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ANIMAL GENETICS

Intraspecific Structure of Sable *Martes zibellina* L. Inferred from Nucleotide Variation of the Mitochondrial DNA Cytochrome *b* Gene

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Abstract—A fragment of the mitochondrial DNA (mtDNA) cytochrome b gene was sequenced in sable from Magadan oblast, Khabarovsk krai, and Kamchatka. Using phylogenetic analysis, the presence of two clusters (A and BC), with the divergence value of 1.4%, was demonstrated. Analysis of the cytochrome *b* gene median networks indicated that split of the ancestral population took place in early Pleistocene (about one Myr ago), while expansion of its more young phylogenetic group A occurred in late Pleistocene, about 120000 years ago.

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INTRODUCTION

Molecular genetic investigations of different species from the family Mustelidae, carried out with the help of phylogenetic analysis of nucleotide sequences of 22 genomic regions (about 12 kbp) made it possible to identify four major clades and three monotypic groups [1]. Eurasian martens from the subfamily Martinae (sable *Martes zibellina* L., common marten *M. martes* L., stone marten *M. foina*, and Indian marten *M. flavigula*) formed a monophyletic group. Among these, the highest interspecific genetic similarity was observed between sable and common marten [1]. At the same time, analysis of the population genetic polymorphism in sable pointed to the existence of intraspecific heterogeneity [2, 3]. Analysis of restriction polymorphism of the mitochondrial DNA (mtDNA) cytochrome b gene in populations of sable from Siberia and the Far East showed the prevalence of three haplotypes, A, B, and C, with each of the haplotypes probably representing a group of monophyletic lineages [2–4]. To confirm these findings, fine analysis of mtDNA variation at the level of nucleotide sequences is required. Earlier studies on the mtDNA cytochrome *b* gene sequence variation were mostly restricted to the insular populations of Japan [5]. The appearance of the mitochondrial haplotype of sable in the gene pool of common marten (in Sweden) was also reported. These data were explained in terms of interspecific hybridization in the contact zone, however, paraphilia of the *M. martes* and *zibellina* group was not excluded [6].

Sable shows high variation of morphological characters, which hampers elaboration of unified intraspecific taxonomy [7, 8]. According to the opinion of many investigators, only the population of sable from Kamchatka can be isolated into Kamchatka subspecies *M. z. kamtschadalica* [8, 9]. The territory of Khabarovsk krai is inhabited by populations of sable belonging to the subspecies *M. z. jakutensis*. At the same time on the most part of Magadan oblast, local hybrid populations of sable subspecies mentioned were formed [10, 11]. Apparently, further investigations of interspecific relationships in martens require new data on mtDNA variation. The present study was focused on the analysis of phylogenetic relationships of the mtDNA cytochrome b gene sequences in sables from Magadan oblast, Kamchatka, and Khabarovsk krai.

MATERIALS AND METHODS

Samples of sable muscle (*M. zibellina* L.) were collected in three localities, including Khabarovsk krai (five specimens), Central Kamchatka (two specimens), and Magadan oblast (10 specimens). The specimens examined are kept in the animal tissue collection of the Laboratory of Genetics, Institute of the Biological Problems of the North, Russian Academy of Sciences, Magadan. Genomic DNA was extracted with the help of standard techniques, which included cell lysis with proteinase K (Sigma, United States) in the presence of 1% sodium dodecyl sulfate, purification of DNA with the mixture of phenol and chloroform, and DNA precipitation with ethanol.

Variation in mtDNA was examined through the analysis of the fragment of cytochrome *b* gene (720 bp), amplified in the conditions of polymerase chain reaction with primers as described earlier [2]. Sequencing of the amplified mtDNA fragments was conducted using a standard method with the DNA Big

Table 1. A list of sable specimens examined in the present study

Specimen number, mtDNA cluster	Population	GenBank accession number
6373, B	Khabarovsk krai, Lazovskii raion	FJ430695
6382, B	The same	FJ430696
5970, C	Khabarovsk krai, Verkhnebureinskii raion	FJ430693
Miau 2, C	Magadan oblast, Myandzha	FJ430707
M 6, A	Kamchatka, Mil'kovskii raion	FJ430705
5980, A	Khabarovsk krai, Verkhnebureinskii raion	FJ430694
M 15, A	Kamchatka, Mil'kovskii raion	FJ430706
5916, A	Khabarovsk krai, Lazovskii raion	FJ430692
B 20, A	Magadan oblast, Balygychan	FJ430697
El 11, A	Magadan oblast, El'gen	FJ430704
Ber 16, A	Magadan oblast, Berelekh	FJ430703
B 82, A	Magadan oblast, Balygychan	FJ430702
Miau 13, A	Magadan oblast, Myandzha	FJ430691
B 27, A	Magadan oblast, Balygychan	FJ430698
B 28, A	The same	FJ430699
B 47, A	"	FJ430701
B 35, A	"	FJ430700

Dye Terminator kit for cyclic sequencing (Applied Biosystems, v. 3.1) and ABI Prism 3130 (Applied Biosystems, United States) genetic analyzer. Sequence alignment and analysis was performed using MEGA 3.1 software package [12].

