomal DNA (Abramson et al. 2012), and the spatial distribution of mtDNA and Y chromosomal DNA haplotypes were slightly different. The present spatial distribution of the haplotypes also differed by each gene. As one hypothesis, this discordance could be caused by behavioral differences between sexes of least weasel. The least weasel has the significant sexual dimorphism in body size (greater male versus female) and sex-biased differences in habitat use (King and Powell 2007). Furthermore, McDevitt et al. (2013) suggested the dispersal of least weasel was sex-biased towards males. The male wide dispersal could be attributed to the phylogeographical status. In fact, the two main clades are supported with high values if the BI trees included *ZFY* sequences in data sets (Figure I-9). Similar conclusions about discordant diversity in multiple loci from the same species have been drawn from other studies, such as Abramson et al. (2012) and Jones and Searle (2015). They also suggested that the discordant may have been caused by the sex biased dispersal in male and female voles and mice. If the male-biased dispersal of least weasel is true, it is easy to suppose that the large males had been strongly affected by cold temperature and could not distribute in high latitude, considering that Zub et al. (2011) suggested larger least weasel had low survival rate in cold environments.

Characteristics of clusters and local populations

The eleven mtDNA clusters were also represented with relatively high support values in the lower-level structure of the mtDNA genealogy (Figure I-4). Three of these clusters (Ib, Ic and If) were newly recognized in the present study. Cluster If, only found in the Northern Urals, has the highest intra-cluster diversity. The tMRCA of this cluster was dated approximately 100 kyBP, close to MIS (marine isotope stage) 5c (105-93 kyBP, Räsänen et al. 2015). The population might then represent a relict that survived the glacial period, and the Ural region would have been one of the cryptic refugia for the species. Actually, based on the paleontological study, Kosintsev et al. (2016) reported least weasel appeared

consistently from MIS 5e in the Ural caves. Likewise, previous studies of other mammalian species also suggested possible refugia in the Ural Mountains. For example, the sables (*Martes zibellina* Linnaeus 1758; Kinoshita et al. 2015) and the bank voles (*Myodes glareolus* Schreber 1780; Deffontaine et al. 2005) from the Ural Mountains had relatively higher genetic diversities, suggesting the polymorphic status of relict populations of them. In addition, the Ural Mountains were reported as refugia for the common shrews (*Sorex araneus* Linnaeus 1758) by Polyakov et al. (2001). Cluster Ib consisted of Finnish individuals, and Cluster Ic consisted of just two individuals from Poland and South Ural.

The remaining eight clusters were previously reported (Kurose et al. 1999, 2005; Lebarbenchon et al. 2010; Rodrigues et al. 2016, 2017). Cluster Ia (=subclade Ia) is now shown to be distributed from Spain to South Urals, and its estimated tMRCA is projected twofold older than by Lebarbenchon et al. (2010) (120 kyBP vs 62kyBP). Kurose et al. (1999, 2005) first reported the cluster Ie (Hokkaido) and this tMRCA was estimated as less than 200 kyBP, but the present study showed around 90 kyBP. The result suggests that ancestors of the Hokkaido population could be prevented to pass the Tsugaru Strait which separates Honshu and Hokkaido, because that strait was formed around 100-150 kyBP (Ohshima 1991).

Remarkably, the Honshu population in Japan has no variation on any loci, and this lineage (C66) was more closely related to the North Ural lineage (C106) than the Hokkaido population in the mtDNA analysis. The *ZFY* haplotype D1, however, was shared among Honshu, Hokkaido, and North Ural. The number of chromosomes was also different between the Honshu population (2n = 38) and the Hokkaido population (2n = 42) (Obara 1991). In addition, all studied populations from Eurasia and North America bear the karyotypes similar to that of the Hokkaido population (Mandhal and Fredga 1980; Zima and Grafodatskij 1985). According to the present results and the previous studies, the migration history could be different between the Honshu and Hokkaido populations.

The Bulgarian individuals were separated to two clusters (IIc and IIe). Cluster IIc is distributed from Serbia in the Balkan Peninsula to Uzbekistan in Central Asia. Cluster IIe includes the individuals from Greece to Poland, and could have experienced the demographic expansion shown by Fu's F_s

statistics. Populations around the Black and Caspian Seas (Turkmenistan, Ukraine, and Georgia) had higher nucleotide diversities, and were differentiated from the other populations. It has been reported that the populations of the Black and Caspian Seas area could be the ancestor type of least weasel, using morphological characters (Abramov and Baryshnikov 2000) and mtDNA *CR* (Kurose et al. 2005). The present results emphasize this possibility.

Chapter II

Fecal DNA analysis revealed local population structure and habitat preference of the feral Japanese marten (*Martes melampus*) in Hokkaido, Japan

Introduction

The Japanese marten *Martes melampus* (Wagner 1841) is an opportunistic omnivore with seasonal variation in food habit (e.g. Okawara et al. 2018). This species is endemic to Japan except Hokkaido; native to Honshu, Shikoku, Kyushu, and Tsushima Islands (Masuda 2009). The Japanese marten is a forest-dweller, showing a habitat preference for broad-leaved and riparian forests (Tatara 1994a; Arai et al. 2003; Hoshino et al. 2014). Genus *Martes* shows a wide/various dietary breadth and food habit depending on geographical and climatic factors (Zhou et al. 2011). Recent reports on various food habits of the Japanese marten have provoked a discussion about biogeographical variation in their diet (Takatsuki 2017; Hisano et al. 2019; Tsuji et al. 2019). Populations in cool-temperate areas with heavy snow fall are likely more dependent on mammalian prey than those in warm-temperate zones, whereas populations in the latter areas are more dependent on fruits (Hisano et al. 2019; Tsuji et al. 2019), suggesting that Japanese marten can survive in the various area by shifting their food habit. In fact, there are persisted populations in their non-native area.

In Hokkaido, the Japanese marten was introduced for fur production around the 1940s, and after the fur industry was closed, fugitive individuals spread to southwestern Hokkaido and competed with sables *Martes zibellina* native to Hokkaido (Hirakawa et al. 2015). Currently, the distribution of the two *Martes* species was divided around the Ishikari low land (Figure II-1a, Murakami and Ohtaishi 2000; Hirakawa et al. 2015). Following this, the Ministry of the Environment of Japan listed Japanese marten to “List of invasive alien species threatening biodiversity, human health and/or economy development in Japan” in 2015 (http://www.env.go.jp/press/100775.html. accessed on 11 March 2020).

It is already known that the Hokkaido population of feral Japanese marten has multiple origins and high genetic diversity (Inoue et al. 2010; Kamada et al. 2013), however, little information on their ecological status make the management challenging, probably due to that the Japanese marten is an elusive species. Previously, feces were often used to research their food habits, because collecting feces is easier than other sampling methods. However, when there are sympatric carnivores inhabiting the study area, it is difficult to distinguish animal species dropping feces with morphological features or

empirical observations (Birks et al. 2005). Recently, Tsuji et al. (2011) reported the criteria of fecal diameters to distinguish the captive animals of Japanese marten and Japanese weasel *Mustela itatsi* (approximately 10.0 mm for the Japanese marten and 6.5 mm for the Japanese weasel). Additionally, Hisano et al. (2017) mentioned that the visual criteria are useful for fresh samples. But further investigation is still necessary, because it has been cautioned that such empirical criteria can lead to misunderstanding of the ecological status (e.g. Martínez-Gutiérrez et al. 2015). In Hokkaido, since six small-medium Mustelidae species (Japanese marten, sable, Japanese weasel, least weasel, stoat and American mink *Neovison vison*) inhabit sympatrically, it is especially important to do monitoring them with accurate methods.

Now-a-days, the application of genetic techniques is essential for the management of the invasive non-native species. Genetic techniques provide us the biological information, such as species distribution, their origins, population structure, and the potential eradication methods of them (Browett et al. 2020). Among various genetic techniques, fecal DNA analysis is a powerful tool to understand the species' habitat preference and density, within the surveyed area and periods (e.g. Balestrieri et al. 2015, 2016).

In the present study, fecal DNA analysis was adopted to investigate the distribution and population structure of the feral Japanese marten in Sapporo, Hokkaido for their management. Particularly, the intensive sampling was conducted in a valley area to research the population size fluctuation and the habitat preference of the local population of the feral Japanese marten. In addition, their ecological difference between native and feral populations was also discussed. Then, the optimal survey number and season to detect the feral Japanese marten presence are suggested for future monitoring and management.

Material and methods

Study area and sampling collection

Surveys were conducted in Sapporo, Hokkaido, Japan. Sapporo is located in the northwest of Ishikari

low land (Figure II-1a). According to the Japan Meteorological Agency, the climatic data in Sapporo during 1981 and 2010 were as follows: mean annual snow depth 597 cm; mean annual precipitation 1106.5 mm; mean annual temperature is 8.9°C (-7.0 to 26.4°C) (http://www.jma.go.jp/jma/index.html. accessed on 11 March 2020).

At first, the valley area (Route 6), located southern part of Sapporo and neighboring residential area, was surveyed intensively (Figure II-1c). This area included the Nishioka Park, marshlands, and an artificial pond (7.35ha), and it is listed as one of important areas for biodiversity conservation in the Ishikari low land (Takada et al. 2010). The Nishioka Park Office have kept various species records from visitors' observations. Three feral Mustelidae species were also recorded with photos: Japanese weasel, Japanese marten, and American mink. In addition, least weasel, racoon dog *Nyctereutes procyonoides*, red fox *Vulpes vulpes*, and racoon *Procyon lotor* were also observed in this area (Kawahara 1998).

Sampling was conducted 66 times along with 4.12km pathway, on June 2016 and between September 2016 to March 2018. Marten-like feces were collected, holding the appearance with twist, less than 15 mm, and/or occurrence with their footprints. Fecal diameters were measured when the fecal shape remained, to verify the above criteria for species identification. Locations of feces were recorded by a digital camera with a built in GPS (TG-835, Olympus) and georeferenced by QGIS version 3.8.3 (QGIS Development Team 2019). When the fecal locations were not plotted on the survey route, most likely due to GPS measurement errors, their plots were moved on the survey route by the shortest distance. Prior to DNA extraction, all feces were directly put into the 15 ml tubes and stored at -80°C for a week or more in the laboratory, to prevent the contamination and infection by parasites. During the sample collection, a part of the boardwalk was removed for the marshland maintenance, hence the feces found in the removed boardwalk were treated as occasional samples and excluded from following habitat analysis and estimation of population sizes.

Secondly, feces were collected in mountainous area in Sapporo, three times between June to November 2018 along the five trails (5.29-12.89 km) (Table II-1 and Figure II-1b): Northern part of Mt. Teine, Route 1; Southern part of Mt. Teine, Route 2; Mt. Moiwa, Route 3; Mt. Toishi, Route 4; Mt.

Shirahata, Route 5. The highest mountain within the study area is Mt. Teine (1023.1 m) and the lowest is Mt. Shirahata (321.2 m). In 1944 year, there used to be one of fur farms of Japanese marten near Route 1 (Kadosaki 1981). Two fecal samples were occasionally collected in Mt. Teine, near Route 1, and these were included to genetical analysis only.

According to Balestrieri et al. (2015), the minimum number of surveys (NS_{min}) to detect the occurrence of the feral Japanese marten were also assessed, using the probability model: $NS_{min} = \log(\alpha) / \log(1 - p)$, where $\alpha = 0.05$ sets the confidence level. Here, the occurrence of Japanese marten was defined when the feces were genetically identified.

DNA extraction and species identification

Total DNA was extracted from the samples with the QIAamp Fast DNA Stool Mini kit (QIAGEN), following the manufacturer's protocols. DNA extracts were stored at 4°C.

In preliminary experiments, the feasibility of the primer set of DS3 (Kurose et al. 2005) and MNE-DR7 (Mizumachi et al. 2017) for the mitochondrial DNA D-loop (mtDNA DL; around 370bp) was confirmed to distinguish seven Mustelidae species (Japanese marten, sable, Japanese weasel, Siberian weasel *Mustela sibirica*, least weasel, stoat, and American mink), using sequencing analysis and BLAST (Altschul et al. 1990). Thus, this primer set was used for the species identification in the present study. However, fox feces were also easily mistaken for feces of Mustelidae species (Davison et al. 2002). To detect and exclude the fox feces from the analysis, the red fox-specific primer set (Shimatani et al. 2008) was also used. PCR and DNA sequencing protocols were the same as Sato et al. (2020).

Individual identification and sex determination

All fecal samples identified as the Japanese marten were used for subsequent individual identification, and successfully genotyped samples were carried on following sex determination. For the valley area samples, the sex determination was performed for all martens' feces to investigate the sex ratio, even if the genotyping failed.

For individual identification, nine microsatellite loci were genotyped (Mar 2, 8, 15, 19, 21, 36, 56 and, 64 from Natali et al. 2010, and Mel106 from Carpenter et al. 2003). The PCR and genotyping protocols were the same as Amaike et al. (2018). To check the reliability of genotyping, identical genotypes were confirmed two times for heterozygosity and three times for homozygosity by repeating separate PCRs. After genotyping, the probabilities of identity for unrelated individuals (PID) and siblings (PID-sibs) (Waits et al. 2001) were calculated using GIMLET ver.1.3.3 (Valière 2002), to test the discrimination power of nine microsatellite set. In addition, individual identification for genotyped fecal samples was performed with Regroup option in GIMLET.

For sex determination, the final intron sequences of zinc finger protein genes on X and Y chromosomes (*ZFX* and *ZFY*) were amplified, following Nagai et al. (2014). At first, three separate PCR amplifications were performed for each sample. When the results were different among the PCRs, additional PCRs were subsequently performed until the repeatability was obtained for least two or three times. If the results of individual identification and sex determination were different, a priority was given to the former.

The kin relationship was analyzed with COLONY v 2.0.6.4 (Jones and Wang 2010). Ten runs were performed for all individuals, based on the following configurations: allele frequencies estimated from all individual data, assuming polygamy for both parents and present of inbreeding, full-likelihood methods and the genotyping error set at 0.01. According to Premachandra et al. (2019), the kin relationships were accepted when the probabilities were more than 95% and checked their mtDNA haplotypes coincided each other for full siblings.

Genetic data analysis

To evaluate the genetic diversity of the Sapporo population (the valley and the mountainous areas), the observed heterozygosity (H_O), the expected heterozygosity (H_E), and the *F*-statistics (F_{IS}, F_{ST}) (Wright 1951) were calculated with ARLEQUIN ver. 3.5.1.2 (Excoffier and Lischer 2010). The deviation from Hardy Weinberg Equilibrium (HWE) for each population was examined using GENEPOP v4.2

(Raymond and Rousset, 1995). The isolation-by-distance (IBD) (Wright 1943) with Mantel test (Mantel 1967) of genetic distance (F_{ST}, R_{ST}) on geographic distance between each individual pair were also examined with GENEPOP. The geographic distance matrix was constructed by Geographic Distance Matrix Generator (version 1.2.3) (http://biodiversityinformatics.amnh.org/open_source/gdmg/, accessed on 11 March 2020).

The Sapporo population's structure was analyzed with STRUCTURE 2.3.4 (Pritchard et al. 2000) implemented on the ADMIXTURE model. Ten runs were performed for each number of genetic clusters (K) from 1 to 8. Each run consisted of 500,000 Markov Chain Monte Carlo (MCMC) iterations after a burn-in of 100,000 iterations. The results were summarized by STRUCTURE HARVESTER (Earl and vonHoldt 2012). The optimal number of K was determined by the mean of log likelihood LnP[K] and Delta K (Evanno et al. 2005). The admixture coefficients of each cluster were mapped using the R function *tessplot* implemented in the plot.admixture.r script (http://membres-timc.imag.fr/Olivier.Francois/plot.admixture.r. accessed on 11 March 2020) in R 3.5.1 (R core team 2018). The locations of individuals were determined by their centroid when multiple feces were from the same individual.

Estimation of density and population size

The numbers of feces and individuals per km were calculated as the number of feces or individuals divided by the transect length multiplied by the number of sampling.

To estimate the effective population size (N_E) and census population size (N_C), COLONY and *capwire* estimator were used, respectively.

The *capwire* implemented two model (the equal capture probability model, ECM; the two innate rates model, TIRM). A model fit of each data was determined by likelihood ratio test, and a parametric bootstrap test with 1000 samples was used to estimate 95% confidence intervals, using the R package *capwire* ver. 1.1.4 (Pennell et al. 2013). For the estimation of mountainous area, the data of all routes was combined because the present sample sizes were too small.

To compare with the native population, the data sets of 2006 and 2007 from Tago et al. (2013) were reffered, who also surveyed the density of Japanese martens in Kyushu (Aso district and Saga city), Japan, using the fecal DNA analysis. Their survey route lengths were not so different from the present study: 5.6 km in Aso and 4.5 km in Saga. To calculate their sampling number, each sampling session and sampling days per session were multiplied (for Aso as 5.2 days and Saga as 4 days), because Tago et al. (2013) conducted their surveys four-six days in a row for each sampling session.

Habitat selection in the valley area

Using QGIS, the vegetation preference of Japanese marten in the valley area (Route 6) was estimated with georeferenced fecal samples and vegetation map, 1:25000 vegetation map of "Kiyota" based on a 1999-2004 census data (Ministry of the Environment of Japan; http://gis.biodic.go.jp/webgis/sc-043.html, accessed on 11 March 2020). Three feces were excluded from the analysis because these were located on the removed boardwalk (see Study area and Sampling collection). In general, the marten prefers woodland area, so the vegetation types were focused in this analysis. The habitat analysis was performed by transect and grid scales in the present study. The statistical analysis was performed with Microsoft Excel 2016 or R, otherwise noted.

For the transect scale, using QGIS, a radius 30 m buffer was made surrounding the transect route and martens' feces, and then each vegetation area was calculated. The transected area was defined as available area and the location of each fecal plot as used area. The number of observations (u) was calculated by dividing the vegetation area of each plot circle. The R package *adehabitatHS* ver. 0.3.15 (Calenge 2006) was used to calculate the Manly's selection ratio (Manly et al. 2002) under the Bonferroni-adjusted 95% confidence intervals. The Log-Likelihood statistic (x_L^2) was also used to check the significance of the observed distribution under the null hypothesis that the selection is random.

For the grid scale analysis, the transect was divided into 45 grids (100 x 100 m / grid). Here, the presence of the Sakhalin fir *Abies sachalinensis* was focused, because the locations where the large number of feces were found included small patches of Sakhalin fir. The presence of Sakhalin fir patches

for each grid was investigated by the author, because they were not recorded in the forementioned vegetation map, and the detail locations were shown in Figure II-2. According to Balestrieri et al. (2015), the marking intensity (MI) of each grid was calculated as number of feces per grid / Transect length per grid, and then the MIs with/without presence of Sakhalin fir patches were compared by the Mann-Whitney *U* test.

The seasonal variation of habitat preference and number of martens was also analyzed. Based on Murakami (2003), the seasons were divided into Spring (March to May), Summer (June to August), Autumn (September to November), and Winter (December to February).

Results

Species identification

Overall, 317 marten-like feces were collected in 81 surveys for a total length of 407.9 km (Table II-1). As a result, 252 out of them were genetically assigned to the Japanese marten, and the other 14 to the Japanese weasel and eight to the red fox. The other 43 feces could not be genetically identified to any species.

In the valley area (Route 6), 245 marten-like feces were collected during 47 out of 66 surveys. Based on the genetic analysis, 225 out of 245 (91.8%) were identified to species (207 feces from the Japanese marten, ten from the Japanese weasel, and eight from the red fox).The American mink was not detected in the present study, although it was observed by the visitor in summer 2015 based on the Nishioka Park Office's record. The fecal diameter of these three species overlapped each other (Japanese marten (n = 166; 4.15-12.55 mm); Japanese weasel (10; 4.15-8.35); red fox (8; 5.25-14.2), even though the pairs of Japanese marten and Japanese weasel, and red fox and Japanese weasel were significantly different from each other in pairwise comparisons using Wilcoxon rank sum test *P*-value adjusted by Bonferroni (Figure II-3).

In mountainous areas (Route 1-5), 72 marten-like feces were collected during all 15 surveys, and 49 of them were genetically identified (success rate of 68%) (45 from the Japanese marten and four

from the Japanese weasel).

In both areas, there were two mtDNA *DL* haplotypes (A and B) for the Japanese marten and one haplotype (C) for the Japanese weasel. These haplotypes were identical with those of the previous reports (A with accession number AB525736, B with AB455647 and AB455649 (these two sequences were collapsed by short length in the present study), and C with AB601574). The haplotype A was the same as MM11 and B as MM12 in Inoue et al. (2010); however, their original reference of MM12 was AB455699 probably due to different citation.

The detection rate of the occurrence of the feral Japanese marten was 68% in the valley area (Route 6), whereas marten's feces were found on every survey at all routes except Route 4 (detection rate 66%) in mountainous areas. The minimum number of surveys (NS_{min}) was 2.62 in the valley area (Route 6), 2.73 in Route 4, and one time in the remained routes (Route 1-3, and 5) (Table II-1).

Individual identification

Genotyping of 102 out of 252 (40%) marten feces was performed for all nine microsatellite loci, which were polymorphic except Mar21. The cumulative PID values of them were low enough to identify the individual (PID = 2.00×10^{-7} and PID-sibs = 1.60×10^{-3}).

In the valley area (Route 6), 17 martens (15males, 1 female and 1 unknown sex) were identified from 73 feces (Table II-2). For sex determination of the valley area, 114 out of 207 feces were identified as 108 males and 6 from females. In mountainous areas (Route 1-5), 19 martens (16 males and 3 females) were confirmed from 29 feces (Table II-3). The spatial distribution patterns of identified individuals were shown in Figure II-4.

The COLONY showed five full siblings in Sapporo: four in the valley area and one in mountainous area at Route 4 (Figure II-4). In the valley area, one individual from full sibships of each year became a resident (NMM8 and NMM11).

Genetic diversity and population structure

The Sapporo population, including all populations, had a high genetic diversity (H_O = 0.646) without

inbreeding (F_{IS} = 0.035, P = 0.29) (Table II-4), although genetic difference within the population was low (F_{ST} = 0.065, P = 0.21). Mantel test showed that there was the isolation by distance (IBD) slightly between all individuals, rejecting the null hypothesis that there is no correlation between genetic (F_{ST}) and geographic distances (Figure II-5; P = 0.017), while the data based on R_{ST} values showed no correlation (Figure II-5; P = 0. 126). The frequency of mtDNA haplotypes was different around Toyohira river: the haplotype A is 15/16 and B is 1/16 in northern part, while A is 12/20 and B is 8/20 in southern part in Sapporo (Figure II-1, Tables II-2 and 3).

Based on the STRUCTURE HARVESTER analysis, the K = 3 was the most optimal K (Figure II-6). The STRUCTURE analysis showed that the valley population was genetically different from the others (Figure II-7), and that was also supported by F_{ST} value (F_{ST} = 0.055, P < 0.001). The valley population changed annually (Figure II-7), and the genetic difference between 2016 and 2017 was low (F_{ST} = 0.033, P = 0.07). The population structure of mountainous area was admixed in a high level, although there was only a weak IBD based on F_{ST} values but not confirmed for R_{ST} values (data not shown).

Density and population size

In the valley area, there was a high density of feces of Japanese marten (0.75 / km) relative to mountainous area (average 0.34 / km), although the density of individuals was not so different (0.184 / km for the valley and 0.178 / km for mountainous area) (Table II-1).

The N_C of Sapporo was estimated as 54 (95%CI 51-70), and the N_E was 40 (95%CI 24-68) (Table II-5). The valley area was 13 (N_C; 95%CI 10-18) and 8-10 (N_E; 95%CI 3-33) respectively, across two years. The both population sizes of mountainous area were almost three times of the valley area (N_C and N_E; 30 and 32, respectively). In contrast, the N_C of Aso and Saga were extremely higher than present results and the density of individuals were also high (0.349 / km on 2006 and 0.361 / km on 2007 in Aso, 0.500 and 0.153 in Saga).

Habitat preference and seasonal variation in the valley population

From the transect scale analysis, the vegetation types were not randomly used, shown by the significant selection ratio (x_L^2 = 107.837, $P < 0.001$). The White birch & Mongolian oak was avoided (Table II-6; $P < 0.001$), and the Japanese elm was preferred significantly (Table II-6; $P < 0.001$). In the grid scale, the MI was significantly higher for with Sakhalin fir than without ($P < 0.005$) (Figure II-8), showing the preference towards Sakhalin fir patches.

The tendency of habitat preference was almost the same in all seasons except winter due to small sample sizes (Table II-7). The N_C was only estimated for autumn due to small sample sizes in other seasons (Table II-8). The number of identified individuals was high in autumns and the NS_{min} was small in summer and autumn (Table II-8). It was difficult to detect the presence of marten during winter, although there were small number of marten-like tracks and snow caverns with their tracks. Actually, no marten-like feces were collected in eight surveys between November 2017 to March 2018.

Discussion

Species identification by fecal DNA

Species identification using fecal DNA in the present study was relatively successful at a high success rate (86 %). The verification of the fecal diameter criteria for species identification showed a drawback because the diameters of Japanese martens, Japanese weasels, and red foxes were overlapped, although the average sizes were significantly different from each other (Figure II-3). Following the fact that feces of Japanese weasel holding over 10mm diameter were found in the other region (data not shown), it is necessary to be aware of this drawback when use of the fecal diameter criteria. In addition, it was reasonable that the Japanese marten was identified at the highest frequency because attention at sample collections was paid on fecal samples with morphology like-marten.

The success rate of the individual identification was moderate (40%) relative to the previous study (e.g. 45% from Ruiz-González et al. 2013), and the success rate in summer was the lowest in the present analysis (18%). The reason was not clear, but the food habit might have affected the success rate, as Murphy et al. (2003) reported that the food habit affects PCR amplification not on mtDNA but on

nuclear DNA. In general, the Japanese marten frequently consumes insects during summer (e.g. Hisano et al. 2019), and insects were reported as one of the reasons for the fecal DNA analysis failure (Idaghdour et al. 2003).

Population genetic structure of the feral Japanese marten in Sapporo

Although the genetic difference among the Sapporo populations was small (F_{ST} = 0.065), the genetic diversity among them was high (H_O = 0.646). This trend was also observed in the valley population. The present mtDNA data indicate occurrence of two lineages in the Sapporo marten population. The results were similar to Kamada et al. (2013), who reported high genetic diversity of feral Japanese marten in Hokkaido with multiple origins. The IBD results showed that Sapporo populations differed in the allele frequency (F_{ST}) but not in the allele size (R_{ST}). It suggests the short time since they were released. Otherwise, the asocial territorial behavior of Japanese martens might have been responsible for these results. In general, genus *Martes* has territorial distribution (Zhou et al. 2011); however, some *Martes* species have their home ranges overlapped (e.g. sable, Miyoshi and Higashi 2005; Japanese marten, Tago et al. 2013). Actually, the present study also showed multiple individuals were observed in a narrow area (Figure II-4).

The population in the valley area was genetically different from the mountainous populations. It might have caused by the occurrence of four presumably full siblings in the valley area (Figure II-4). Otherwise, the Toyohira River and its surrounding urban environments may act as geographic barriers for Japanese martens between the valley and mountainous populations, because Toyohira river was reported as one of the geographic barriers for red fox in Sapporo (Kato et al. 2017). In fact, the frequency of mtDNA haplotypes was different between northern and southern parts of the Toyohira River, in the present study.

On the other hand, the population structure of the valley area changed annually. The valley area is not geographically isolated from southern mountainous areas, so Japanese marten can migrate freely. Indeed, most of the individuals were found only once during the present surveys. This suggests

that the valley area is used as one of the gathering points for migration of the Japanese martens.

Density and population size

Despite the large number of marten's feces were found, the number of individuals were not so large in the valley area, relative to mountainous areas. It is in congruence with that Tatara's (1994a) report that Japanese marten used the valley more frequently. The survey routes in mountainous areas were mostly ridges but longer, probably resulting in small number of feces and moderate number of individuals per km. It was also supported by the values of the census population size (N_C) in mountainous areas, where were surveyed wider range than the valley area, resulting the larger number of individuals. In contrast, the N_C and density of martens in native populations in Kyushu were extremely higher than the feral population in Sapporo (Table II-5). Tago et al. (2013) also discussed the reason of high density and concluded that it was due to the different sampling methods from the previous studies of *Martes* and it was not due to the regional specificity. Compared with the other pine martens' (*Martes martes*) studies based on the fecal DNA analysis (Balestrieri et al. 2016; Kubasiewicz et al. 2017), however, the density of the Kyushu populations was still remarkably high.

Only few feces of females were found in the present study. It might be caused by the different behaviors between males and females. Buskirk and Lindstedt (1989) suggested that male bias sampling is common among Mustelidae species, because the male is larger in body size than female (sexual dimorphism). Higher activities and wider home ranges for male make it easy to encounter male's field signs. Croose et al. (2019) reported the effectiveness combining feces and hair tubes sampling, to investigate the density of pine martens for both sexes. If not only fecal sampling but hair tubes sampling was simultaneously used in the present study, more female samples might have been collected.

Habitat preference and seasonal variation in the valley population

The feral Japanese marten preferred the riparian forests and valley in the present study areas, similar to native populations (Tatara 1994a; Arai et al. 2003). The broad-leaved forest was also preferred by native populations (Tatara 1994a; Tsujino and Yumoto 2014), even if that trees were younger and lower

(Hoshino et al. 2014). However, in the present study, the feral Japanese marten population completely avoided the broad-leaved forests of the white birch and Mongolian oak (Table II-6). Moreover, the locations, where the large number of feces and individuals were found, included small patches of the Sakhalin fir, suggesting that this tree species is an important resource for the feral Japanese martens. In fact, the coniferous forests such as the Sakhalin fir are important for foraging routes to sables (Miyoshi and Higashi 2005). In addition, the coniferous forests provide the cover and high prey densities to the American marten (*Martes americana*) and fisher (*Pekania pennati*) (Seip et al. 2018; Suffice et al. 2020). Assuming that the feral Japanese marten preferred the coniferous forest, they may consume the mammalian prey frequently in Hokkaido, because the relative frequency of the mammalian prey occurrence was significantly higher in cool-temperate, heavy snow depth zones, and/or in coniferous forests (Hisano et al. 2019; Tsuji et al. 2019). This consume behavior might be one of the reasons why the competition between the feral Japanese marten and sable is strong in Hokkaido, because sables also depend on mammalian prey in Hokkaido (Murakami 2003). It is needed to further study of home range of the feral Japanese marten and their food habit to clarify this hypothesis.

For seasonal variation in the valley area, the observed numbers of martens increased during autumn. This result might reflect their dispersal pattern because the dispersal season of Japanese martens is predicted as autumn from the results of Tatara (1994b). The values of the minimum number of surveys (N_{min}) were small during summer and autumn (1.17-1.67) but large in winter (4.32) (Table II-8). This may result from their activity patterns different among seasons. Kawauchi et al. (2003) reported the high activity of Japanese martens during summer, and Bartolommei et al. (2016) also showed that movements of pine martens were high during summer to autumn and low during winter to spring. To detect the elusive carnivores during winter, the environmental DNA analysis methods were lately developed (Franklin et al. 2019), which use the footprints on snow. It is already revealed the analysis to be useful for Japanese marten (Kinoshita et al. 2019). These methods must be a powerful tool and promising for winter surveys to detect the presence of feral Japanese martens.

The present fecal DNA analysis revealed population structure, population size, and habitat

preference of the feral Japanese marten in Sapporo. In addition, the intensively surveys between small areas showed the importance of coniferous forests for them. According to the present values of the minimum number of survey (N_{min}), the optimal monitoring method is that the survey should be conducted at least three times during summer and autumn, to detect the presence of the feral Japanese marten for the management.

General Discussion

The present phylogeographic study showed the migration history of the least weasel in Palearctic. The least weasel could have diverged from the Black-Caspian Sea area to two main clades, across northern Palearctic (clade I) and the Southwestern part of the Palearctic (clade II). The expansion of clade I was remarkably rapid, although it was poorly supported the assemblage. The similar expansion histories were also reported in the closely related species stoat (Dawson et al. 2014). The data showed low genetic differences and suggested a rapid expansion of stoats from Spain across the continent and through Beringia to North America. The continent-wide expansions have also reported in other carnivorans. Especially, among them, the Eurasian lynx has the most similar trend with those of the least weasel (Lucena-Perez et al. 2020). The data showed the homogeneous genetic pattern among Asian populations, and there was a gene flow from the Ural population to Asian populations until 100 kyBP. In the present study, the northern clade (clade I) was too complicated to read the phylogeographical relationships on the haplotype network with many loops indicating the homoplasies, and the Ural and Honshu lineages built a sister group comprised on around 110 kyBP. These finding leads to that the gene flow of least weasels might have occurred until relatively recent years as same as Eurasian lynx. The dispersal histories of these three species might resemble each other, because they are hypercarnivore species depending on the mammalian prey. Furthermore, according to the present results and the previous studies, the migration patterns to Japan of the least weasel could be different between the Honshu and Hokkaido populations. The red fox also has a similar migration history to Japan (Kutschera et al. 2013); however, their migration from the continent to Hokkaido was occurred at three times as same as those of the brown bear (Matsuhashi et al. 1999; Hirata et al. 2013). In contrast, the migration of the least weasels to Hokkaido could be once, based on the present data. It was also suggested in sables (Kinoshita et al. 2015), however the coalescent times of Hokkaido populations of sables and least weasels were different. These results show that the rough migration history could be similar in some species, but the details were depending on the adaptations and/or ecological niches of each species.

Secondly, in the present study, the local population of the feral Japanese marten was focused to reveal how they survive in non-native range of them. Based on the fecal DNA analysis, 36 individuals were observed in Sapporo, and their genetic diversity was high presumably caused by their multiple origins (Kamada et al. 2013). Although it was not general results for non-native species, some non-native species also have similar results; the introduced populations of American mink have also high genetic diversity caused by the multiple introductions in the Iberian Peninsula (García et al. 2017). In addition, the present intensive survey figured out four full sibling sets and one of them remained in the study area, suggesting that the other individuals had dispersed to the other area. The most of dispersed individuals were males, suggesting the male-biased dispersal of the Japanese marten. This male-biased disperse is commonly observed in many mustelids. The present analyses also demonstrated the vegetation preference of the feral Japanese marten. As same as the native population, the riparian forest was also preferred by the feral Japanese martens, but the coniferous forest, which is avoided by the native population, was also preferred in Hokkaido. It suggests that the feral Japanese martens could have changed their behavior along to the local habitat to adapt to non-native area of them.

The present study showed the migration history of the least weasel in Palearctic on the continental scale and geographical time scale by the phylogeographical study. Furthermore, the present fecal DNA analyses showed the movement patterns of local populations of the feral Japanese marten. These biogeographic studies based on wide and local scales will contribute to elucidate the complex Mustelidae evolutionary history.

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Figures and Tables

Figure I-1. Sampling locations. The samples examined in the present study are shown as diamonds, and the detailed localities are shown in Table I-1. According to the previous studies (Hosoda et al. 2000; 2011, Kurose et al. 1999; 2000; 2005, Lebarbenchon et al. 2006; 2010, Mcdevitt et al. 2012, and Rodrigues et al. 2016), the references localities are shown as circles and the detailed localities were indicated in Table I-2. If the samples did not have detailed sampling localities in the previous studies, their plots were located at the metropolis of each country or prefecture. The gray color means the distribution range of least weasel in the Palearctic, modified from McDonald et al. (2016).

Figure I-2. A Bayesian phylogenetic tree of mtDNA control region (*CR*). The posterior probabilities and bootstrap values are shown on branches. The haplotype names are coincided with Table I-2 and 5.

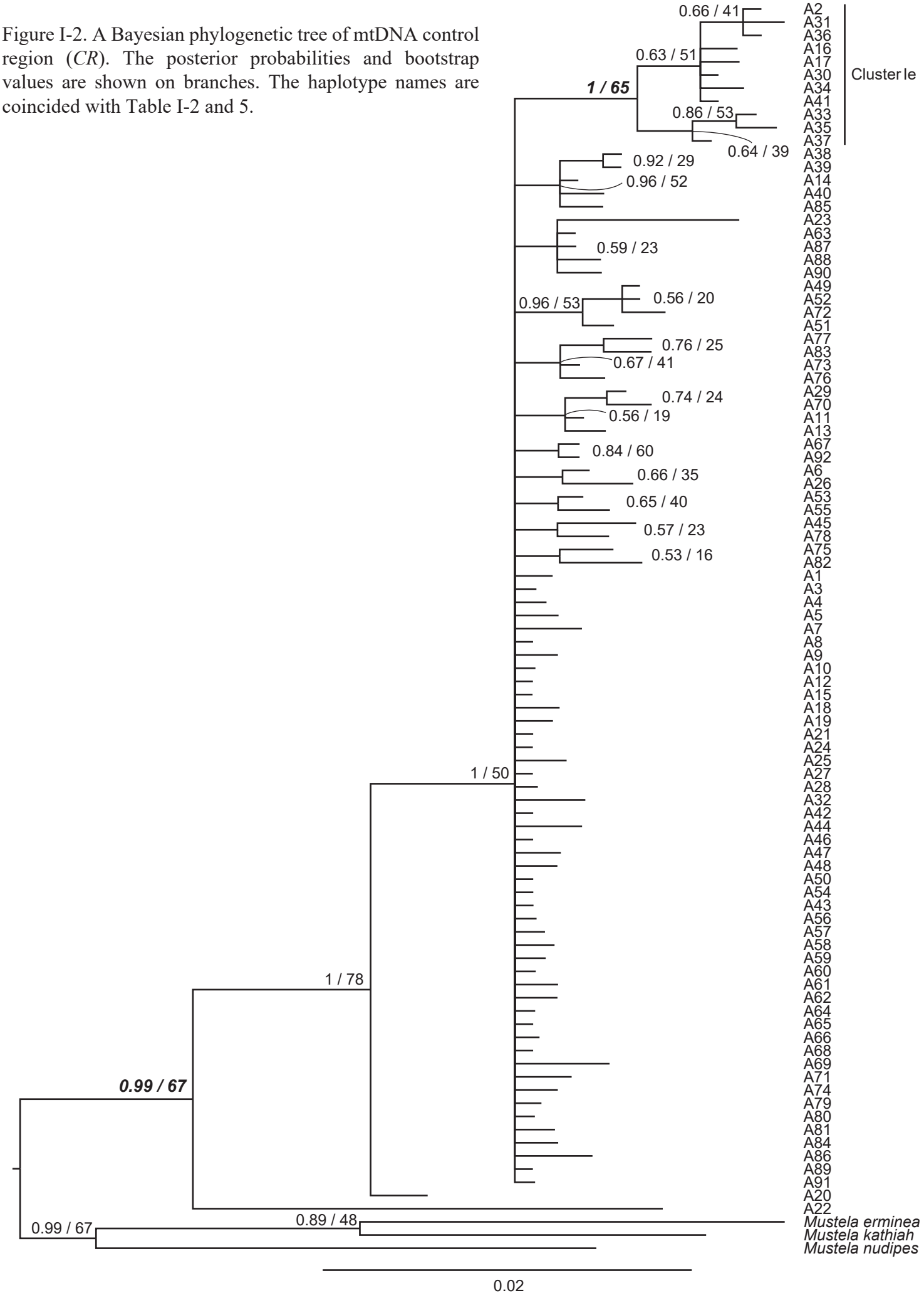


Figure I-3. A Bayesian phylogenetic tree of mtDNA cytochrome *b* (*Cytb*). The posterior probabilities and bootstrap values are shown on branches. The haplotype names are coincided with Table I-2 and 5.

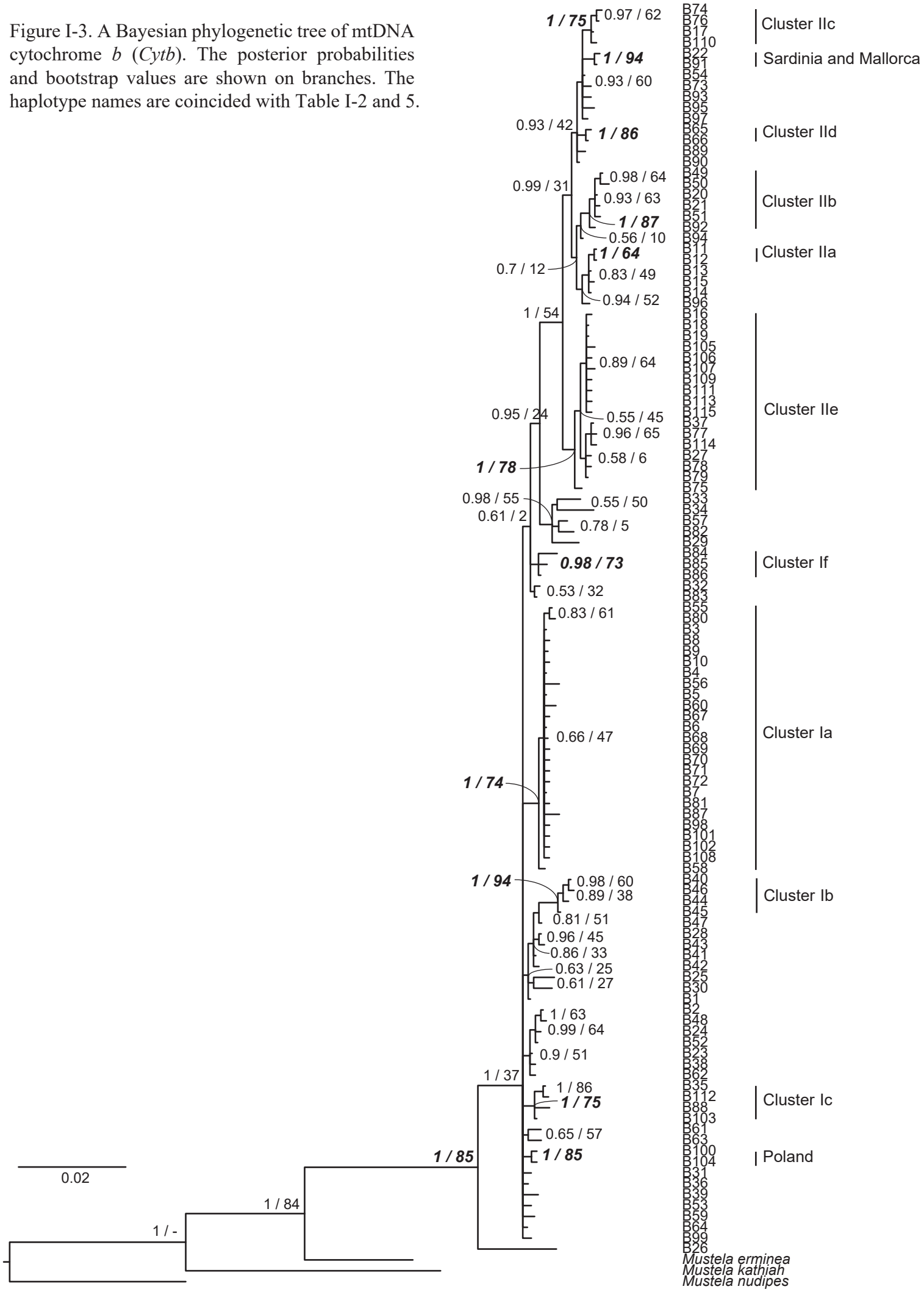


Figure I-4. A Bayesian phylogenetic tree of concatenated mtDNA *CR* and *Cytb*. Posterior probabilities and bootstrap values are shown on branches. Haplotype names are coincided with Table I-2 and 5 and Figure I-7. The novel haplotypes are shown with bold. The cluster names are given at the terminal nodes. The symbols and colors are corresponding to Figure I-6. These cluster-supporting values are shown by bold and italic. The distribution and coalescent time are also indicated. The x marks indicate the individuals from the Black Sea-Caspian Sea region.