Phylogenetic analysis was conducted using the neighbor-joining (NJ) technique and Kimura's two-parameter model (MEGA 3.1 software package). For phylogenetic analysis, Bayesian Inference (BI), using Monte Carlo algorithm and Markov chains, was also applied (BEAST v. 1.4.7 software package) [13]. For the analysis, the HKY+I+G, i.e., the model of Hasegawa, Kishino, and Yano (HKY) was used. The model assumes that different nucleotide positions can evolve at different rates and can be invariant (I), and nucleotide substitution rate variation follows gamma distribution (G). For BI analysis, Markov chains were run for 10 million generations sampling every 500 generations. The first 3000 trees were excluded from the analysis as unstable. The information on the remaining 10000 trees was summarized in the form of a single phylogenetic tree ("target" tree) using the TreeAnnotator v. 1.4.7 software program [13].

Median networks were constructed using the MJ (median-joining) algorithm (the Network 4.5 software program) [14]. The degree of the mtDNA divergence was evaluated using the ρ distance, which corresponds to the mean distance from ancestral haplotype to all derivate haplotypes, including the hypothetic ones (the so-called median vectors, mv) [14]. The divergence time was calculated using the value of mutation accumulation rate in the cytochrome *b* gene, equal to 0.95% divergence/Myr. This rate was calculated based on the divergence time between sable and common marten, which constituted 1.1 Myr (0.5 to 1.7 Myr within the 95% confidence interval) according to the data of Bayesian analysis of molecular clock model, calibrated with paleontological data for the family Mustelidae, as described in [1].

RESULTS AND DISCUSSION

In the present study, partial sequences (720 bp) of the mtDNA cytochrome *b* gene were determined in 17 sables from the populations of Magadan oblast, Kamchatka, and Khabarovsk krai (Table 1). For phylogenetic analysis, additional data on these gene sequences from the GenBank were used. Specifically, nucleotide sequences of common marten *M. martes* L., stone marten *M. foina*, and Indian marten *M. flavigula* were tested. Some data on the mtDNA variation in sable *Martes zibellina* L., from Japan were also incuded into the analysis. Taken together, these data provided phylogenetic analysis of molecular data within subfamily Martinae (Fig. 1). Interspecific differences in this subfamily (in the form of *p* distances showing the proportion of nucleotide positions, which are different in the DNA sequences compared) varied in the range from 3.1%, between sable and common marten, to 13.3%, between sable and Indian marten (Table 2). Intraspecific sequence divergence in sable constituted 0.55%, on average. At the same time, the difference range was rather wide, from 0 to 1.5%. This finding is explained by the existence of two phylogenetic groups of sable mtDNA (Fig. 1). These groups are designated as A and