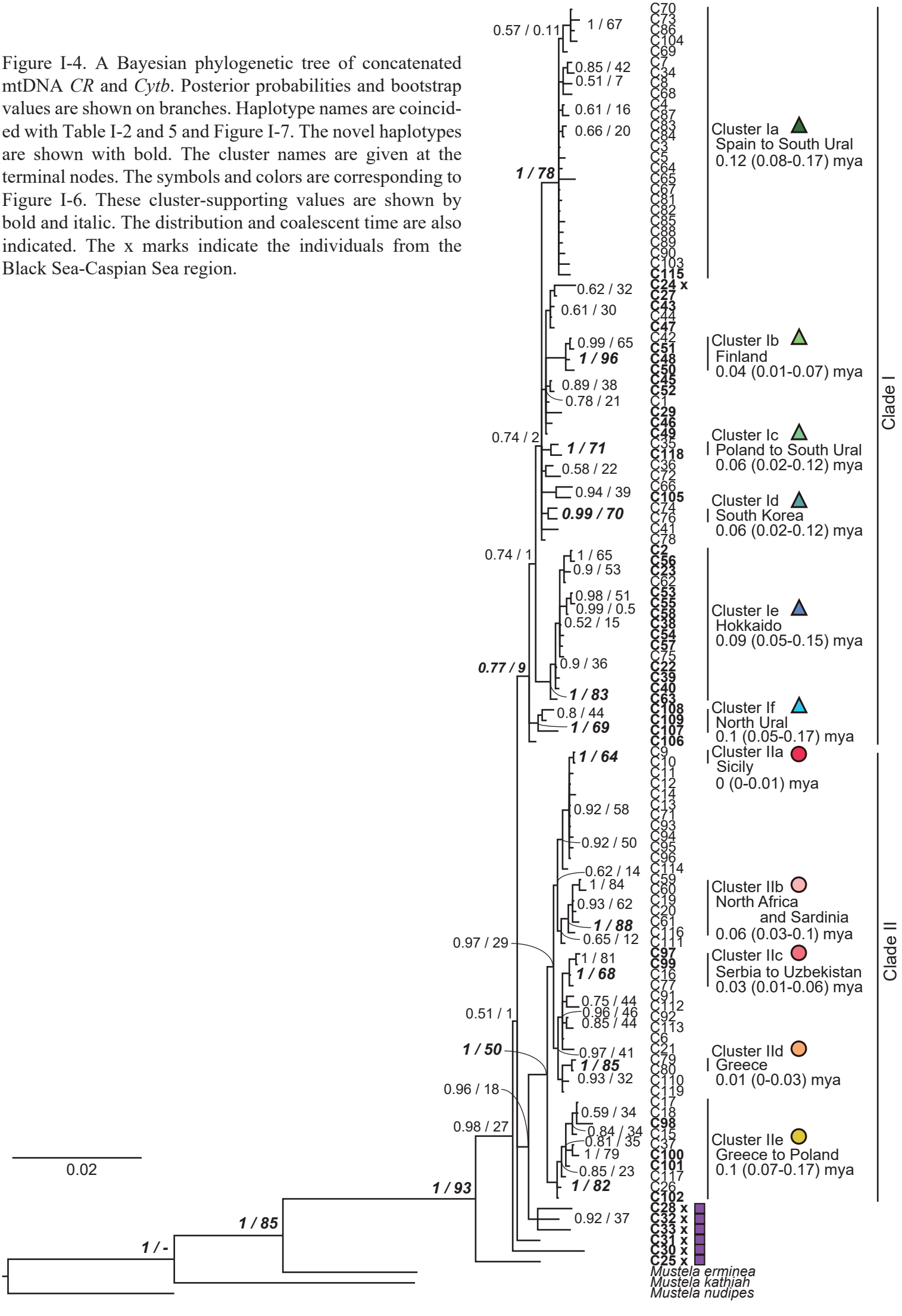
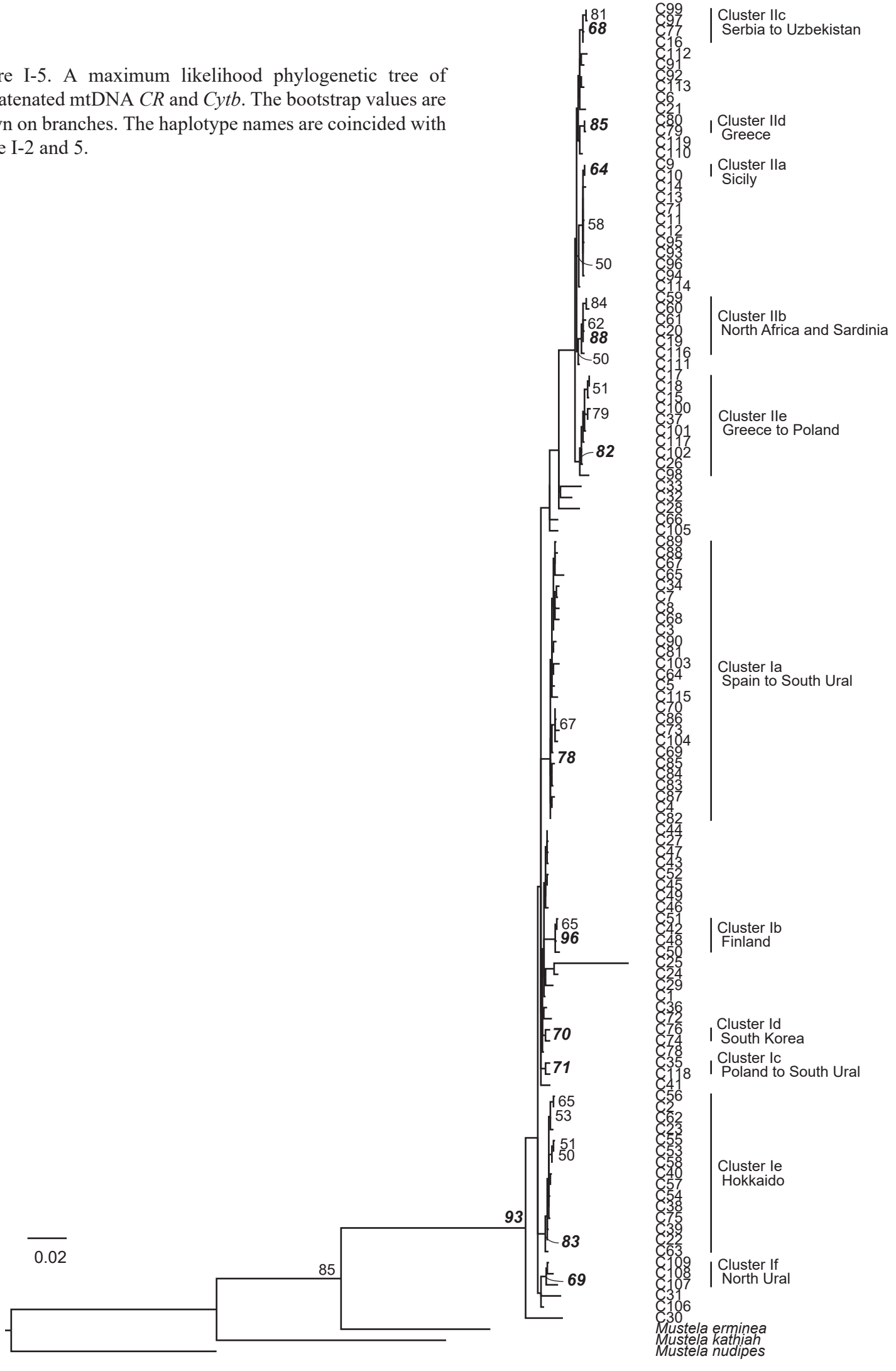


Figure I-5. A maximum likelihood phylogenetic tree of concatenated mtDNA *CR* and *Cytb*. The bootstrap values are shown on branches. The haplotype names are coincided with Table I-2 and 5.



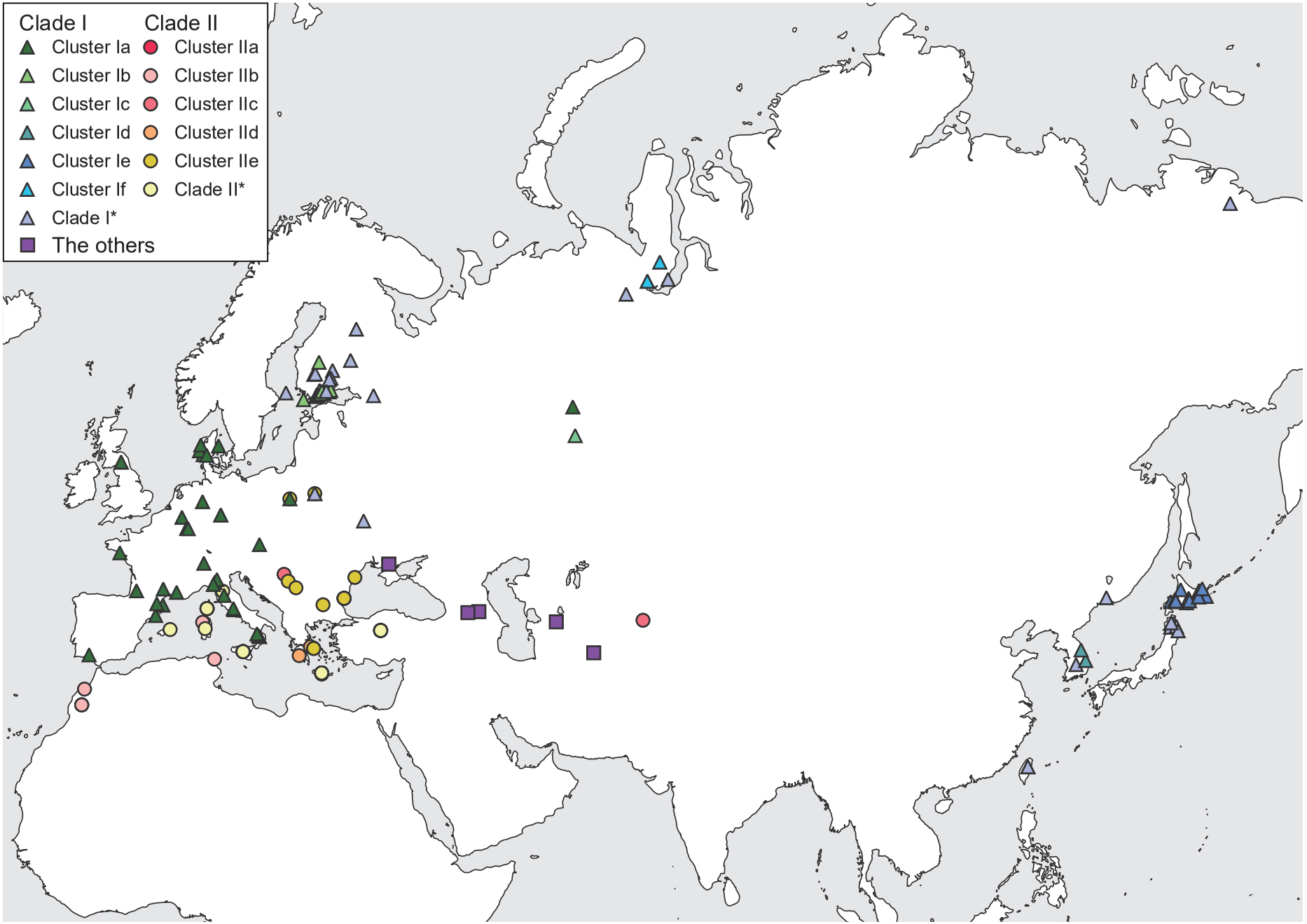


Figure I-6. Locations of clades and clusters in the Palearctic region. The clade and cluster locations are shown by symbols and colors. Information on localities is the same as Figure I-1. The asterisks mean the haplotypes were not grouped to any clusters but included to each clade. The purple means the other haplotypes, which were not included to any clades in Figure I-4.

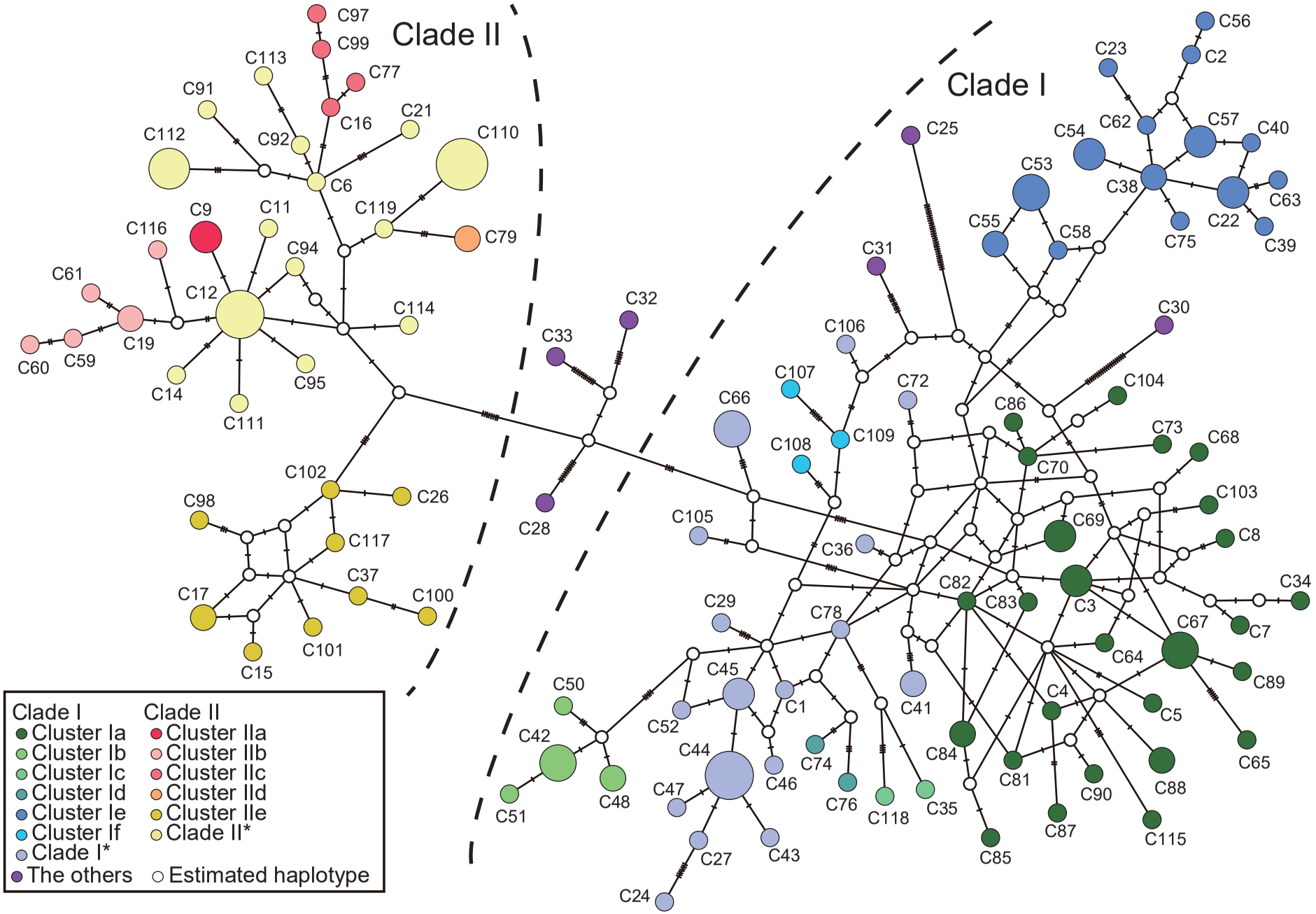


Figure I-7. A haplotype network of concatenated mtDNA *CR* and *Cytb*. The sizes of circles indicate the proportion of haplotype frequencies. Open circles are estimated haplotypes, and a hatch mark between haplotypes means a nucleotide substitution. Haplotype names are the same as in Figure I-4, Table I-2 and 5. The colors are coincided with Figure I-6. All samples were divided to each clade or the others, based on Figure I-4. The asterisks mean the haplotypes were not grouped to any clusters but included to each clade. The purple circles mean the other haplotypes, which were not included to any clades in Figure I-4. If nucleotide sequences of the haplotypes differ by only indels or unresolved nucleotides, they were treated as the same haplotypes in the POPART analysis: C9 and C10; C12, C13, C71, C93 and C96; C17 and C18; C19 and C20; C45 and C49; C79 and C80.

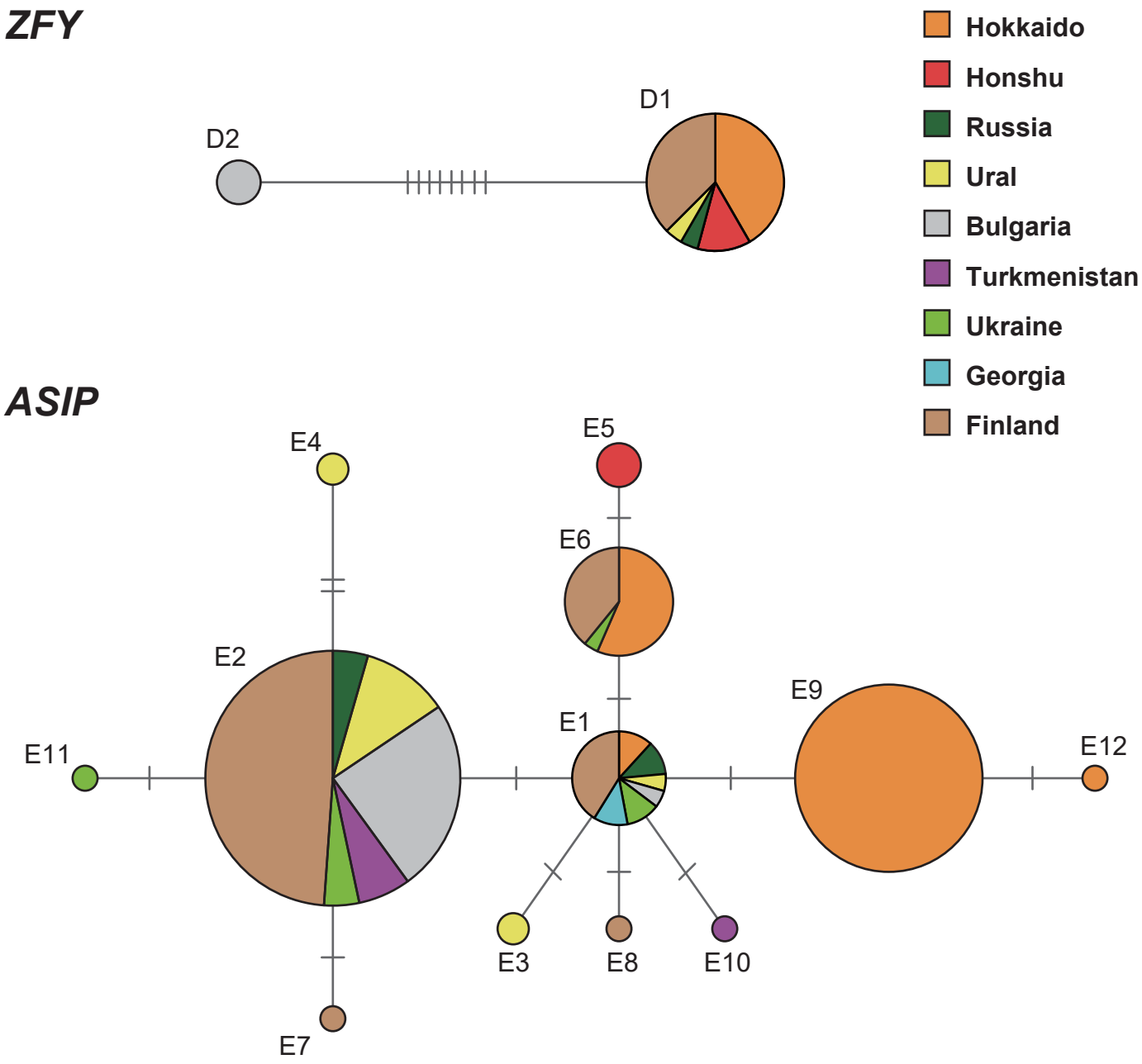
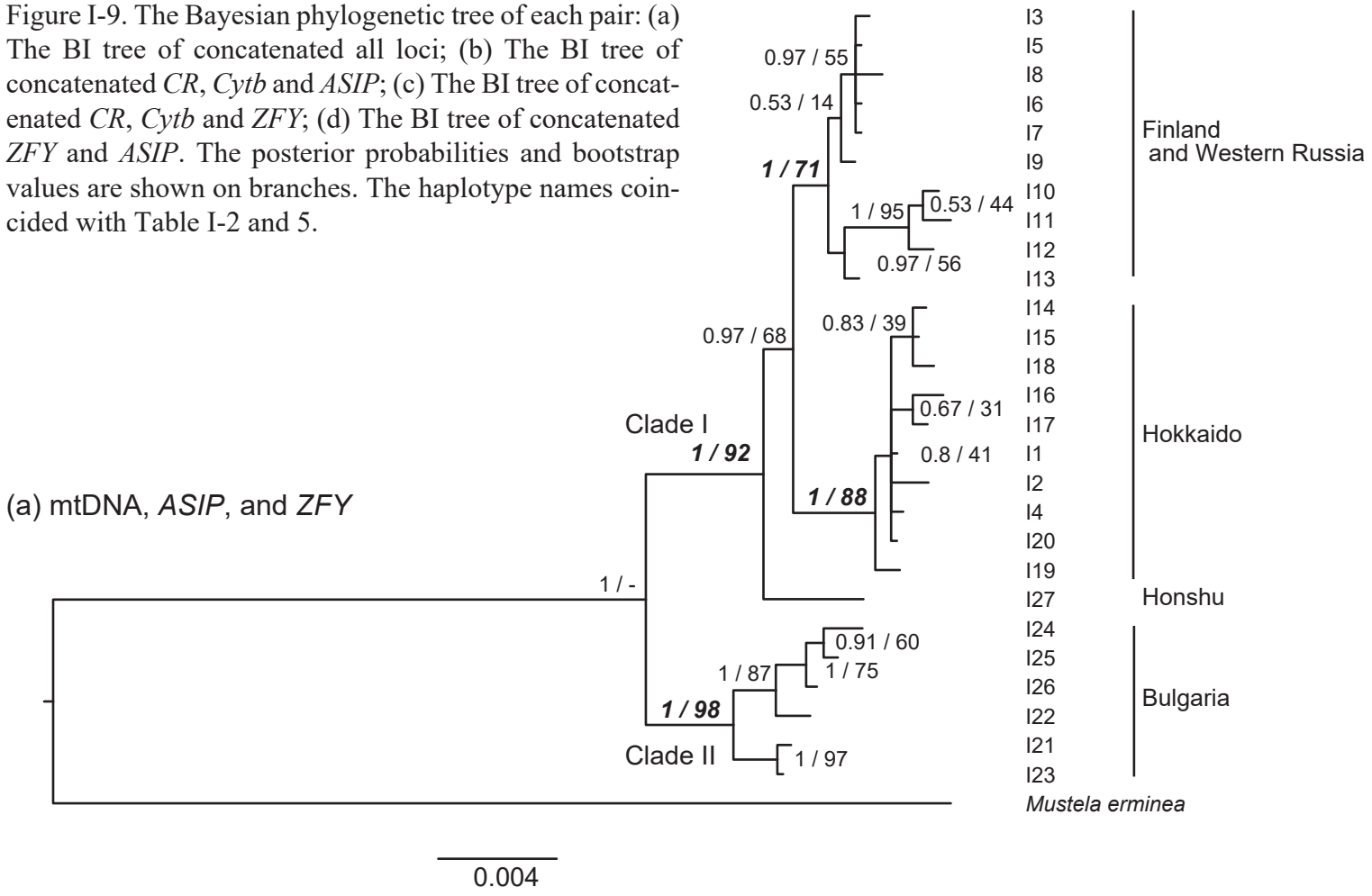


Figure I-8. Haplotype networks of *ZFY* (a) and *ASIP* (b). The sizes of circles indicate the proportion of haplotype frequencies. Open circles are estimated haplotypes, and a hatch mark between haplotypes means a nucleotide substitution. The haplotype names are the same as Table I-5. Individuals from each region are distinguished by using different colors: Hokkaido, orange; Honshu, red; Russia, green; Ural, yellow; Bulgaria, gray; Turkmenistan, purple; Ukraine, light green; Georgia, sky blue; Finland, light brown.

Figure I-9. The Bayesian phylogenetic tree of each pair: (a) The BI tree of concatenated all loci; (b) The BI tree of concatenated *CR*, *Cytb* and *ASIP*; (c) The BI tree of concatenated *CR*, *Cytb* and *ZFY*; (d) The BI tree of concatenated *ZFY* and *ASIP*. The posterior probabilities and bootstrap values are shown on branches. The haplotype names coincided with Table I-2 and 5.