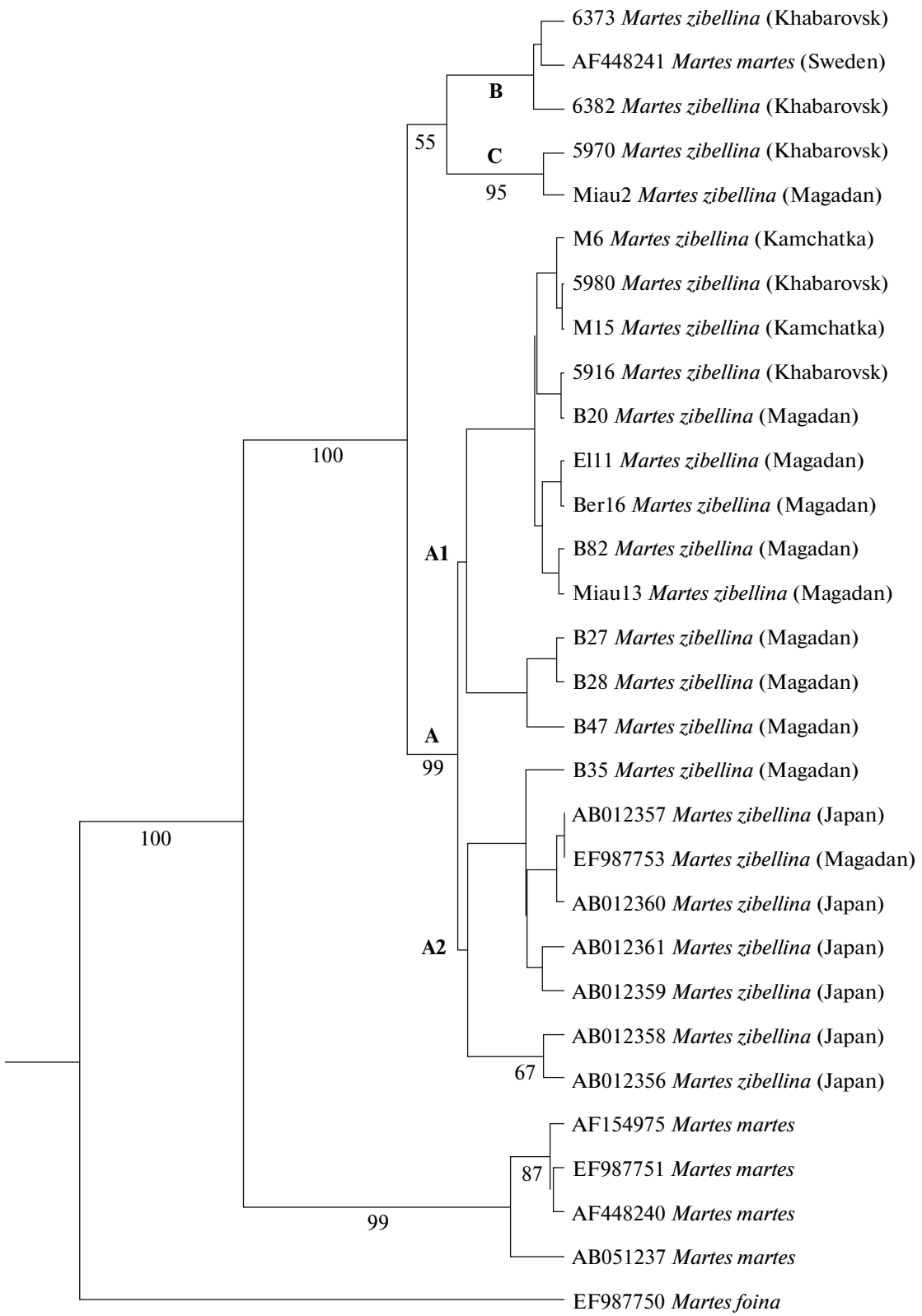

Fig. 1. Bayesian consensus phylogenetic tree based on the mtDNA cytochrome *b* gene variation data in the representatives of subfamily Martinae. The tree is rooted relative nucleotide sequence of Indian marten *M. flavigula* (GenBank accession number AB051235) (not shown). On the branches are the bootstrap values over 50%. The mtDNA clusters of sable are designated by Latin letters, A (A1, A2), B, and C. More detailed information about sable mtDNA specimens examined is demonstrated in Table 1.

BC. These designations correspond to the earlier suggested groupings of sable, which were identified based on the data on restriction fragment length polymorphism described for some mtDNA genes of sable [2–4]. Sequence divergence of the cytochrome *b* gene in cluster A constituted only 0.15% (in terms of *p* distances), while in cluster BC the value of this index was 0.42%. Cluster BC is differentiated into two subclusters, B and C. The differences between clusters A and B constituted 1.3%, and 1.5%, between clusters A and C. In cluster A, two subclusters can be also identified, however, they are weakly statistically supported (bootstrap support values below 50%). Nevertheless, in respect of phylogeography, subclusters A1 and A2 were different. This is because subcluster A2 was mostly represented by sables from Hokkaido. The exclusion was two specimens from Srednekanskii raion of Magadan oblast, B35 from the outskirts of the settlement of Balygychan, and EF987753, from the outskirts of the settlement of Seimchan. At the same time, subcluster A1 was represented by sables from the different regions of Northeast Asia, including Kamchatka, Khabarovsk krai, and Magadan oblast.

In the phylogenetic tree (Fig. 1), clusters B and C were formed by haplotypes of sables from Magadan oblast and Khabarovsk krai. Interestingly, common marten individual from Sweden, carrying sable haplotype AF448241 [6], belonged to subcluster B. Furthermore, haplotype of this individual was identical to that in Khabarovsk sable 6373. Phylogenetic tree clearly shows how far sable subcluster B was from haplotypes of common marten. These findings pointed to the appearance of sable maternal lineage in the common marten gene pool as a result of interspecific hybridization rather than paraphyletic origin of *M. martes* L.

Earlier studies on sable mtDNA variation showed that haplogroups A, B, and C occurred at different population frequencies [2, 4]. For instance, in the populations from Central Kamchatka, all sable individuals examined belonged to haplogroup A. In Khabarovsk krai, sables tested belonged to haplorgoups A (33%), B (57%), and C (10%). In the introduced populations of Magadan oblast, the animals belonged to haplogroups A (63%), B (22%), and C (15%) [4]. It was demonstrated [5, 15] that all examined *Martes zibellina* L. individuals from Hokkaido (n = 10) belonged to haplogroup A (more precisely, to A2). At the same time, high sequence divergence values between haplogroups A and BC (1.4%, on average) implies that ancestral gene pool of sable was once split into two parts, probably, as a result of cover glaciation. Later on, in the process of warming, the two gene pool parts were reunified. It seems likely that morphological differences existed between sables belonging to haplogroups A and BC still have certain contribution to contemporary phenotypic diversity of sable populations. This suggestion is supported by morphologic specificity of Kamchatka sable, which is treated as a separate subspecies *M. z. kamtschadalica*, and characterized by the presence of mitochondrial haplotypes of A1 group [4, 10]. It should be noted in this respect that A1 haplotypes are associated with haplotypes from groups B and C in the gene pool of sable inhabiting Khabarovsk krai and Magadan oblast and belonging to another subspecies, *M. z. jakutensis*.

Table 2. Interspecific differences in the marten subfamily Martinae (in % of sequence divergence of the cytochrome *b* gene)

	Martes zibellina L.	*M. martes* L.	*M. foina*
M. martes L.	3.1		
M. foina	8.5	7.4	
M. flavigula	13.3	12.6	13.0

In order to evaluate the time of split of sable mitochondrial gene pool into two groups (A and BC), median network of sable haplotypes was examined in comparison with the haplotypes of common marten (Fig. 2). The analysis showed that haplotype B was ancestral relative to haplotypes C and A. Moreover, the mean distance from hypothetical ancestral haplotype (mv2) to haplotypes of groups C and A constitutes 7.4 ± 0.6 mutations. This value roughly corresponds to the coalescence time of 1.08 ± 0.09 Myr. Analysis of the median network of 20 sequences belonging to haplogroup A showed that evolutionary age of this cluster was 123 ± 61 thousand years. It should be noted that derivative state of haplogroup C relative to more ancient haplogroup B deserves certain interest, since higher frequencies of haplogroup C, constituting 20.5%, on average (ranging from 13 to 40% in different populations), were observed in sable populations from the Kolyma area [4]. It was reported that to the beginning of 20th century, aboriginal sable

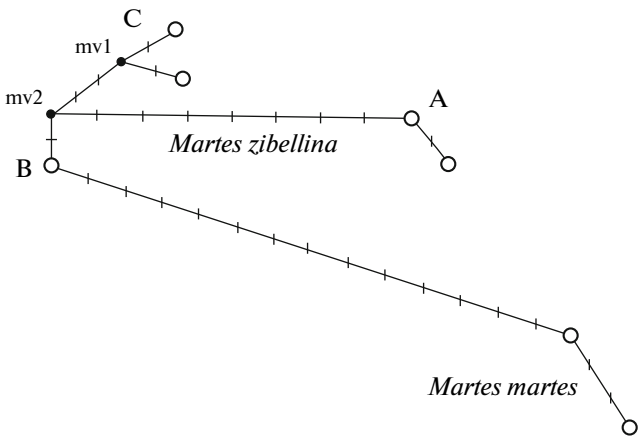

Fig. 2. Median network of the cytochrome *b* gene haplotypes for *M. zibellina* L. in comparison with common marten *M. martes* L. Shown are the phylogenetic relationships between haplotypes A, B, and C of sable and haplotypes of common marten (designated by circles); median vectors (*mv*) represent hypothetical haplotypes. On the branches are the numbers of mutations.

in these territories was almost or completely expired. According to reconstructions, Kolyma sable was larger and darker, and it was phenotypically different from Kamchatka sable, as well as from the more southern Bureinskii sable [8]. Taxonomic status of Kolyma sable is uncertain. However, it seems likely that haplogroup C could be the marker of Kolyma subspecies of sable.

In conclusion, the data obtained suggest that the split of ancestral population of sable could take place in Early Pleistocene, while expansion of its phylogenetically young group A, probably, occurred during more favorable climate conditions in Late Pleistocene (about 120 thousand years ago). Elucidation of more detailed genetic history of this species requires extension of the investigation area along with comprehensive analysis of variation in the phylogenetic groups of mtDNA.

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