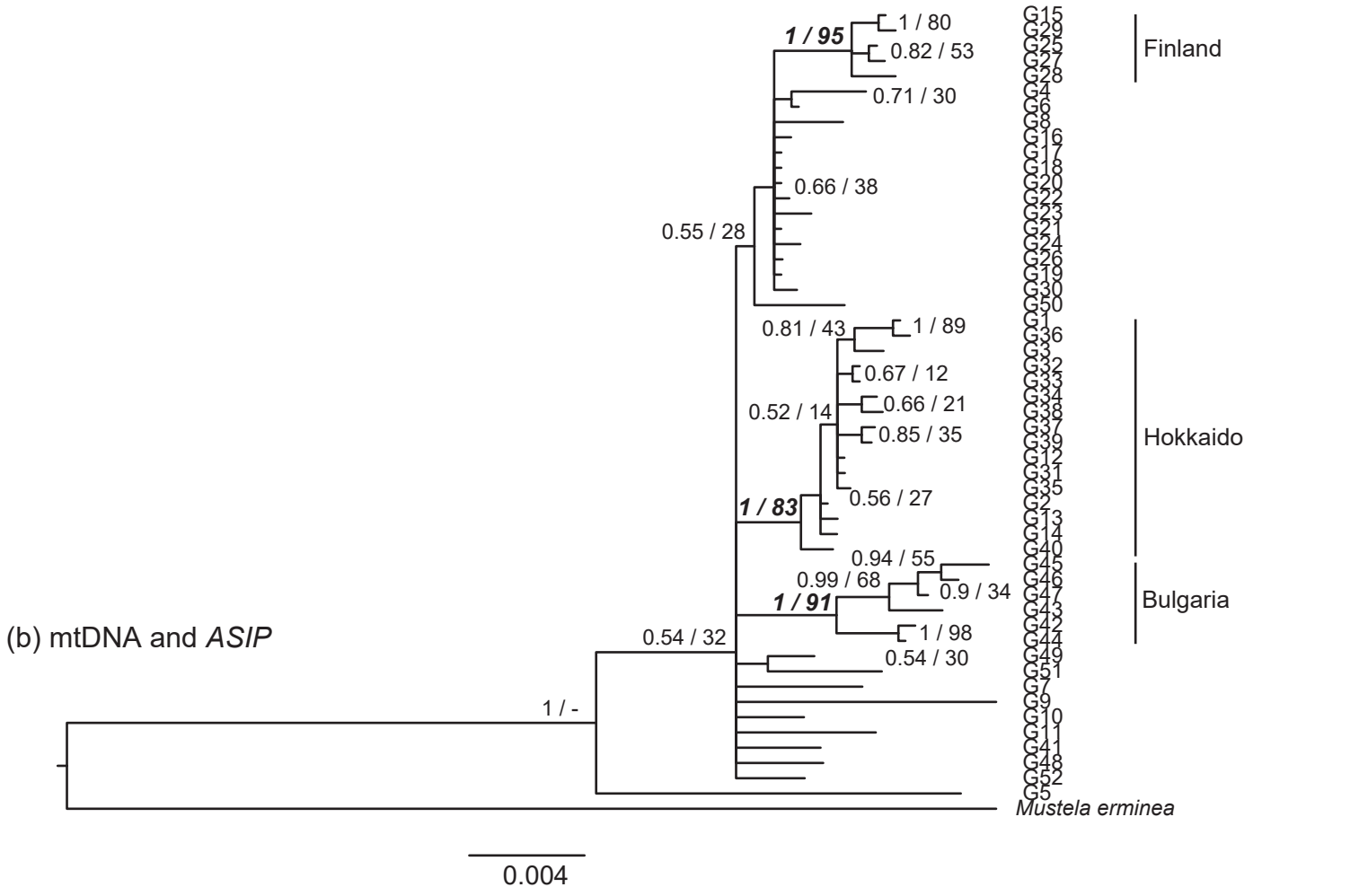
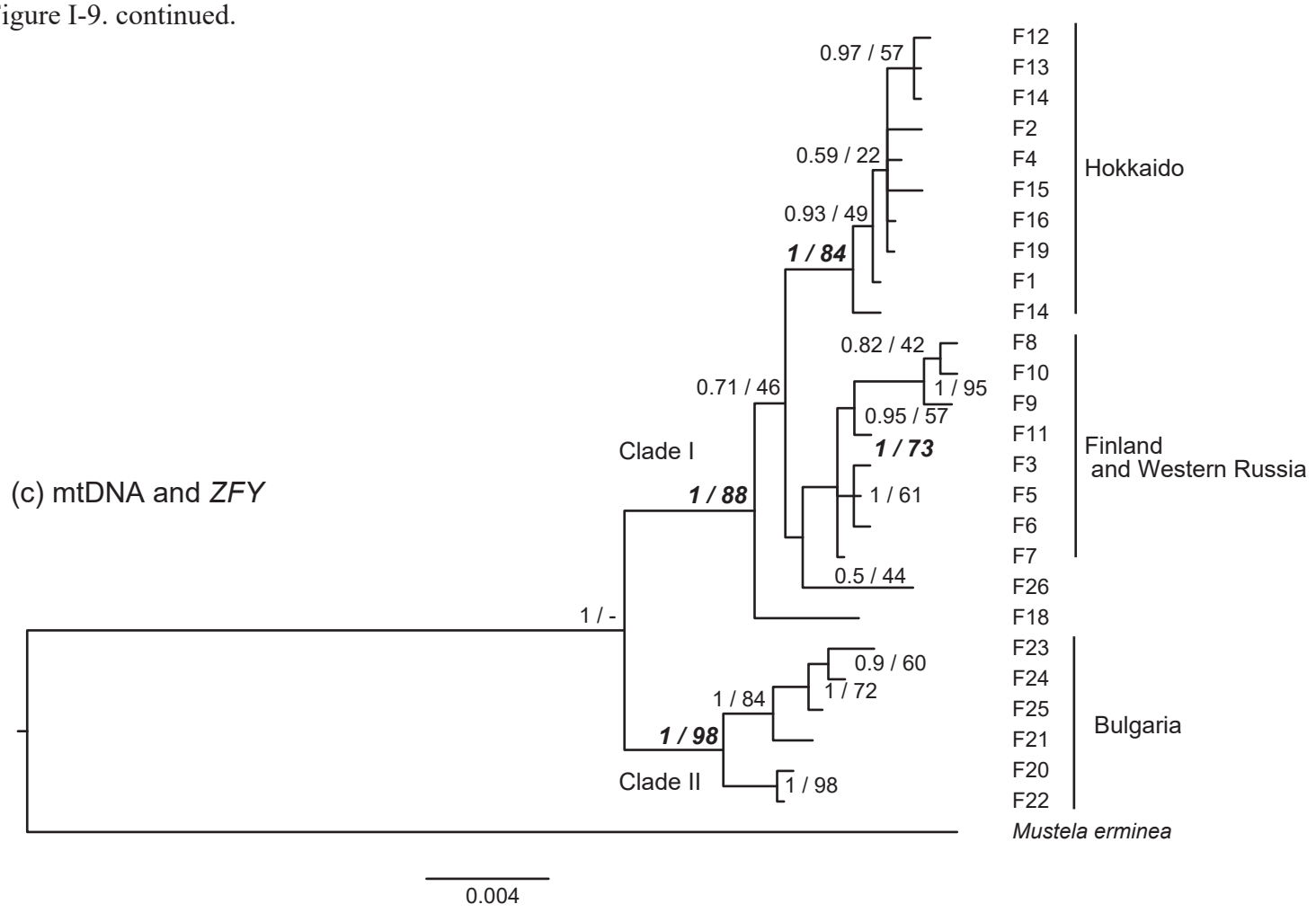
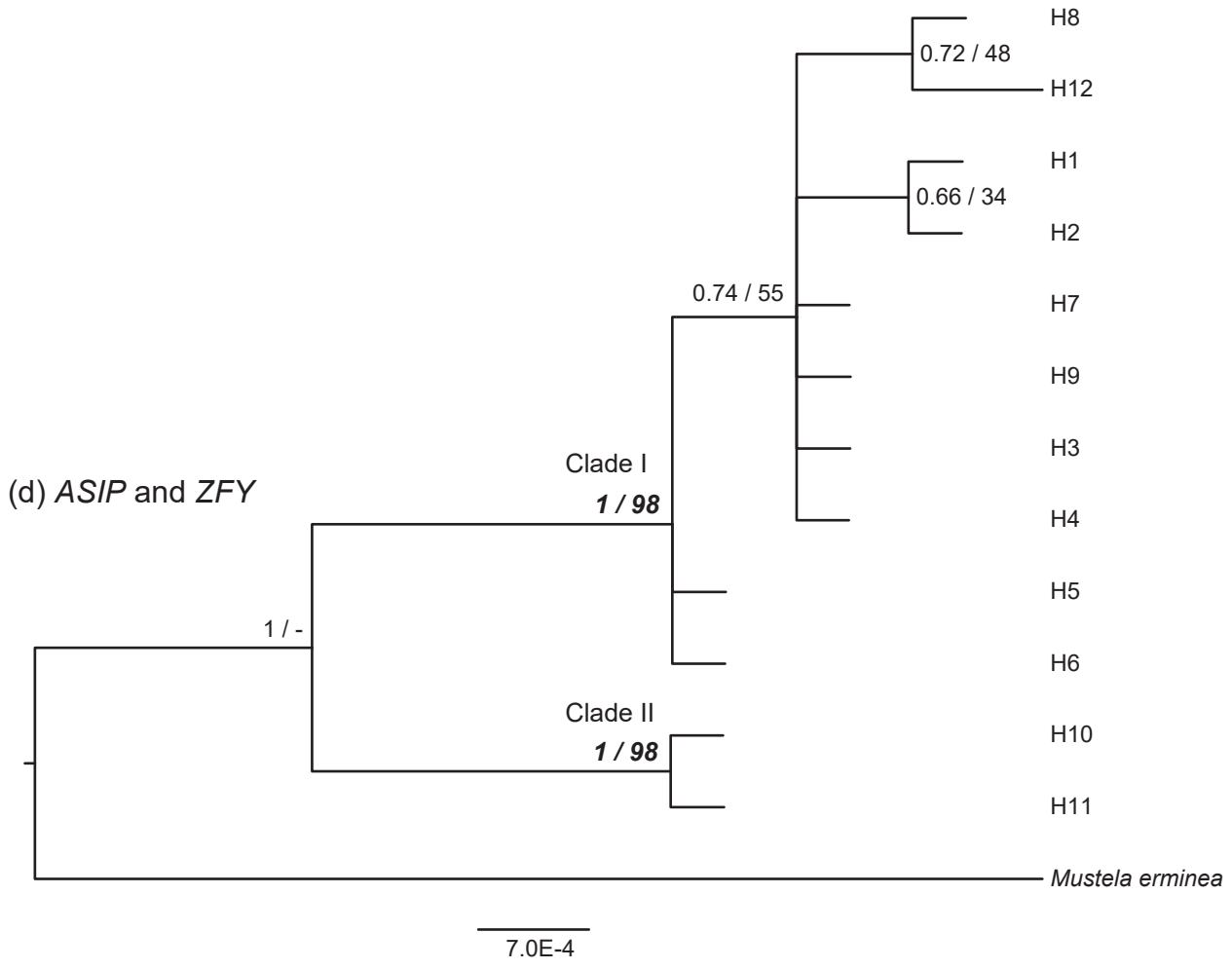


Figure I-9. continued.





Node No.	mya(median)	95%HPD
1	0.12	0.08-0.17
2	0.04	0.01-0.08
3	0.02	0.01-0.05
4	0.01	0-0.03
5	0.01	0-0.03
6	0.04	0.01-0.07
7	0.06	0.02-0.11
8	0.06	0.02-0.12
9	0.11	0.06-0.18
10	0.01	0-0.02
11	0.01	0-0.02
12	0.02	0-0.05
13	0.09	0.05-0.15
14	0.1	0.05-0.17
15	0.16	0.09-0.24
16	0	0-0.01
17	0.06	0.03-0.11
18	0.01	0-0.03
19	0.06	0.03-0.1
20	0.01	0-0.02
21	0.03	0.01-0.06
22	0.15	0.1-0.2
23	0.08	0.05-0.12
24	0.11	0.07-0.15
25	0.2	0.14-0.27
26	0.06	0.02-0.1
27	0.48	0.37-0.6
28	0.01	0-0.03
29	0.02	0-0.04
30	0.07	0.04-0.11
31	0.74	0.56-0.94
32	0	0-0.02
33	0.11	0.07-0.17
34	0.02	0-0.05
35	2.48	1.95-3.06
36	3.87	3.06-4.76
37	4.82	3.83-6.01

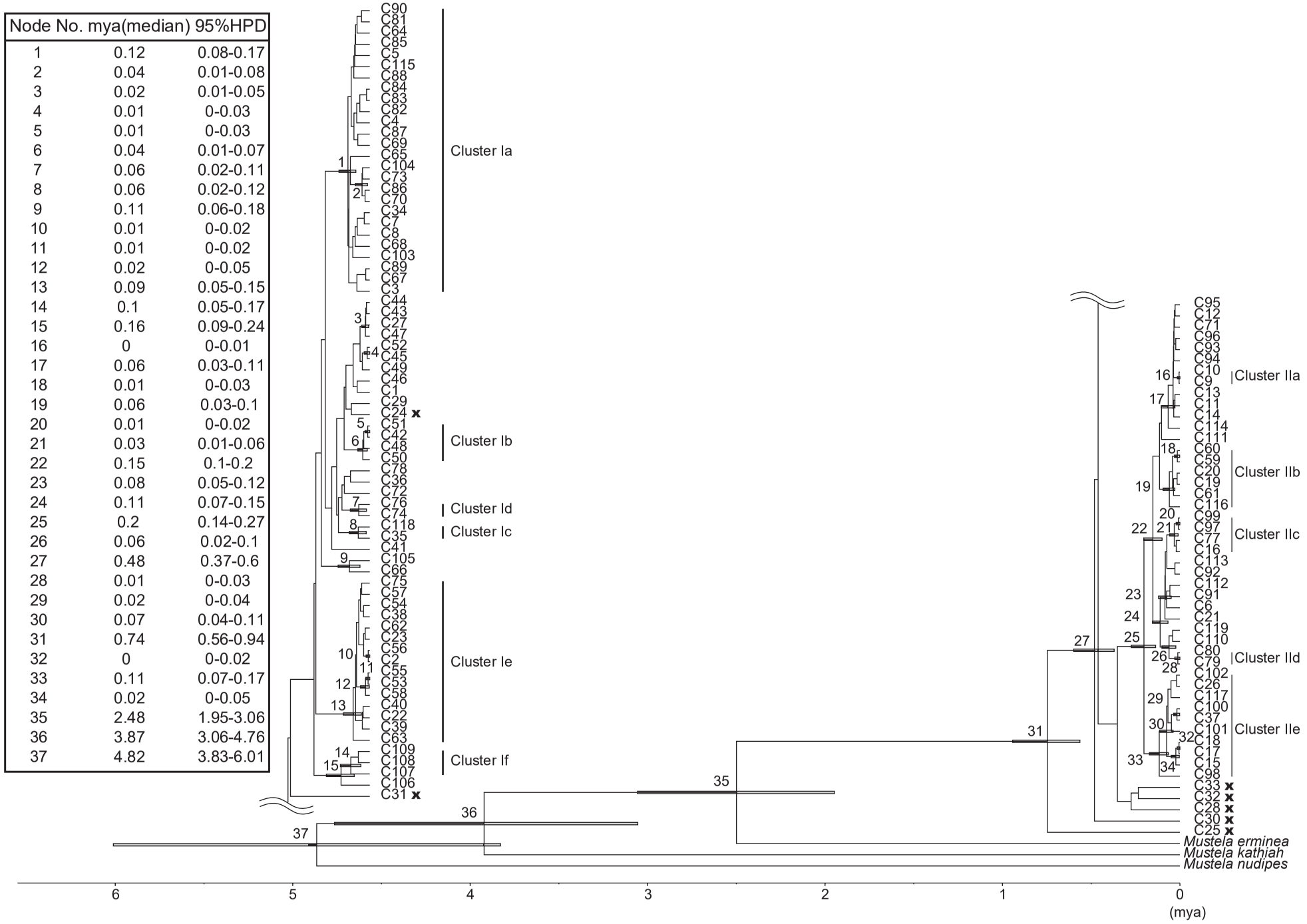


Figure I-10. A Bayesian chronogram of mtDNA. The numbers at nodes are the clade numbers for clades with ≥ 0.95 posterior probabilities, and the coalescent times are represented in Table (inset). Node bars show the 95% highest posterior density of nodal age estimates. The x marks indicate the individuals from the Black Sea-Caspian Sea region.

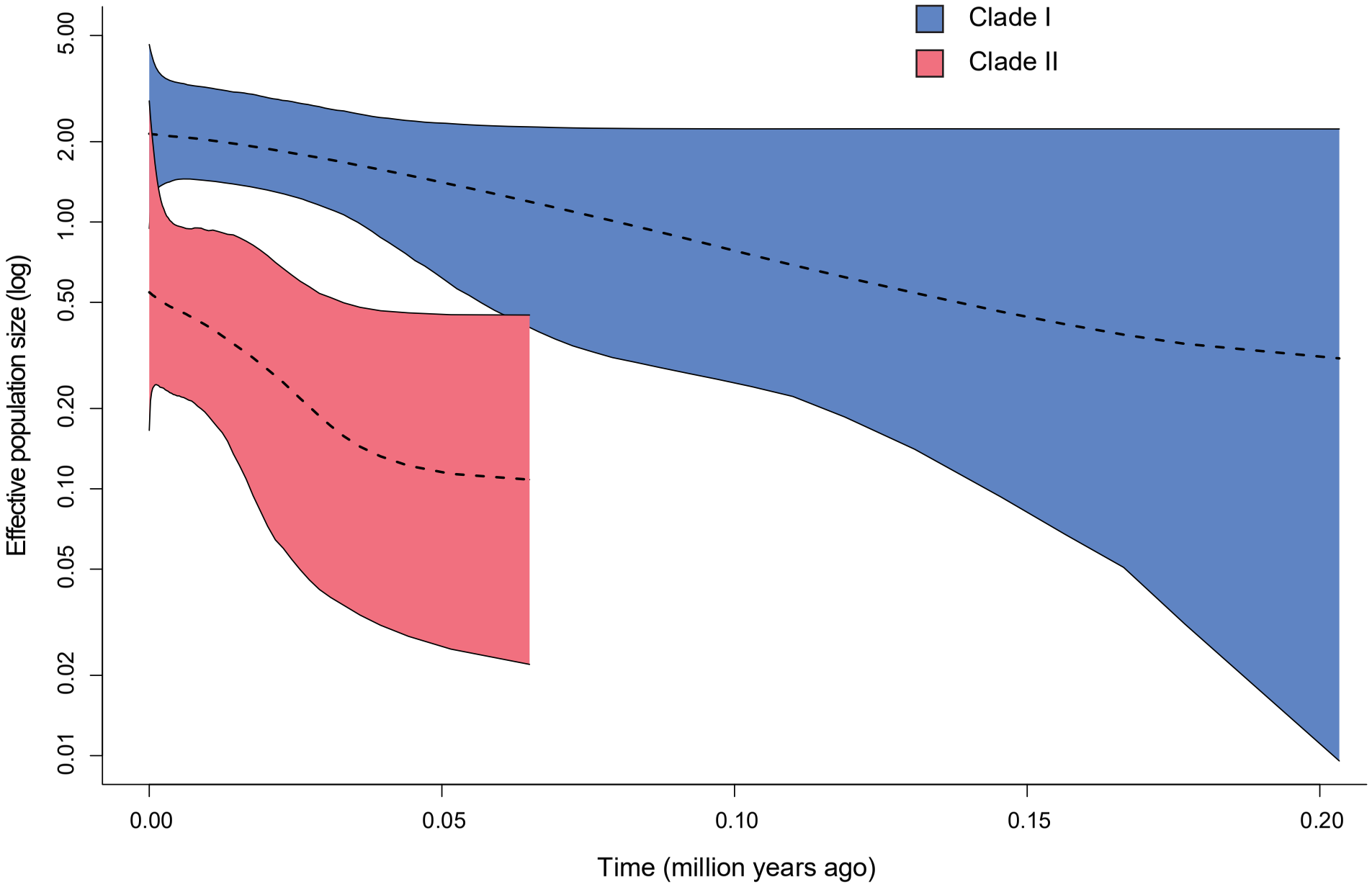


Figure I-11. Extended Bayesian skyline plots using concatenated mtDNA *CR* and *Cytb* of the least weasels (Clade I and II). Two lines show the range of 95% Central Posterior Density (CPD), and the broken line indicates the median. Plots indicate the population expansion.

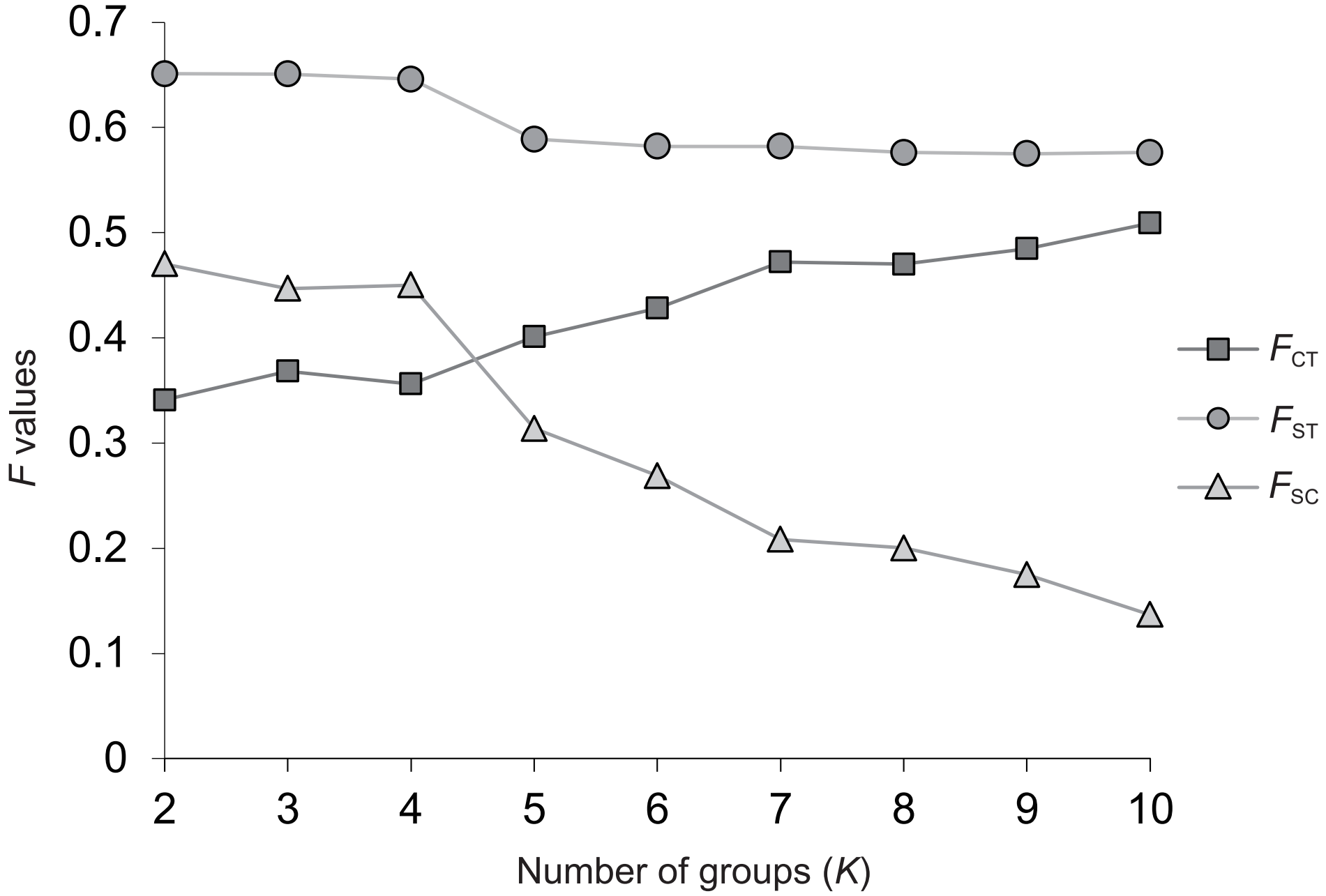


Figure I-12. The results of SAMOVA analysis for mtDNA data set. The number of group (K) is shown on the x-axes. The F values were shown on the y-axes. F_{ct}; square, F_{st}; circle, F_{sc}; triangle.

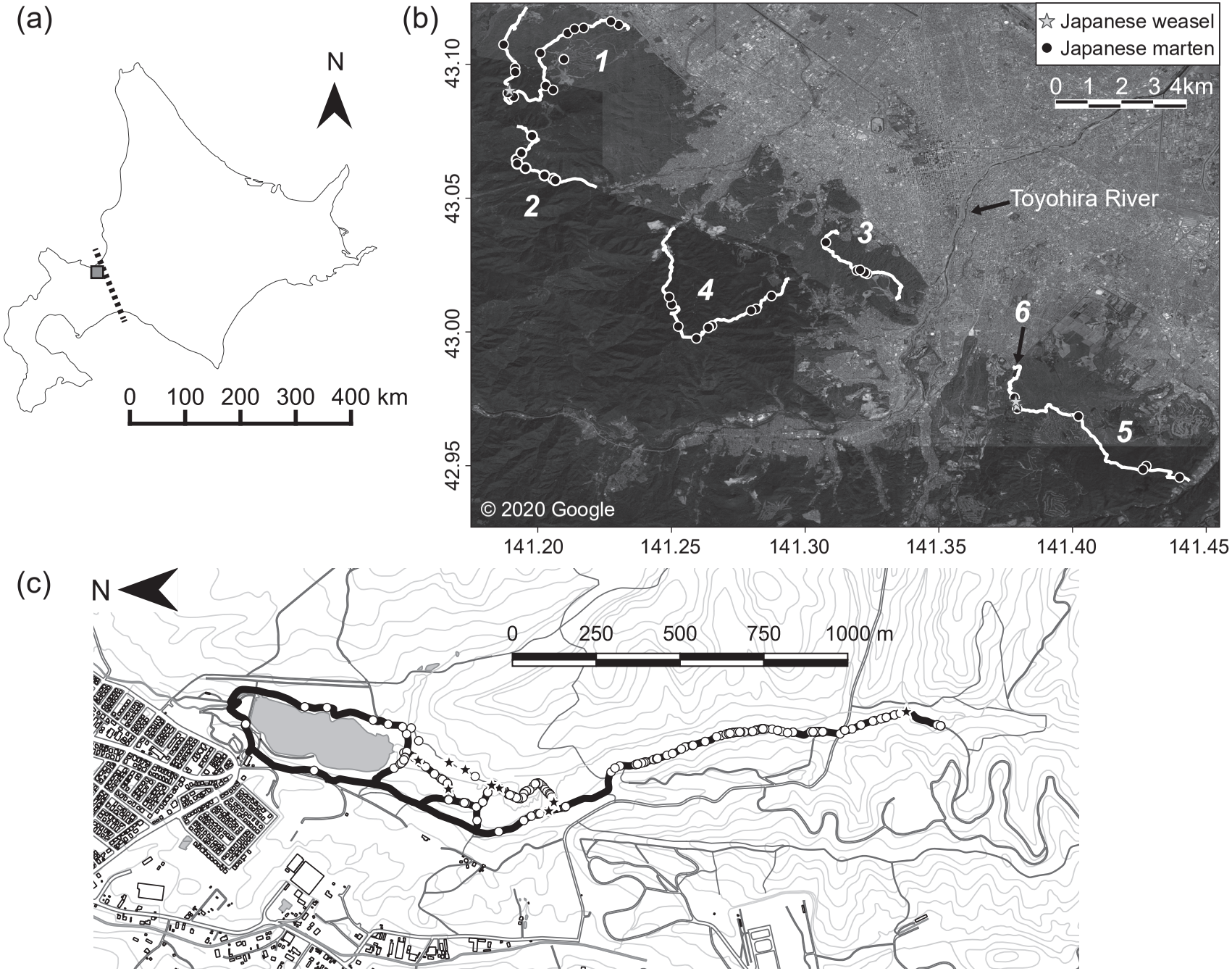


Figure II-1. Study area in Hokkaido islands (a), genotyped fecal samples in Sapporo (b), and the valley (c). Maps were made based on Geospatial Information Authority of Japan (a, c), and background from Google Maps (b; Map data © 2020 Google). The dash line in (a) shows the boundary line (Ishikari low land) of distributions of Japanese marten (west) and sable (east), and a rectangle shows the location of Sapporo. The numbers in (b) show Route IDs: 1, Northern part of Mt. Teine; 2, Southern part of Mt. Teine; 3, Mt. Moiwa; 4, Mt. Toishi; 5, Mt. Shirahata; 6, Nishioka. The survey routes were shown by the white (b) and black (c) lines. The locations of feces were shown by circles (Japanese marten) and stars (Japanese weasel) in (b) and (c).

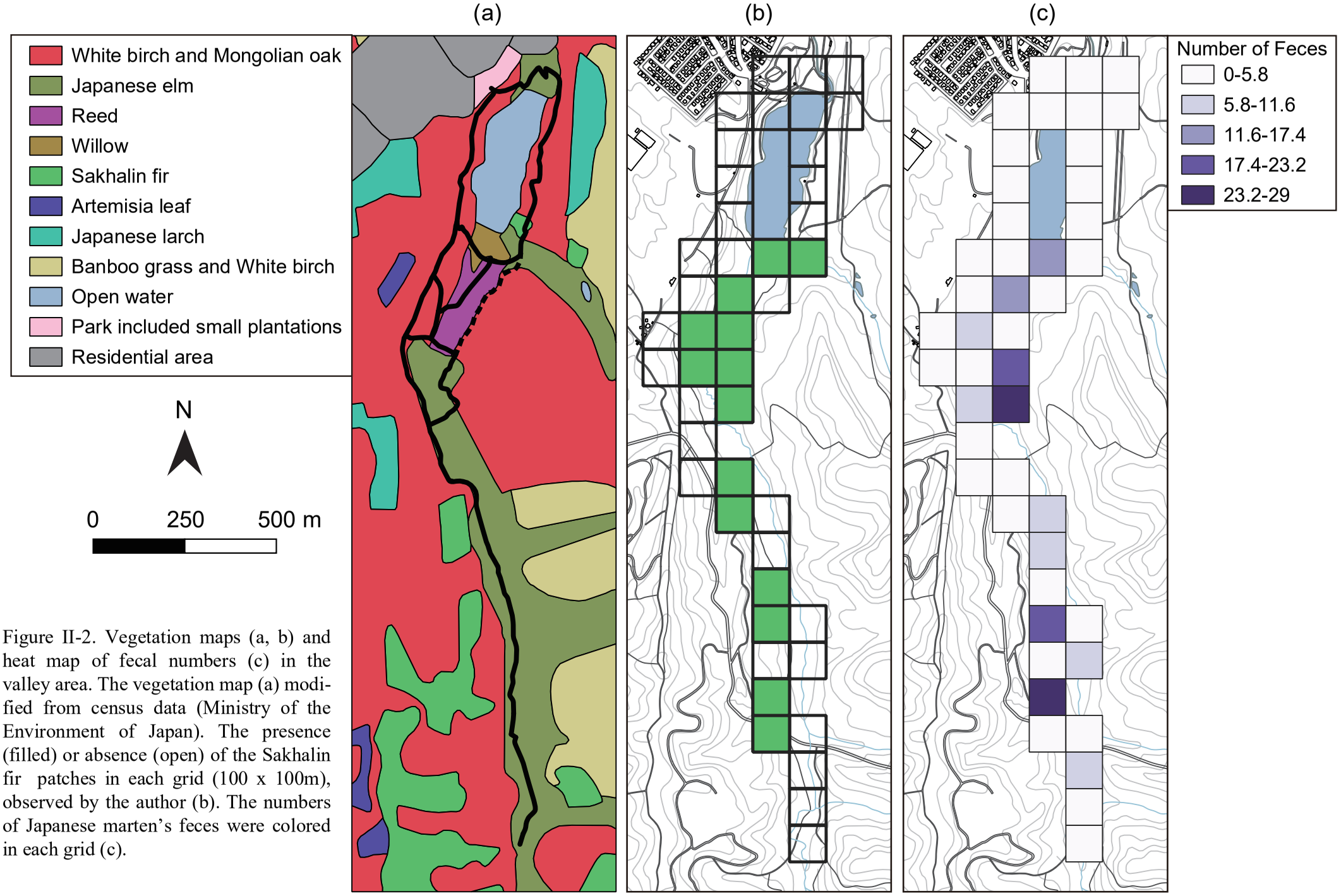
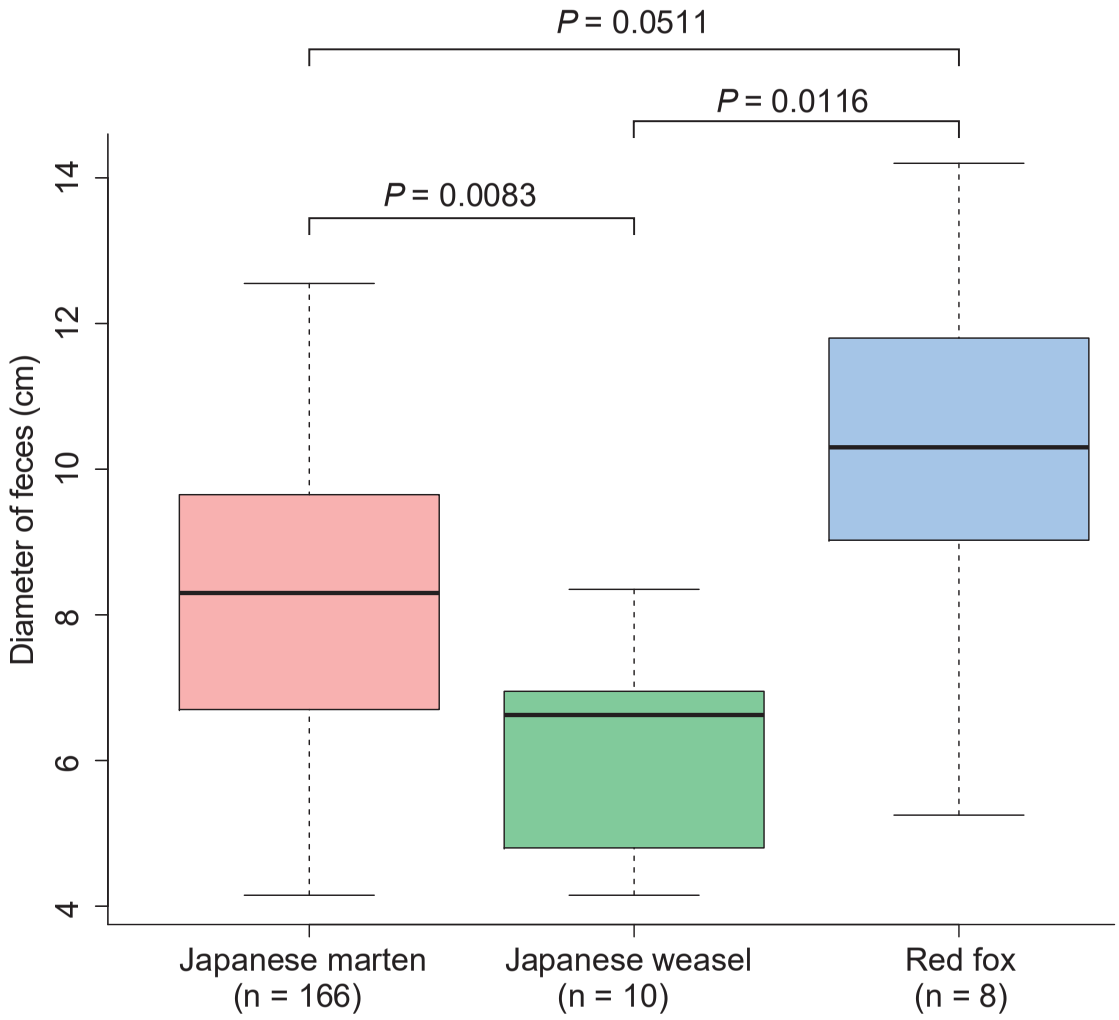


Figure II-2. Vegetation maps (a, b) and heat map of fecal numbers (c) in the valley area. The vegetation map (a) modified from census data (Ministry of the Environment of Japan). The presence (filled) or absence (open) of the Sakhalin fir patches in each grid (100 x 100m), observed by the author (b). The numbers of Japanese marten's feces were colored in each grid (c).



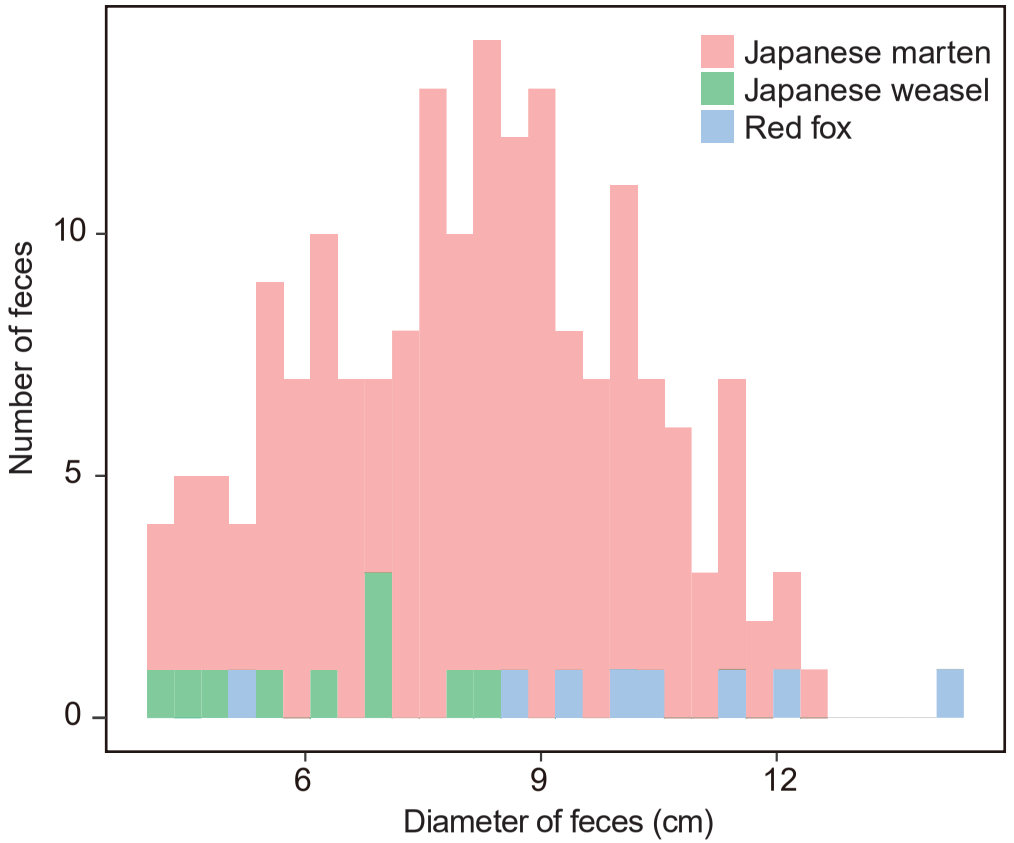


Figure II-3. The box plots and histograms of fecal diameter for different species. Pairwise comparisons were conducted to differences in fecal diameter among species, using Wilcoxon rank sum test and *P* values were adjusted by Bonferroni.

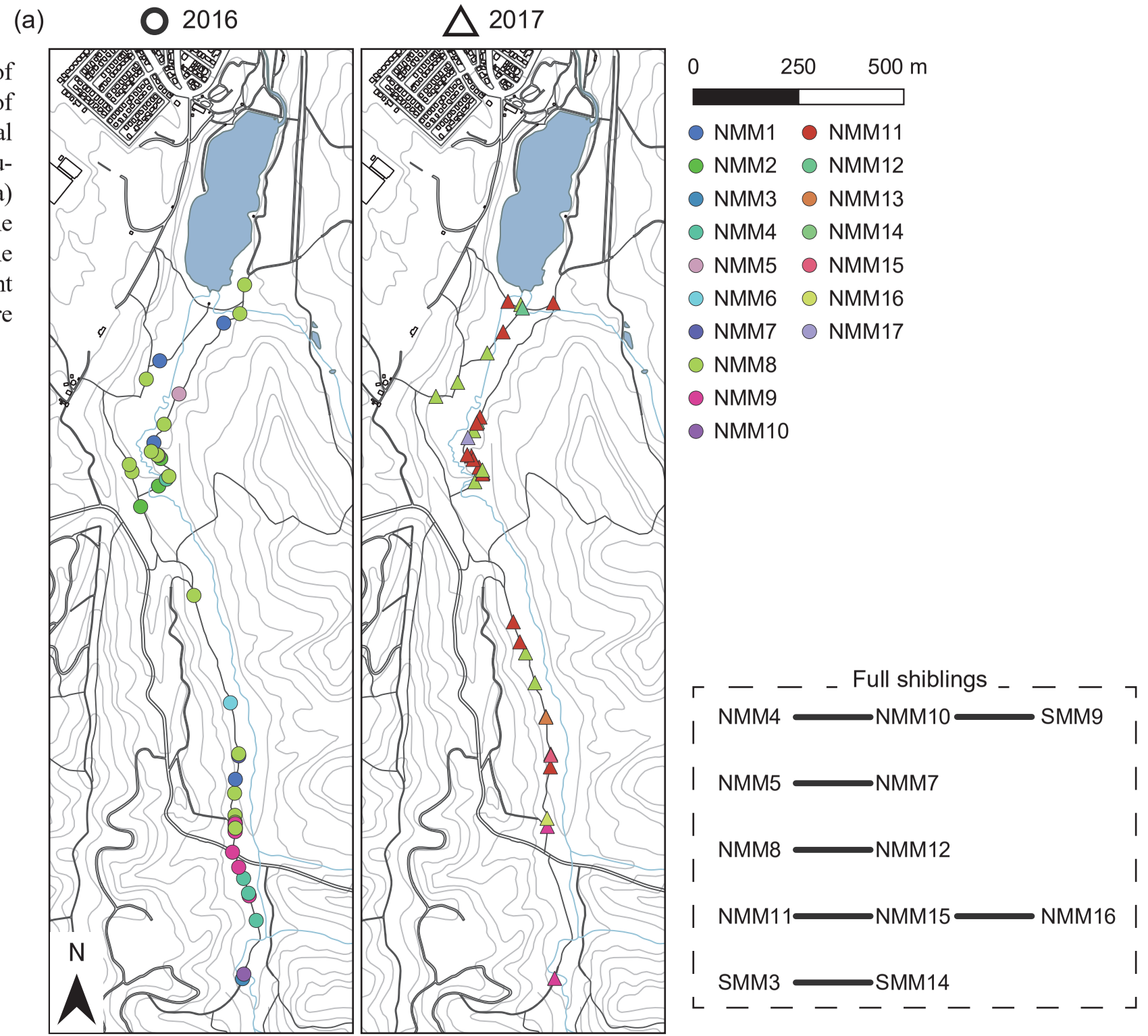


Figure II-4. Spatial distribution of individuals and full siblings of feral Japanese martens. Spatial distribution of identified individuals was plotted on the valley (a) and mountainous (b) areas. The different shapes of plots mean the collected samples in different years. The full siblings were shown in bottom right of (a).

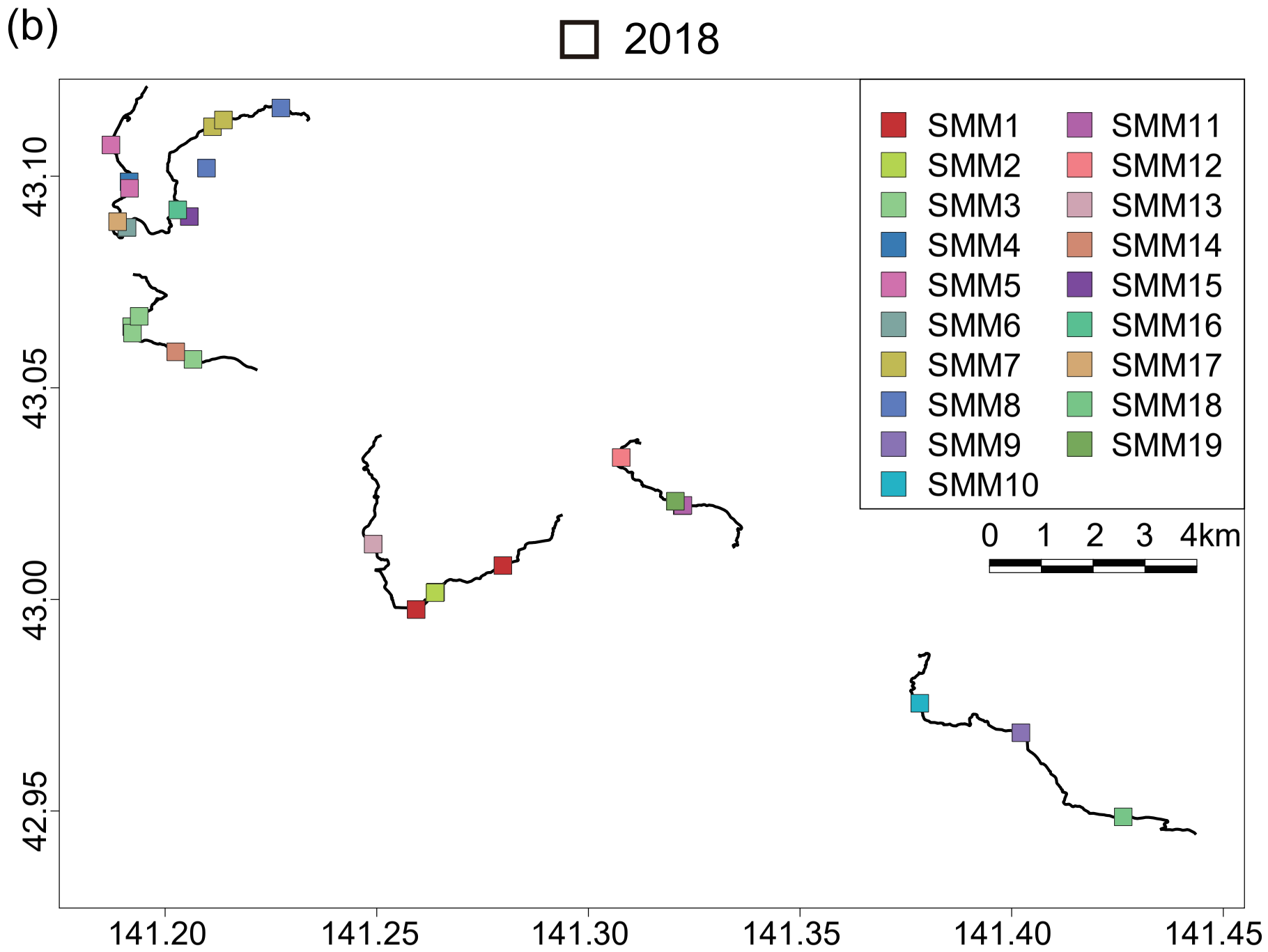


FIgure II-4. continued.

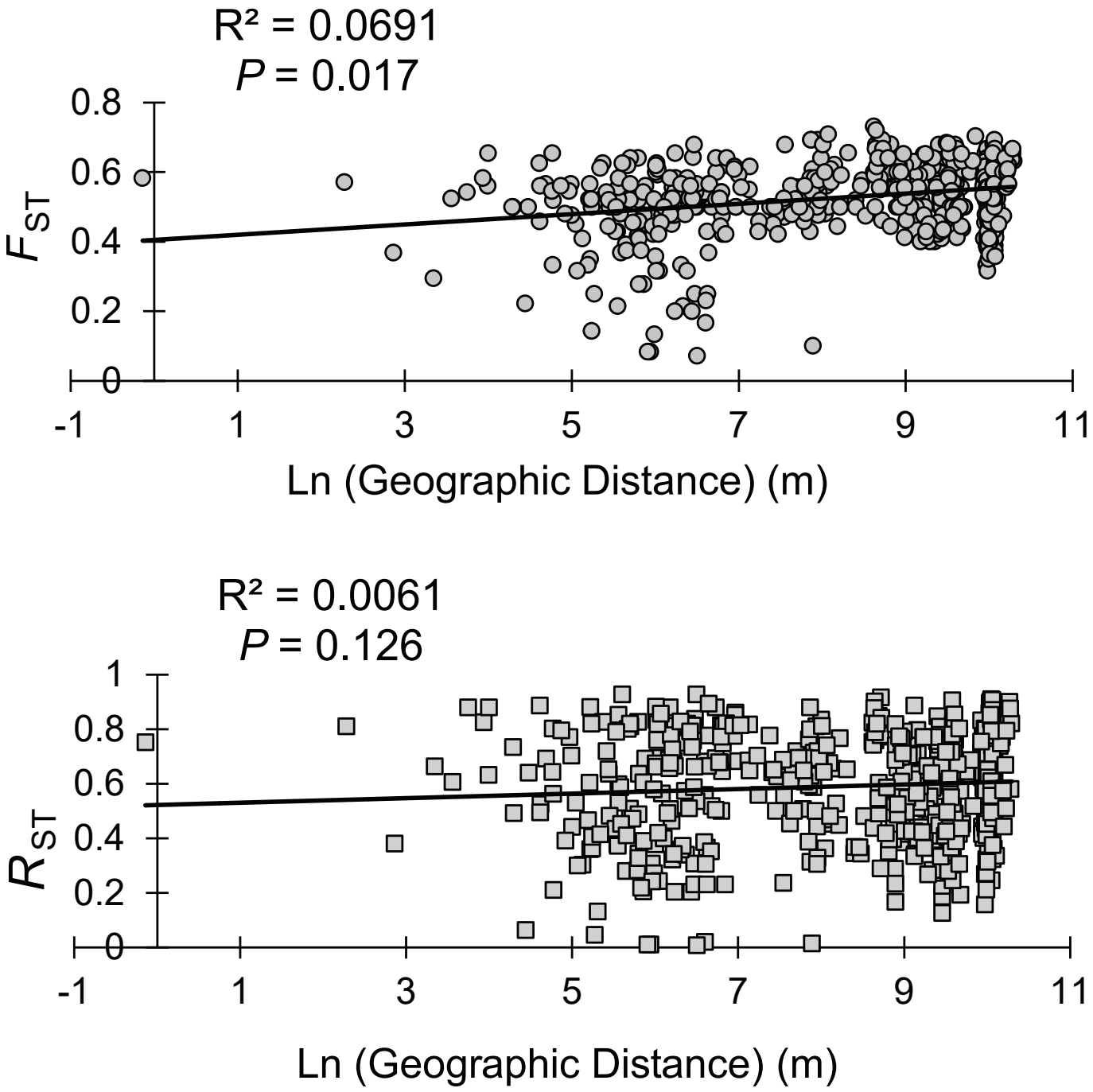
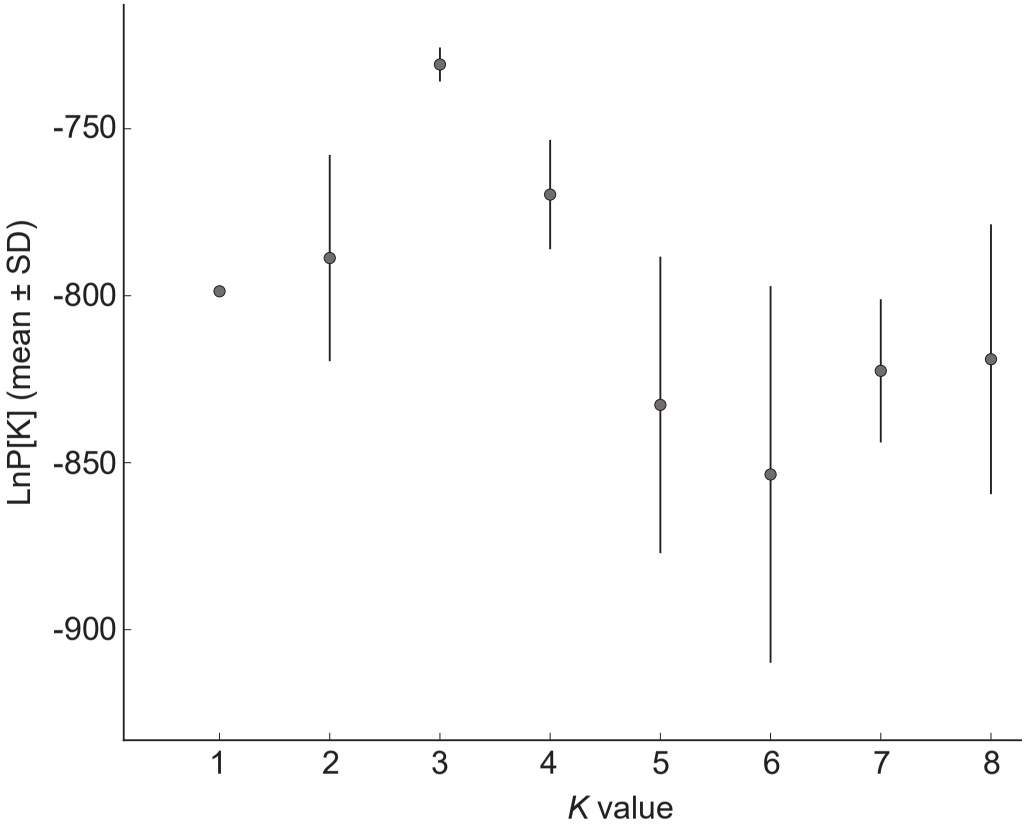


Figure II-5. The results of Mantel test (IBD). The upper panel is based on F_{ST} values (circles) and lower is R_{ST} values (rectangles).



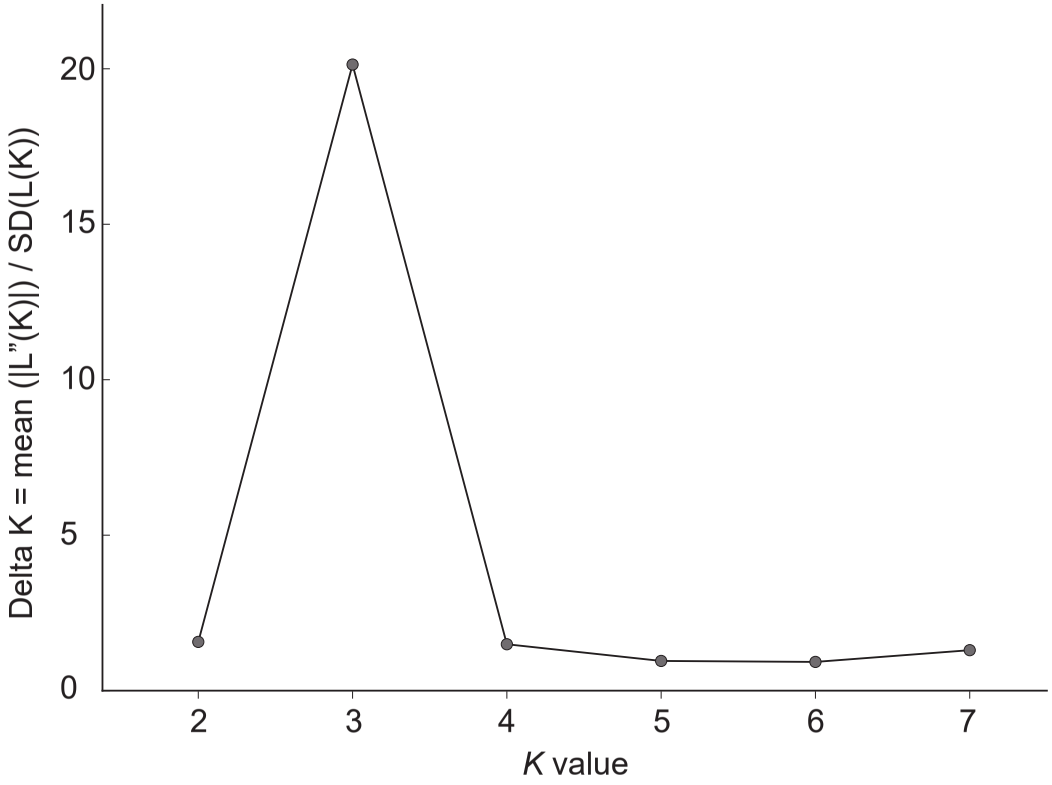


Figure II-6. The LnP [K] and Delta K provided by the STRUCTURE HARVESTER analysis.

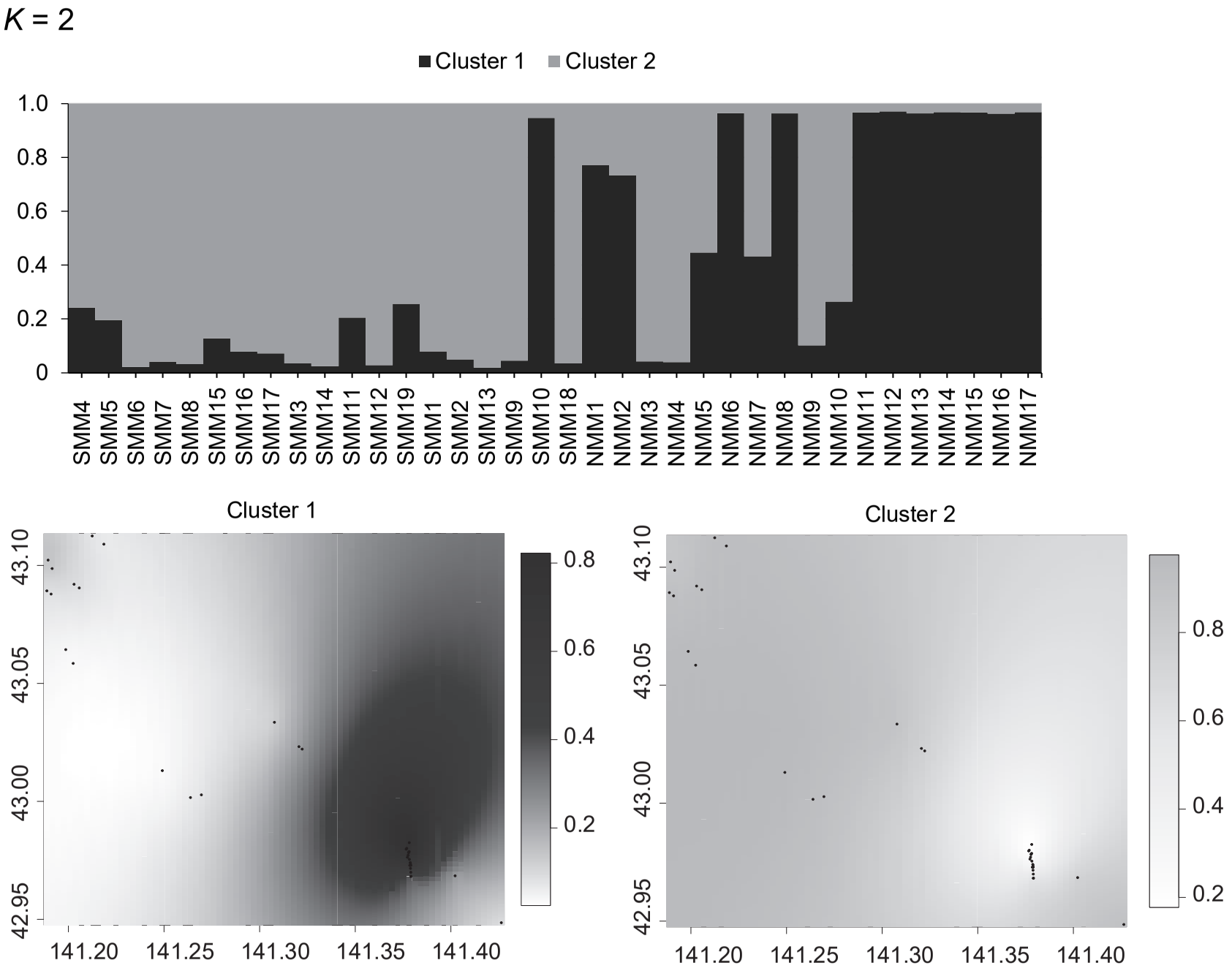


Figure II-7. Results of the STRUCTURE analysis of the Sapporo population. The results of K=2 and K=3 were shown (a and b). The upper panels show bar plots and lower panels show the spatial distribution of the admixture coefficient. Slide bars show the percentage distribution for each one of the components.

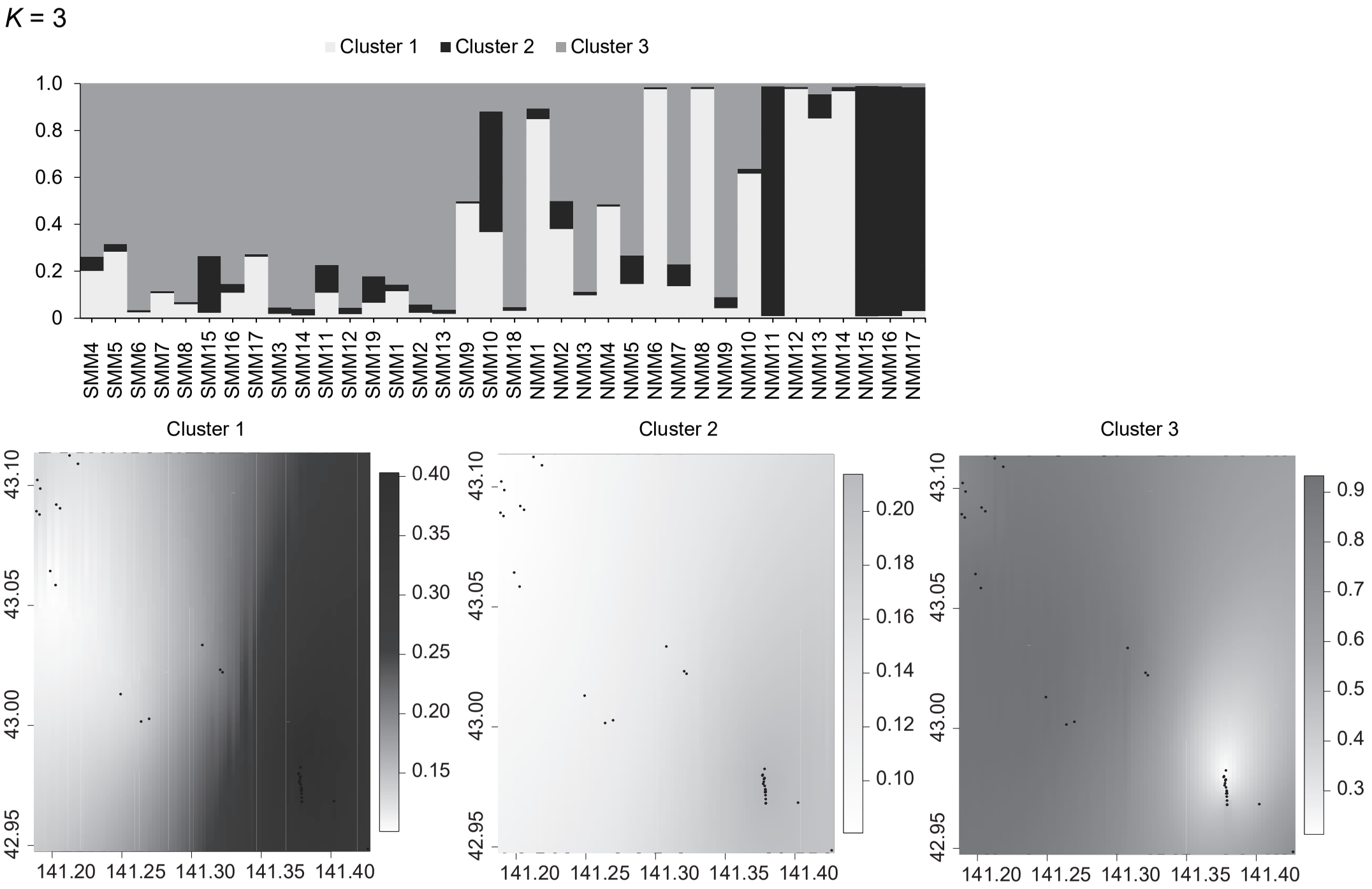


Figure II-7. continued.

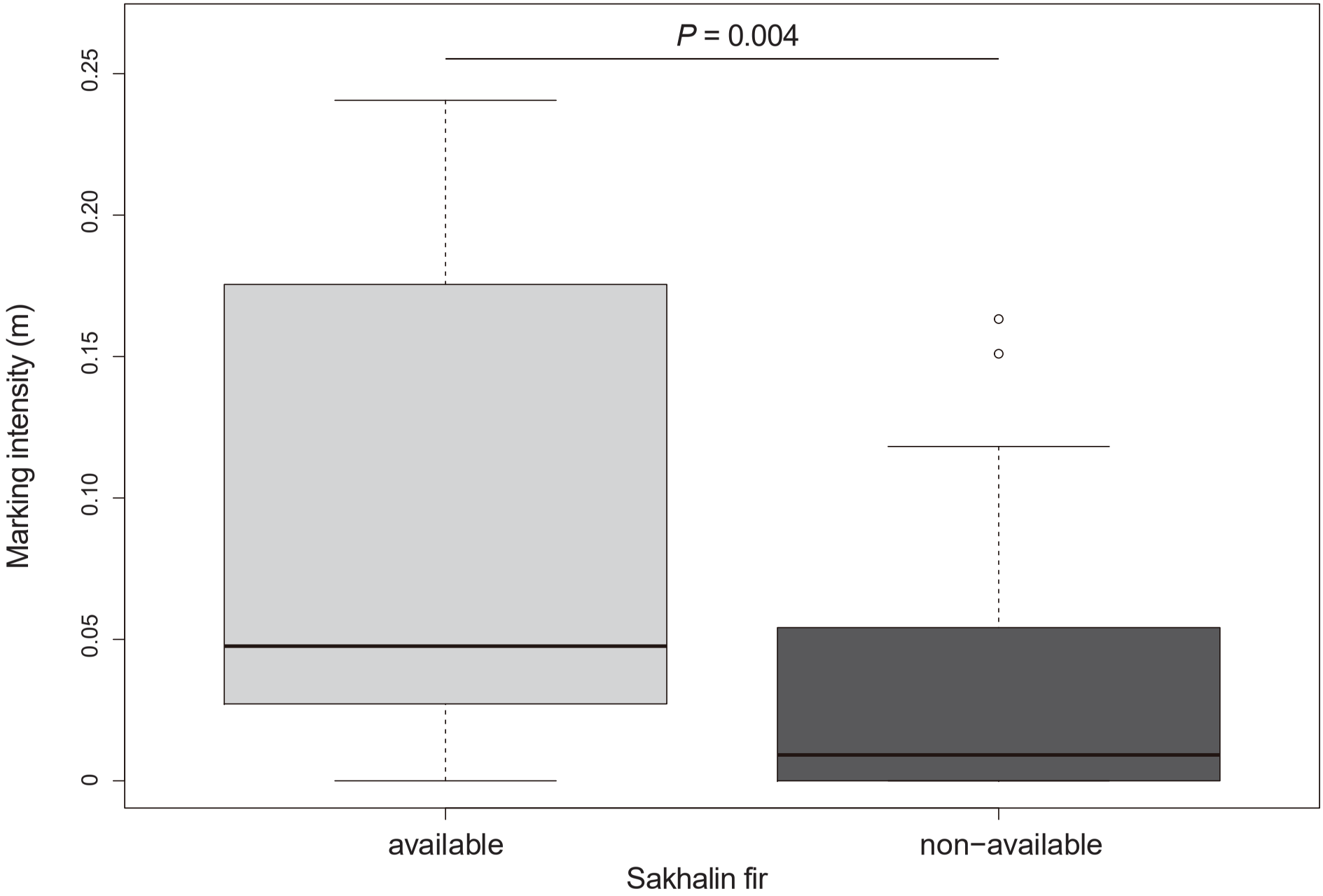


Figure II-8. The comparison of marking intensity with and without available Sakhalin fir patches.

Table I-1. Geographic origins and Accession numbers of samples examined in this study.

Voucher No.	Geographic origin	Accession No.			
		CR	*Cytb*	*ZFY*	*ASIP*
Mn4	Japan, Hokkaido, Shari-cho	LC314620	LC324904	LC325040	LC324973
Mn5	Japan, Hokkaido, Shari-cho	LC314621	LC324905	-	LC324974
Mn6	Japan, Hokkaido, Shari-cho	LC314624	LC324906	-	LC324975
Mn7	Japan, Hokkaido, Shari-cho	LC314622	LC324907	-	LC324976
NEM01	Japan, Hokkaido, Nemuro	LC314625	LC324908	-	LC324977
OBH01	Japan, Hokkaido, Obihiro	LC314623	LC324909	-	LC324978
S12	Japan, Hokkaido, Shari-cho	AB006720[a]	LC324910	LC325041	LC324979
S13	Japan, Hokkaido, Shari-cho	AB006721[a]	-	-	LC324980
S14	Japan, Hokkaido, Shari-cho	same as S13[a]	LC324911	LC325042	LC324981
YOT1	Japan, Hokkaido, Mt.Youtei	AB006719[a]	LC324912	-	LC324982
OB1	Japan, Hokkaido, Makubetu-cho	AB006718[a]	LC324913	LC325043	LC324983
OB2	Japan, Hokkaido, Shihoro-cho	same as S13[a]	LC324914	-	LC324984
HIT1	Japan, Hokkaido, Sapporo	AB006722[a]	LC324915	-	LC324985
HIT2	Japan, Hokkaido, Sapporo	AB006723[a]	LC324916	LC325044	LC324986
N26	Japan, Hokkaido, Unknown	AB006724[a]	LC324917	LC325045	LC324987
N27	Japan, Hokkaido, Sapporo	AB006725[a]	LC324918	LC325046	LC324988
N28	Japan, Hokkaido, Sapporo	same as N27[a]	LC324919	-	LC324989
N29	Japan, Hokkaido, Sapporo	same as N27[a]	LC324920	-	LC324990
N30	Japan, Hokkaido, Sapporo	AB006726[a]	LC324921	-	LC324991
N31	Japan, Hokkaido, Sapporo	same as YOT1[a]	LC324922	LC325047	LC324992
N32	Japan, Hokkaido, Shibecha-cho	AB006727[a]	LC324923	LC325048	LC324993
N33	Japan, Hokkaido, Sapporo	same as N30[a]	LC324924	LC325049	LC324994
N34	Japan, Hokkaido, Sapporo	same as HIT1[a]	LC324925	-	LC324995
N35	Japan, Hokkaido, Shibecha-cho	same as N32[a]	LC324926	-	LC324996
N37	Japan, Hokkaido, Sapporo	same as HIT1[a]	LC324927	-	LC324997
AKI1	Japan, Akita, Kazuno-shi	AB006728[a]	LC324928	LC325050	LC324998
IWA1	Japan, Iwate, Kunohe-gun	same as AKI1[a]	LC324929	AB491594[c]	LC324999
IWA2	Japan, Iwate, Iwaizumi-cho	same as AKI1[a]	LC324930	LC325051	LC325000
151646	Russia, Staroutkinsk	LC314601	LC324931	-	LC325001
278252	Russia, Karpinsk	LC314602	LC324932	-	LC325002
79252	Russia, Priuralsky District	LC314603	LC324933	-	-
79253	Russia, Priuralsky District	LC314604	-	-	-
79254	Russia, Priuralsky District	LC314605	-	-	-
79255	Russia, Priuralsky District	LC314606	LC324934	-	LC325003
79271	Russia, oz. Yarato 2-e	LC314607	LC324935	LC325052	-
79272	Russia, oz. Yarato 2-e	LC314608	-	-	-
79273	Russia, oz. Yarato 2-e	LC314609	-	-	-
79274	Russia, Yamalsky District	LC314610	-	-	-
79304	Russia, Yamalsky District	LC314611	LC324936	-	LC325004
79307	Russia, Priuralsky District	LC314612	LC324937	-	LC325005
RLEN3	Russia, Leningrad Province	same as RALTI[b]	LC324938	LC325053	LC325006
RIND1	Russia, lndigirka	AB049772[b]	LC324939	-	LC325007
ROMS1	Russia, Omsk, West Siberia	LC314613	-	-	-
BUSG1	Bulgaria, Sredna Gora	LC314614	LC324940	LC325054	LC325008
BUV1	Bulgaria, Varna	LC314615	LC324941	LC325055	LC325009
BUV2	Bulgaria, Varna	LC314616	LC324942	LC325056	LC325010
BUV3	Bulgaria, Varna	LC314617	LC324943	LC325057	LC325011
BUV4	Bulgaria, Varna	LC314618	LC324944	LC325058	LC325012
BUV5	Bulgaria, Varna	LC314619	LC324945	LC325059	LC325013
RTUR2	Turkmenistan, Murgab	same as RCAU1[b]	LC324946	-	LC325014
RKAR2	Turkmenistan, South East Karakum	AB049774[b]	LC324947	-	LC325015
RASK1	Ukraine, Askania, Nova	AB049765[b]	LC324948	-	LC325016
RASK2	Ukraine, Askania, Nova	AB049768[b]	LC324949	-	LC325017
RUMA1	Ukraine, Kiev Province	same as RALTI[b]	LC324950	-	LC325018
RCAC1	Georgia, Tbilisi	AB049770[b]	LC324951	-	-
RCAU2	Georgia, Lagodekhi	AB049771[b]	LC324952	-	LC325019
KS.KN 39356	Finland, Porvoon mlk	LC314626	LC324953	LC325060	LC325020
KS.KN 39357	Finland, Joutsa	LC314627	LC324954	LC325061	LC325021
KS.KN 34388	Finland, Siuntio	LC314628	LC324955	-	LC325022
KS.KN 34462	Finland, Helsinki, Vuosaari	LC314629	LC324956	-	LC325023
KS.KN 34463	Finland, Inkoo	LC314630	LC324957	-	LC325024
KS.KN 39310	Finland, Heinolan mlk	LC314631	LC324958	-	LC325025
KS.KN 34644	Finland, Vantaa, Korso	LC314632	LC324959	-	LC325026
KS.KN 38719	Finland, Siuntio	LC314633	LC324960	-	LC325027
KS.KN 34740	Finland, Vihti, Selki	LC314634	LC324961	-	LC325028
KS.KN 33901	Finland, Vantaa, Riipilä	LC314635	LC324962	-	LC325029
KS.KN 47736	Finland, Finström	LC314636	LC324963	LC325062	LC325030
KS.KN 47078	Finland, Dragsfjärd	LC314637	LC324964	LC325063	LC325031
KS.KN 47020	Finland, Keuruu, Haapamäki	LC314638	LC324965	LC325064	LC325032
KS.KN 47872	Finland, Espoo	LC314639	LC324966	LC325065	LC325033
KS.KN 47874	Finland, Tampere	LC314640	LC324967	LC325066	LC325034
KS.KN 47882	Finland, Asikkala	LC314641	LC324968	LC325067	LC325035
KS.KN 47906	Finland, Pernaja	LC314642	LC324969	-	LC325036
KS.KN 47907	Finland, Porvoo	LC314643	LC324970	LC325068	LC325037
KS.KN 48312	Finland, Heinävesi	LC314644	LC324971	-	LC325038
KS.KN 48372	Finland, Kuhmo	LC314645	LC324972	-	LC325039

[a] Kurose et al. (1999). [b] Kurose et al. (2005). [c] Yamada & Masuda (2010).

Table I-2. References from GenBank.

Voucher No.	Geographic origin	Accession No.		Haplotype No.		
		CR	*Cytb*	*CR*	*Cytb*	*CR & Cytb*
Au19	Austria, Grinzling	AM258893	AM258847	A72	B81	C104
Be65	Belgium, Blegny Trembleur	AM258921	AM258871	A71	B80	C103
Dk100	Denmark, Billund, Vandel Dam	AM258942	AM258890	A62	B67	C90
Dk97	Denmark, Glesborg	AM258939	AM258887	A46	B3	C67
Dk98	Denmark, Lemvig	AM258940	AM258888	A61	B7	C89
Dk101	Denmark, Olgod	AM258943	AM258891	A60	B72	C88
Dk99	Denmark, Tyvmose	AM258941	AM258889	A60	B72	C88
Fi35	Finland, Helsinki	AM258901	AM258859	A27	B28	C44
Fi36	Finland, Tampere	AM258902	AM258860	A59	B40	C42
Fr03	France, Bouches du Rhône	AJ698491	AM258837	A58	B72	C87
Fr01	France, Aveyron, La Couvertoirade	AJ698489	-	A56	-	-
Fr14	France, Aveyron, St Martin du Larzac	AJ698501	AM258843	A56	B68	C84
Fr02	France, Bouches du Rhône, Saintes	AJ698490	-	A49	-	-
Co24	France, Corsica	AJ698505	AM258852	A8	B13	C12
Co22	France, Corsica	AJ698503	AM258850	A50	B13	C71
Co23	France, Corsica	AJ698504	AM258851	A65	B13	C93
Co13	France, Corsica	AJ698500	AM258842	A66	B13	C94
Co12	France, Corsica	AJ698499	AM258841	A67	B13	C95
Co11	France, Corsica	AJ698498	AM258840	A68	B13	C96
Co27	France, Corsica Corse du sud, Bonifacio	AJ698506	-	A8	-	-
Co28	France, Haute Corse, Lucciana	AJ698507	-	A92	-	-
Fr06	France, Hérault, Le Caroux	AJ698494	-	A56	-	-
Fr05	France, Hérault, Mireval	AJ698493	-	A56	-	-
Fr07	France, Hérault, Mireval	AJ698495	-	A49	-	-
Fr04	France, Hérault, Murviel	AJ698492	-	A56	-	-
Fr87	France, Landes, Mont de Marsan	AM258930	AM258880	A57	B68	C83
Fr08	France, Morbihan, Sarzeau	AM258892	AM258838	A49	B70	C86
Fr21	France, Pyrénées Orientales, Banyuls	AJ698502	AM258849	A56	B68	C84
Fr09	France, Pyrénées Orientales, Nohedes	AJ698496	AM258839	A43	B69	C85
Fr10	France, Vaucluse, Vaugines	AJ698497	-	A91	-	-
RCAU1	Georgia, Lagodekhi	AB049764	-	A19	-	-
Ge66	Germany, Bielefeld	AM258922	AM258872	A43	B67	C81
Ge113	Germany, Konz	AM932880	-	A56	-	-
Ge114	Germany, Konz	AM932881	AM932885	A56	B6	C82
HS1758	Germany, Thuringia	AB601582	AB564127	A3	B3	C3
TH229	Germany, Thuringia	AB601586	AB564130	A5	B8	C5
185_Gre	Greece	KP456003	KP455998	A84	B79	C117
11_Gre	Greece	KP456012	KP456001	A53	B90	C119
51_Cre	Greece, Crete	KP456008	-	A64	-	-
52_Cre	Greece, Crete	KP456008	-	A64	-	-
53_Cre	Greece, Crete	KP456008	-	A64	-	-
55_Cre	Greece, Crete	KP456008	-	A64	-	-
57_Cre	Greece, Crete	KP456008	-	A64	-	-
59_Cre	Greece, Crete	KP456013	-	A63	-	-
60_Cre	Greece, Crete	KP456008	-	A64	-	-
65_Cre	Greece, Crete	KP456013	-	A63	-	-
67_Cre	Greece, Crete	KP456008	-	A64	-	-
68_Cre	Greece, Crete	KP456008	-	A64	-	-
69_Cre	Greece, Crete	KP456008	-	A64	-	-
71_Cre	Greece, Crete	KP456013	-	A63	-	-
72_Cre	Greece, Crete	KP456013	-	A63	-	-
Cr33	Greece, Crete	AM258900	-	A53	-	-
56_Cre	Greece, Crete	KP456012	KP456000	A53	B89	C110
58_Cre	Greece, Crete	KP456012	KP456000	A53	B89	C110
61_Cre	Greece, Crete	KP456012	KP456000	A53	B89	C110
62_Cre	Greece, Crete	KP456012	KP456000	A53	B89	C110
63_Cre	Greece, Crete	KP456012	KP456000	A53	B89	C110
64_Cre	Greece, Crete	KP456012	KP456000	A53	B89	C110

Table I-2. continued.

Voucher No.	Geographic origin	Accession No.		Haplotype No.		
		CR	*Cytb*	*CR*	*Cytb*	*CR & Cytb*
66_Cre	Greece, Crete	KP456012	KP456000	A53	B89	C110
70_Cre	Greece, Crete	KP456012	KP456000	A53	B89	C110
Cr32	Greece, Crete Karteros Iraklion	AM258899	AM258858	A63	B73	C91
Cr31	Greece, Crete Nida Platau	AM258898	AM258857	A64	B54	C92
Gr102	Greece, Filiatra, Mesinia	AM749057	AM749048	A55	B66	C80
Gr103	Greece, Vravrova, Athènes	AM749058	AM749049	A55	B65	C79
It83	Italy, Cosenza, Cellara	AM258926	AM258876	A48	B3	C69
It82	Italy, Cosenza, Rende	AM258925	AM258875	A48	B3	C69
It81	Italy, Cosenza, Valle Capra	AM258924	AM258874	A48	B3	C69
It16	Italy, Montelibretti	AJ849682	AM258844	A52	B60	C73
It84	Italy, Parma, Collecchio	AM258927	AM258877	A46	B3	C67
It86	Italy, Parma, Fugazzolo	AM258929	AM258879	A47	B58	C68
It85	Italy, Parma, Sala Baganza	AM258928	AM258878	A46	B3	C67
It89	Italy, Plain of Po	AM258932	AM258882	A46	B3	C67
It17	Italy, San Polo Dei Cavaliere	AJ849683	AM258845	A51	B59	C72
Sa29	Italy, Sardinia West center	AM258896	AM258855	A15	B22	C21
Sa63	Italy, Sardinia, Sassari	AM258920	AM258870	A14	B20	C19
Si107	Italy, Sicily	AM749062	AM749053	A8	B12	C10
Si109	Italy, Sicily	AM749064	AM749055	A9	B13	C11
Si108	Italy, Sicily	AM749063	AM749054	A8	B13	C12
Si104	Italy, Sicily	AM749059	AM749050	A8	B14	C13
Si105	Italy, Sicily	AM749060	AM749051	A10	B15	C14
Si106	Italy, Sicily	AM749061	AM749052	A8	B11	C9
Si110	Italy, Sicily	AM749065	AM749056	A8	B11	C9
It20	Italy, Toscana	AJ849685	AM258848	A49	B5	C70
It18	Italy, Toscana, Pratolino	AJ849684	AM258846	A50	B13	C71
HS686	Japan, Aomori	AB601578	AB564123	A45	B57	C66
HS597	Japan, Hokkaido, Eniwa	AB601577	AB564122	A30	B62	C75
5	Japan, Hokkaido, Horokanai-cho	AB006717	AB026106	A41	B52	C62
Lu112	Luxembourg, Berbourg	AM932879	AM932884	A42	B55	C64
Lu111	Luxembourg, Berbourg	AM932878	AM932883	A44	B56	C65
Mo91	Morocco, Casablanca region	AM258933	-	A38	-	-
Mo92	Morocco, Casablanca region	AM258934	-	A38	-	-
Mo93	Morocco, Casablanca region	AM258935	AM258883	A38	B49	C59
Mo55	Morocco, Marrakech region	AM258916	-	A39	-	-
Mo54	Morocco, Marrakech region	AM258915	AM258868	A14	B21	C20
Mo53	Morocco, Marrakech region	AM258914	AM258867	A38	B50	C60
Mo25	Morocco, Marrakech region	AM258894	AM258853	A40	B51	C61
MD29	Poland, Barwik	-	AM258865	-	B35	-
MD32	Poland, Barwik	-	AM258865	-	B35	-
MD33	Poland, Barwik	-	AM258865	-	B35	-
MD31	Poland, Barwik	-	AM258863	-	B37	-
Po40	Poland, Bialowieza	AM258905	-	A27	-	-
Po41	Poland, Bialowieza	AM258906	-	A27	-	-
MD35	Poland, Bialowieza	-	JN819541	-	B111	-
MD38	Poland, Bialowieza	-	JN819541	-	B111	-
MD39	Poland, Bialowieza	-	JN819541	-	B111	-
MD40	Poland, Bialowieza	-	JN819541	-	B111	-
MD42	Poland, Bialowieza	-	JN819541	-	B111	-
MD41	Poland, Bialowieza	-	JN819540	-	B112	-
MD36	Poland, Bialowieza	-	AM258865	-	B35	-
MD37	Poland, Bialowieza	-	AM258863	-	B37	-
Po44	Poland, Bialowieza	AM258909	AM258865	A27	B35	C35
Po43	Poland, Bialowieza	AM258908	AM258864	A28	B36	C36
Po42	Poland, Bialowieza	AM258907	AM258863	A29	B37	C37
Po47	Poland, Biebrza	AM258912	-	A27	-	-
Po48	Poland, Biebrza	AM258913	-	A29	-	-
MD20	Poland, Bieszczady (Kalnica)	-	JN819543	-	B106	-
MD21	Poland, Bieszczady (Kalnica)	-	AM258885	-	B17	-
MD16	Poland, Bogdaniec	-	AM258864	-	B36	-
MD49	Poland, Buczkowice	-	JN860931	-	B114	-

Table I-2. continued.

Voucher No.	Geographic origin	Accession No.		Haplotype No.		
		CR	*Cytb*	*CR*	*Cytb*	*CR & Cytb*
MD48	Poland, Czaniec, gm. Porąbka	-	JN819548	-	B115	-
MD5	Poland, Darz˙lubie	-	JN819536	-	B99	-
MD23	Poland, Famułki K.	-	AM258866	-	B19	-
MD3	Poland, Goleniów	-	AM258864	-	B36	-
MD18	Poland, Górowo, Iławeckie	-	AM258865	-	B35	-
MD30	Poland, Gugny	-	AM258863	-	B37	-
MD34	Poland, Gugny	-	AM258863	-	B37	-
MD10	Poland, Iława	-	JN819549	-	B102	-
Po46	Poland, Kampinos	AM258911	AM258866	A13	B19	C18
Po45	Poland, Kampinos	AM258910	AM932882	A26	B4	C34
MD1	Poland, Krosno Odrzańskie	-	AM258865	-	B35	-
MD24	Poland, Łąki Famułkowskie	-	JN819552	-	B108	-
MD17	Poland, LeSńo	-	JN819549	-	B102	-
MD7	Poland, Międzychód	-	AM258864	-	B36	-
MD6	Poland, Międzyzdroje	-	JN819537	-	B100	-
MD46	Poland, Morskie, Oko	-	JN819547	-	B113	-
MD27	Poland, Niepust	-	JN819545	-	B109	-
MD22	Poland, Nowa Słupia	-	JN819544	-	B107	-
MD12	Poland, Płaska	-	JN819538	-	B103	-
MD28	Poland, Pozary	-	JN819553	-	B110	-
MD26	Poland, Pozary	-	AM258865	-	B35	-
MD2	Poland, Rogalice	-	AM258866	-	B19	-
MD14	Poland, Rozdoły	-	JN819549	-	B102	-
MD15	Poland, Ryjewo	-	AM258865	-	B35	-
MD25	Poland, Sieraków	-	AM258866	-	B19	-
MD47	Poland, Słotwina, gm. Lipowa	-	JN860931	-	B114	-
MD13	Poland, Słubice (Zielony Bór)	-	JN819539	-	B104	-
MD4	Poland, Sobibór	-	JN819550	-	B98	-
MD19	Poland, Świerzawie	-	JN819542	-	B105	-
MD9	Poland, Świętokrzyski PN	-	AM258866	-	B19	-
MD43	Poland, Tychów, gm. Czarnocin	-	AM258864	-	B36	-
MD8	Poland, Wymiarki	-	JN819551	-	B101	-
MD11	Poland, Żytkiejmy	-	AM258865	-	B35	-
Ro30	Romania, Danube Delta	AM258897	AM258856	A15	B27	C26
RALT1	Russia, Altai	AB049769	-	A18	-	-
HS1119	Russia, Kedrovaya, Primorye	AB601580	AB564125	A54	B64	C78
RROS1	Russia, Rostov	same as RASK2	-	A24	-	-
Se95	Serbia, Backo Dobro Polje	AM258937	AM258885	A12	B17	C16
Se94	Serbia, Dobanovci	AM258936	AM258884	A13	B18	C17
Se96	Serbia, Svilajnac, Sedlare	AM258938	AM258886	A11	B16	C15
YP1	South Korea	HM106319	HM106319	A1	B1	C1
HS1913	South Korea, Mt. Odaesan	AB601583	AB564128	A1	B63	C76
HS928	South Korea, Yeongyang	AB601579	AB564124	A1	B61	C74
Mi88	Spain, Baleares, Minorca	AM258931	AM258881	A15	B54	C6
Sp67	Spain, Barcelona	AM258923	AM258873	A6	B9	C7
Sp60	Spain, Granada	AM258917	-	A7	-	-
Sp62	Spain, Huelva	AM258919	AM258869	A7	B10	C8
Sp61	Spain, Malaga	AM258918	-	A7	-	-
77_Bal	Spain, Mallorca	-	KP456002	-	B91	-
78_Bal	Spain, Mallorca	-	KP456002	-	B91	-
Sw26	Switzerland, Tessin, Cadagno lake	AM258895	AM258854	A3	B3	C3
NMNS3302	Taiwan, Mt. Hohuanshan, Nantou County	AB601584	AB046612	A32	B39	C41
NMNS5244	Taiwan, Mt. Hohuanshan, Nantou County	AB601585	AB564129	A32	B39	C41
186_Tun	Tunisia	KP456004	KP455993	A85	B92	C116
320_Tur	Turkey	KP456007	-	A10	-	-
322_Tur	Turkey	KP456009	-	A86	-	-
331_Tur	Turkey	KP456008	-	A64	-	-
334_Tur	Turkey	KP456011	-	A90	-	-
326_Tur	Turkey	-	KP455998	-	B79	-
329_Tur	Turkey	-	KP455998	-	B79	-
330_Tur	Turkey	-	KP455998	-	B79	-

Table I-2. continued.

Voucher No.	Geographic origin	Accession No.		Haplotype No.		
		CR	*Cytb*	*CR*	*Cytb*	*CR & Cytb*
327_Tur	Turkey	-	KP455999	-	B97	-
325_Tur	Turkey	KP456010	KP455997	A87	B94	C111
313_Tur	Turkey	KP456005	KP455994	A88	B95	C112
317_Tur	Turkey	KP456005	KP455994	A88	B95	C112
318_Tur	Turkey	KP456005	KP455994	A88	B95	C112
319_Tur	Turkey	KP456005	KP455994	A88	B95	C112
324_Tur	Turkey	KP456005	KP455994	A88	B95	C112
321_Tur	Turkey	KP456008	KP455996	A64	B93	C113
314_Tur	Turkey	KP456006	KP455995	A89	B96	C114
RETU1	Turkey, Kars Province	AB049776	-	A64	-	-
UK38	United Kingdom, Kielder	AM258904	AM258862	A3	B3	C3
UK37	United Kingdom, Kielder	AM258903	AM258861	A4	B3	C4
HS1745	Uzbekistan, Chizchik River	AB601581	AB564126	A53	B17	C77

Table I-3. Primers for PCR amplification and sequencing.

Locus	Primer name	Forward / Reverse	Sequence (5'-3')	Reference
mtDNA control region	Cb-z	F	ATGAATTGGAGGACAACCAGT	Kurose et al. (1999)
	D4	R	AGGCATTTTCAGTGCCTTGCTTTG	Kurose et al. (1999)
	DS3	F	GCTGACATTCTAACTAAACTATTCC	Kurose et al. (2005)
	EQCR3bi	R	GAACCAGATGCCAGGTATAG	Lorenzen. et al. (2011)
	MNE-DR7	R	ATGTGTGATCATGGGCTG	Mizumachi et al. (2017)
	LCR2	F	ACCTCTTCTCGCTCCGGG	Patou et al. (2009)
	MNE-D5b	F	GACATCTCGATGGACTAATGAC	This study
	MSD2	R	TATGTCCTGTGACCATTGACTGAA	Kurose et al. (2005)
mtDNA cytochrome *b*	L7	F	ACCAATGACATGAAAAATCATCGTT	Montgelard et al. (2002)
	H6	R	TCTCCATTTCTGGTTTACAAGAC	Montgelard et al. (2002)
	Cb-M6	F	AGACGTCAACTACGGCTGAAT	Kurose et al. (2000)
	Cb-M7	F	GAATGAATCTGAGGCGGATTTTC	Kurose et al. (2000)
	Cb-M8	F	CAACCCCCTCAACACACCT	Kurose et al. (2000)
	Cb-M9	F	CTATTAGTATTATTCTCACCCGA	Kurose et al. (2000)
	Cb-MR6	R	GCCGATGTTTCATGTTTCGG	Kurose et al. (2000)
	Cb-MR7	R	GATCCTGTTTCGTGGAGGAATA	Kurose et al. (2000)
	Cb-MR8	R	GATCGTAGAATAGCATATGCGAA	Kurose et al. (2000)
ZFY	MNIZFY_F	F	GTAAGGGTGCACAAGTTCCAC	This study
	MNIZFY_R	R	CCATGAGTGACTGAGCCAAA	This study
	MNIZFY_F2	F	GAAATGGGTTTTGAGTCAGACA	This study
	MELSN-R2	R	CATTTAAAGCCACATGTATTTGG	Tashima et al. (2011)
	MENZFY_F	F	GTCAACATGAAGCAGGCAC	This study
	MNIZFY_R2	R	GCTTCCGTCATGATCCCAC	This study
	MNIZFY_F3	F	GAGCAAGGAGCCCAATGC	This study
ASIP	MNASIPf	F	CCCTGCTGGTCTCCATGT	This study
	MNASIPf2	F	TAGTCTCTGGACTCTGGCCC	This study
	MNASIPf3	F	GAGAAGGGACTACAGGATGTC	This study
	MNASIPr	R	CCCACACTCAGTTATATTCTGC	This study
	MNASIPr2	R	GGAGCTCTGACTTATGTCAC	This study

Table I-4. Nucleotide substitution models for reconstructing phylogenetic trees.

Partition	Figure 2	Figure S1	Figure S2	Figure S4 (a)	Figure S4 (b)	Figure S4 (c)	Figure S4 (d)
CR	HKY+I+G	HKY+I+G	-	HKY+I+G	HKY+I+G	HKY+I+G	-
C1	K80+G	-	K80+G	HKY+I+G	HKY+I+G	HKY+I+G	-
C2	HKY	-	HKY	F81	F81	F81	-
C3	GTR+G	-	GTR+G	HKY	GTR+G	HKY	-
ASIP	-	-	-	HKY+I+G	HKY+I+G	-	HKY
ZFY	-	-	-	HKY	-	HKY	HKY

C1, C2 and C3 mean the first, second and third codons for *Cytb* .

Table I-5. Haplotype numbers of samples examined in this study.

Voucher No.	Haplotype No.									
	CR	*Cytb*	*CR & Cytb*	*ZFY*	*ASIP*- 1	*ASIP*- 2	*mtDNA &ZFY*	*mtDNA & ASIP*	*ZFY & ASIP*	*ALL*
Mn4	A16	B53	C63	D1	E9	E9	F17	G40	H2	I19
Mn5	A16	B23	C22	-	E9	E9	-	G2	-	-
Mn6	A33	B23	C53	-	E9	E9	-	G31	-	-
Mn7	A16	B23	C22	-	E9	E9	-	G2	-	-
NEM01	A33	B23	C53	-	E9	E9	-	G31	-	-
OBH01	A30	B23	C38	-	E9	E9	-	G12	-	-
S12	A17	B24	C23	D1	E9	E9	F2	G3	H2	I2
S13	A16	-	-	-	E9	E9	-	-	-	-
S14	A16	B23	C22	D1	E9	E12	F1	G2	H1	I1
YOT1	A2	B2	C2	-	E9	E9	-	G1	-	-
OB1	A31	B23	C40	D1	E9	E9	F4	G14	H2	I4
OB2	A16	B38	C39	-	E9	E9	-	G13	H11	-
HIT1	A34	B23	C54	-	E1	E9	-	G33	H11	-
HIT2	A30	B23	C38	D1	E1	E9	F19	G12	H4	I20
N26	A37	B23	C58	D1	E6	E6	F14	G39	H8	I18
N27	A36	B23	C57	D1	E6	E6	F16	G38	H8	I17
N28	A36	B23	C57	-	E6	E6	-	G38	H11	-
N29	A36	B23	C57	-	E6	E6	-	G38	H11	-
N30	A35	B23	C55	-	E6	E6	-	G37	H11	-
N31	A2	B48	C56	D1	E9	E6	F15	G36	H3	I16
N32	A33	B23	C53	D1	E9	E9	F13	G31	H2	I15
N33	A35	B23	C55	D1	E9	E9	F12	G35	H2	I14
N34	A34	B23	C54	-	E6	E6	-	G34	H12	-
N35	A33	B23	C53	-	E9	E9	-	G31	H12	-
N37	A34	B23	C54	-	E9	E9	-	G32	H12	-
AKI1	A45	B59	C66	D1	E5	E5	F18	G41	H12	I27
IWA1	A45	B59	C66	D1	E5	E5	F18	G41	H12	I27
IWA2	A45	B59	C66	D1	E5	E5	F18	G41	H12	I27
151646	A27	B88	C118	-	E1	E2	-	G52	-	-
278252	A43	B87	C115	-	E2	E2	-	G51	-	-
79252	A77	B86	C109	-	-	-	-	-	-	-
79253	A79	-	-	-	-	-	-	-	-	-
79254	A80	-	-	-	-	-	-	-	-	-
79255	A76	B85	C108	-	E3	E3	-	G50	-	-
79271	A75	B84	C107	D1	-	-	F26	-	-	-
79272	A81	-	-	-	-	-	-	-	-	-
79273	A82	-	-	-	-	-	-	-	-	-
79274	A83	-	-	-	-	-	-	-	-	-
79304	A74	B83	C106	-	E2	E2	-	G49	-	-
79307	A73	B82	C105	-	E4	E4	-	G48	-	-
RLEN3	A18	B27	C27	D1	E2	E2	F3	G6	H5	I3
RIND1	A21	B30	C29	-	E1	E1	-	G8	-	-
ROMS1	A74	-	-	-	-	-	-	-	-	-
BUSG1	A15	B79	C102	D2	E2	E2	F25	G47	H11	I26
BUV1	A11	B78	C101	D2	E2	E2	F24	G46	H11	I25
BUV2	A70	B77	C100	D2	E2	E2	F23	G45	H11	I24
BUV3	A64	B76	C99	D2	E2	E2	F22	G44	H11	I23
BUV4	A69	B75	C98	D2	E2	E2	F21	G43	H11	I22
BUV5	A64	B74	C97	D2	E1	E2	F20	G42	H10	I21
RTUR2	A19	B26	C25	-	E10	E2	-	G5	-	-
RKAR2	A20	B29	C28	-	E2	E2	-	G7	-	-
RASK1	A25	B34	C33	-	E1	E2	-	G11	-	-
RASK2	A24	B33	C32	-	E1	E6	-	G10	-	-
RUMA1	A18	B25	C24	-	E2	E11	-	G4	-	-
RCAC1	A23	B32	C31	-	-	-	-	-	-	-
RCAU2	A22	B31	C30	-	E1	E1	-	G9	-	-
KS.KN 39356	A73	B47	C52	D1	E1	E1	F11	G30	H9	I13
KS.KN 39357	A27	B28	C44	D1	E2	E7	F5	G21	H6	I5
KS.KN 34388	A73	B41	C45	-	E2	E2	-	G22	-	-
KS.KN 34462	A27	B28	C44	-	E2	E2	-	G20	-	-
KS.KN 34463	A27	B28	C44	-	E1	E1	-	G18	-	-
KS.KN 39310	A27	B28	C44	-	E1	E8	-	G17	-	-
KS.KN 34644	A59	B28	C43	-	E2	E2	-	G16	-	-
KS.KN 38719	A59	B40	C42	-	E2	E2	-	G15	-	-
KS.KN 34740	A59	B40	C42	-	E2	E2	-	G15	-	-
KS.KN 33901	A59	B46	C51	-	E2	E2	-	G29	-	-
KS.KN 47736	A27	B28	C44	D1	E2	E2	F6	G20	H5	I7
KS.KN 47078	A59	B40	C42	D1	E2	E2	F10	G15	H5	I12
KS.KN 47020	A78	B45	C50	D1	E6	E6	F9	G28	H8	I11
KS.KN 47872	A59	B44	C48	D1	E6	E6	F7	G27	H8	I10
KS.KN 47874	A27	B28	C44	D1	E6	E2	F6	G19	H7	I6
KS.KN 47882	A27	B41	C49	D1	E2	E2	F8	G26	H5	I9
KS.KN 47906	A59	B44	C48	-	E1	E1	-	G25	-	-
KS.KN 47907	A27	B43	C47	D1	E6	E6	F6	G24	H8	I8
KS.KN 48312	A73	B41	C45	-	E2	E2	-	G22	-	-
KS.KN 48372	A1	B42	C46	-	E6	E6	-	G23	-	-

Table I-6. Statistical data of the least weasel in Palearctic.

Gene	Group	*n*	*h*	*Hd*	π	*D*	F_s
mtDNA	All	168	119	0.992	0.0123	-1.598*	-23.780*
	Hokkaido (Cluster Ie)	26	15	0.948	0.0023	-0.898	-6.035*
	Honshu	4	1	0.000	0.0000	0.000	NA
	Russia†	3	3	1.000	0.0053	0.000	0.987
	Ural	7	7	1.000	0.0090	-1.057	-0.923
	Bulgaria	6	6	1.000	0.0062	0.592	-0.947
	Turkmenistan	2	2	1.000	0.0283	0.000	3.829
	Ukraine	3	3	1.000	0.0251	0.000	2.591
	Georgia	2	2	1.000	0.0258	0.000	3.738
	Finland	22	11	0.875	0.0035	0.591	-0.831
	Clade I	106	71	0.988	0.0084	-1.590*	-24.152*
	Cluster Ia	35	26	0.977	0.0030	-1.614*	-18.823*
	Cluster Ib	8	4	0.750	0.0012	-0.705	0.119
	Cluster Ic	2	2	1.000	0.0037	0.000	1.792
	Cluster Id	2	2	1.000	0.0037	0.000	1.792
	Cluster If	3	3	1.000	0.0053	0.000	0.987
	Clade II	56	41	0.973	0.0059	-1.185	-19.949*
	Cluster IIa	3	2	0.667	0.0000	0.000	NA
	Cluster IIb	6	6	1.000	0.0023	-0.978	-0.905
	Cluster IIc	4	4	1.000	0.0017	0.650	-1.322
	Cluster IId	2	2	1.000	0.0006	0.000	0.000
	Cluster IIe	10	9	0.978	0.0031	-0.301	-3.619*
ASIP	All	67	12	0.780	0.003	-0.702	-3.367
	Hokkaido	25	4	0.478	0.002	0.591	0.479
	Honshu	3	1	0.000	0.0000	0.000	NA
	Russia†	2	2	0.667	0.0014	1.633	0.540
	Ural	5	4	0.733	0.0032	0.324	0.017
	Bulgaria	6	2	0.167	0.0003	-1.141	-0.476
	Turkmenistan	2	2	0.500	0.0021	-0.710	1.099
	Ukraine	3	4	0.867	0.0026	-0.185	-1.350
	Georgia	1	1	1.000	0.0000	0.000	NA
	Finland	20	5	0.631	0.0020	0.047	-0.443
ZFY	All	30	2	0.331	0.0077	0.946	13.074

n, number of individuals; h, number of haplotypes; Hd, haplotype diversity; π, nucleotide diversity; D, Tajima's D; F_s, Fu's F_s; NA, not analysis. Asterisks show that values of D or F_s are statistically significant ($P < 0.05$). A dagger means excluded the Ural individuals.

Table I-7. Population grouping of the least weasels in Palearctic by SAMOVA.

	K								
	2	3	4	5	6	7	8	9	10
Hokkaido	I	I	I	I	I	I	I	I	I
Honshu	I	I	II	II	II	II	II	II	II
Russia	I	I	I	II	II	III	III	III	III
Bulgaria	II	II	III	III	III	IV	IV	IV	IV
Turkmenistan	I	III	IV	IV	IV	V	V	V	V
Ukraine	I	III	I	IV	IV	V	V	V	V
Georgia	I	I	I	II	II	III	V	V	VI
Finland	I	I	I	II	II	III	III	III	III
France	I	I	I	V	V	VI	VI	VI	VII
Italy	I	I	I	V	V	VI	VI	VI	VII
Austria	I	I	I	V	VI	VII	VII	VII	VIII
Morocco	II	II	III	III	III	IV	VIII	VIII	IX
Switzerland	I	I	I	V	VI	VII	VII	VII	VIII
Romania	II	II	III	III	III	IV	IV	IV	IV
Greece	II	II	III	III	III	IV	VIII	IX	X
United Kingdom	I	I	I	V	VI	VII	VII	VII	VIII
Poland	I	I	I	V	V	VI	VI	VI	VII
Spain	I	I	I	V	V	VI	VI	VI	VII
Belgium	I	I	I	V	VI	VII	VII	VII	VIII
Germany	I	I	I	V	VI	VII	VII	VII	VIII
Serbia	II	II	III	III	III	IV	IV	IV	IV
Denmark	I	I	I	V	VI	VII	VII	VII	VIII
Luxembourg	I	I	I	V	VI	VII	VII	VII	VIII
South Korea	I	I	I	II	II	III	III	III	III
Uzbekistan	I	I	I	III	II	IV	V	V	III
Taiwan	I	I	I	II	II	III	III	III	III
Tunisia	II	II	III	III	III	IV	VIII	VIII	IX
Turkey	II	II	III	III	III	IV	VIII	IX	X
F_{CT}	0.3410	0.3685	0.3563	0.4010	0.4282	0.4722	0.4702	0.4849	0.5092
F_{ST}	0.6510	0.6507	0.6461	0.5890	0.5820	0.5821	0.5762	0.5751	0.5764
F_{SC}	0.4704	0.4470	0.4502	0.3138	0.2690	0.2083	0.2002	0.1750	0.1368

K is the number of groups.

Table II-1. The information of survey routes and sampling effort.

Route name	Route ID	Transect length (km)	NS	N_{MLF}	N_{MF}	MF / km	NS_{min}	N_{ind}	N_{ind} / km
Northern part of Mt. Teine	1	12.82	3	25 (2)	15 (2)	0.338	NA	8 (1)	0.260
Southern part of Mt. Teine	2	5.29	3	14	8	0.505	NA	2	0.189
Mt. Moiwa	3	6.00	3	9	6	0.333	NA	3	0.222
Mt. Toishi	4	10.71	3	13	10	0.311	2.73	3	0.124
Mt. Shirahata	5	10.44	3	11	6	0.192	NA	3	0.096
Nishioka	6	4.12	66	245 (6)	207 (3)	0.750	2.62	17	0.184
Total		407.90	81	317 (8)	252	0.618	2.68	36 (1)	0.179

NS : number of surveys; N_{MLF}: number of marten-like feces; N_{MF}: martens' feces; N_{ind}: number of individuals.The numbers in the parenthesis show occasional samples, not included in the analysis. Route IDs correspond to Figure II-1.

Table II-2. Identified individual information of Japanese marten in the valley area (Route 6).

Individual ID	Sex	MtDNA haplotype	Year		2016					2017						
			2016	2017	Sep.	Oct.	Nov.	Dec.	Jan.	May	Jun.	Jul.	Aug.	Sep.	Oct.
NMM1	Male	B	4 (1)		1	1 (1)	2								
NMM2	Male	A	4		1	3									
NMM3	Male	A	1			1									
NMM4	Male	A	4			2	2								
NMM5	Male	A	3 (1)			2 (1)	1								
NMM6	Male	B	1			1									
NMM7	Male	A	1			1									
NMM8	Male	B	15 (1)	9		1	13 (1)	1	1	3	2		1	1	1
NMM9	Male	B	6	2		1	5		1						1
NMM10	Unknown	A	1				1								
NMM11	Male	A		16						6	5	1	2		2
NMM12	Male	B		1						1					
NMM13	Female	B		1							1				
NMM14	Male	B		1							1				
NMM15	Male	A		1											1
NMM16	Male	A		1											1
NMM17	Male	A		1											1
			10	9	2	7	5	1	2	3	4	1	2	1	6

For each identified individual, sex, mtDNA haplotype and number of feces collected in each year and month are shown. The number in the parenthesis indicates occasional samples.

Table II-3. Identified individual information of Japanese marten in mountainous area (Route 1-5).

Route ID	Individual ID	Sex	MtDNA haplotype	Year				
				2018	Jun.	Sep.	Oct.	Nov.
1	SMM4	Male	A	1		1		
1	SMM5	Male	A	2		1	1	
1	SMM6	Male	A	2		2		
1	SMM7	Female	A	2		2		
1	SMM8	Male	B	2 (1)		1	1 (1)	
1	SMM15	Male	A	1 (1)			1 (1)	
1	SMM16	Male	A	1			1	
1	SMM17	Female	A	1			1	
2	SMM3	Male	A	4		1	3	
2	SMM14	Male	A	1			1	
3	SMM11	Male	A	2		1		1
3	SMM12	Male	A	1		1		
3	SMM19	Male	A	1				1
4	SMM1	Male	A	2	1		1	
4	SMM2	Female	A	2	2			
4	SMM13	Male	A	1			1	
5	SMM9	Male	A	1		1		
5	SMM10	Male	B	1		1		
5	SMM18	Male	A	1				1
				19	3	10	9 (2)	3

For each identified individual, sex, mtDNA haplotype and number of feces collected in year 2018 and each month are shown. The number in the parenthesis indicates occasional samples.

Table II-4. Genetic variation of feral Japanese marten in Sapporo.

Population	N_{ind}	H_O	H_E	F_{IS}	*HWE* (*P*- value)
Sapporo (all)	36	0.646	0.702	0.035	0.277
Mountainous area (Route1-5)	19	0.618	0.683	**0.097**	**0.041**
Route1	8	0.656	0.659	0.005	0.582
Route2	2	0.929	0.643	-0.857	1.000
Route3	3	0.619	0.648	0.055	0.358
Route4	3	0.542	0.708	0.278	**0.026**
Route5	3	0.542	0.717	0.288	**0.011**
Valley area (Route 6)	17	0.676	0.682	0.008	0.452
Route 6 (2016)	10	0.713	0.701	-0.018	0.707
Route 6 (2017)	9*	0.653	0.637	-0.026	0.620

N_{ind}, number of individuals; H_O, observed heterozygosity; H_E, expected heterozygosity; F_{IS}, inbreeding coefficient; *HWE* , significant deviation from Hardy Weinberg Equilibrium test. An asterisk shows the value including 2 individuals' data, which were also observed on 2016 too. The bolded number indicates statistical significance ($P < 0.05$).

Table II-5. The estimation of population size of Japanese marten.

	Year	N_{min}	N_C (95% CI)	N_E (95% CI)
Valley area	2016	10	11 (10-12)	10 (4-33)
	2017	9	15 (11-18)	8 (3-24)
Mountainous area	2018	19	30 (20-36)	32 (18-67)
Sapporo (total)		36	54 (51-70)	40 (24-68)
Aso*	2006	61	86 (85-102)	NA
	2007	42	84 (80-84)	
Saga*	2006	36	56 (54-72)	
	2007	11	12 (11-13)	

N_{min}: minimum population size; N_C: census population size; N_E: effective population size. Data from Tago et al. (2013) for Aso and Saga are indicated by the asterisk (*).

Table II-6. The results of habitat selection in the valley area.

Vegetation type	π	u	o	w ($\pm SE$)	B
White birch and Mongolian oak	0.442	25.599	0.125	**0.284 ± 0.052**	0.052
Japanese elm	0.442	148.953	0.730	**1.653 ± 0.070**	0.304
Reed	0.069	21.037	0.103	1.484 ± 0.306	0.273
Willow	0.023	7.646	0.037	1.621 ± 0.575	0.298
Sakhalin fir	0.009	0.762	0.004	0.401 ± 0.458	0.074
Park included small plantations	0.012	0	0	**0**	0
Residential area	0.002	0	0	**0**	0
Total	1	204*	1	5.44	1

The relative frequency of available area (π), number of observations (u), the relative frequency of used vegetation area (o), selection ratio (w), the standardized selection ratio (B) are summarized for each vegetation type. The statistically significant ($P < 0.007$) results are bolded. An asterisk shows that the total value does not become 204, because the number of observations is from division value.

Table II-7. The seasonal variation of habitat selection in the valley area.

		Autumn 1 (n=46)	Winter (n=7)	Spring (n=38)	Summer (n=74)	Autumn 2 (n=37)
x^2_L		27.228	1.924	16.559	58.193	23.712
x^2_L (P-value)		$P < 0.001$	$P = 0.927$	$P = 0.011$	$P < 0.001$	$P < 0.001$
Vegetation type	Birch and Oak	**0.326 (0.117)**	NA	**0.406 (0.141)**	**0.155 (0.066)**	**0.244 (0.115)**
(Selection ratio and SE)	Japanese elm	**1.811 (0.113)**	NA	**1.515 (0.173)**	**1.711 (0.113)**	**1.523 (0.175)**
	Reed	0.761 (0.475)	NA	0.738 (0.515)	2.087 (0.589)	2.306 (0.868)
	Willow	0	NA	3.633 (1.946)	1.325 (0.866)	2.556 (1.677)
	Sakhalin fir	0.329 (0.875)	NA	1.752 (2.206)	0	0
Rank of B	1st	Japanese elm (0.561)	NA	Willow (0.395)	Reed (0.395)	Willow (0.386)
	2nd	Reed (0.236)	NA	Sakhalin fir (0.218)	Japanese elm (0.324)	Reed (0.348)
	3rd	Sakhalin fir (0.101)	NA	Japanese elm (0.188)	Willow (0.251)	Japanese elm (0.230)

SE, standard error; B, standardized selection ratio; NA, not analyzed. Summer of 2016 and 2017 was distinguished by adding 1 and 2. For each season, the number of used samples were shown (n).The values of log-likelihood statistic test and its statistically significant results were shown (x^2_L and x^2_L (P-value), respectively). The bold means statistically sighnificant ($P < 0.007$).

Table II-8. The seasonal variation of the sampling collection and population size in the valley area.

	Autumn 1	Winter 1	Spring	Summer	Autumn 2	Winter 2
NS	10	10	12	13	12	6
N_{MLF}	59 (6*)	8	54	80	41	0
N_{MF}	49 (3*)	7	38	74	37	0
NS_{min}	1.30	4.32	2.73	1.17	1.67	NA
Number of genotyped feces	36	3	10	13	8	0
Genotyping rate	0.78	0.43	0.26	0.18	0.22	NA
N_{ind}	10	2	3	4	6	0
Avereage number of feces / Individual	3.6	1.50	3.33	2.80	1.33	NA
Model used for *Capwire*	TIRM	NA	NA	NA	ECM	NA
N_C (95% CI)	12 (10-14)	NA	NA	NA	11 (6-12)	NA

NS, number of surveys; N_{MLF}, number of marten-like feces; N_{MF}, martens' feces; N_{ind}, number of individuals; N_C, census population size; NA, not analyzed. Summer and winter of 2016 and 2017 was distinguished by adding 1 and 2. An asterisk shows occasional samples.