BIOCHEMISTRY, BIOPHYSICS AND MOLECULAR BIOLOGY

Phylogeography of the Siberian Flying Squirrel (*Pteromys volans* L., 1785) and the History of the Formation of the Modern Species Range: New Data

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Siberian flying squirrel (*Pteromys volans* L., 1758) is the only representative of the subfamily Pteromyinae, occurring on the territory of Northern Eurasia. The habitats of this species are confined to the forest area (mainly conifers, partly the forest-steppe and forest-tundra zones). The range of this species covers the territory of Eurasia from Scandinavia, Finland, and the Baltic Sea to the Eastern Siberia and the Korean Peninsula as well as Sakhalin and Hokkaido islands [1, 2]. Although the results of phylogeographic studies using cytochrome *b* (*cyt b*) gene of mitochondrial DNA as a marker were presented in several papers [3, 4], the central part of the range—the territory of the Urals and Western Siberia—remains unstudied (Fig. 1). In addition, the history of the formation and intraspecific differentiation of *P. volans* during the dispersal of this species to the forest zone of Eurasia is not entirely clear.

In this context, the objective of this work was to determine the position of the Siberian flying squirrel in the Urals in the phylogeographic structure of the species, to compare the phylogeographic and paleontological data, and to describe the history of the formation and intraspecific differentiation of *P. volans* using the molecular clock method.

The relevance of this study is determined not only by its theoretical significance for understanding the history of the formation of the modern range of Siberian flying squirrel and the role of the Ural region in the formation of the genetic diversity of modern representatives of the order Rodentia, but also in terms of biodiversity conservation. Despite the wide distribution area, the abundance of *P. volans* declines everywhere, primarily due to the increased forest logging, as a result of which this species was given the nature-conservation status in the major part of the range. Under-

standing of the processes underlying the formation of the intraspecific genetic structure, the assessment of the genetic diversity of populations and the degree of relationship between them are directly related to the protection of rare species.

To analyze the position of the flying squirrel from the Urals in the phylogeographic structure of the species, the complete *cyt b* sequence (1140 bp) was determined for five *P. volans* individuals from three localities of the Middle Urals (Fig. 1).

In this study, we used the collection materials of the museum of the Institute of Plant and Animal Ecology, Ural Branch, Russian Academy of Sciences. DNA was extracted from muscle tissue by the salt extraction method [5]. The *cyt b* gene was amplified using primers L7 and H6 [6]. Sequencing was performed using the Big Dye Terminator Cycle Sequencing Kit V. 3.1 (Applied Biosystems, United States) following manufacturer's protocol on the ABI Prism 3130 sequencer (Applied Biosystems). 63 sequences dataset of the *P. volans cyt b* gene (five sequences were original data from the Urals and 58 sequences were retrieved from GenBank [3, 4, 7]) were used in the analysis. Two *cyt b* gene sequences of *P. momonga* were used as an outgroup.

Among the 63 analyzed sequences of the *cyt b* gene of *P. volans*, we identified 48 unique haplotypes, two of which were identified during the analysis of the original data (the flying squirrels inhabiting the Urals: acc. num. KR063240-44 in GenBank).

The results of phylogeographic analysis are consistent with the results of previous studies [3, 4]. Within the continuous range of *P. volans*, three phylogroups with a clear geographic location can be differentiated—"Hokkaido," "Far East," and "Northern Eurasia;" within the latter, the "Northwestern Eurasia" subgroup widespread in the European part of the species range and in the southeastern part of West Siberia was distinguished (Figs. 1–3). The haplotypes of the flying squirrels from the Urals belong to the "Northwestern Eurasia" subgroup of the "Northern Eurasia" group (Fig. 2). The affiliation of the samples from the

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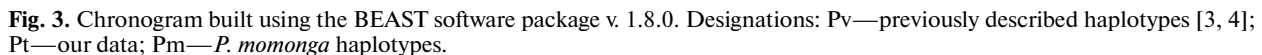*e-mail: lida@ipae.uran.ru*
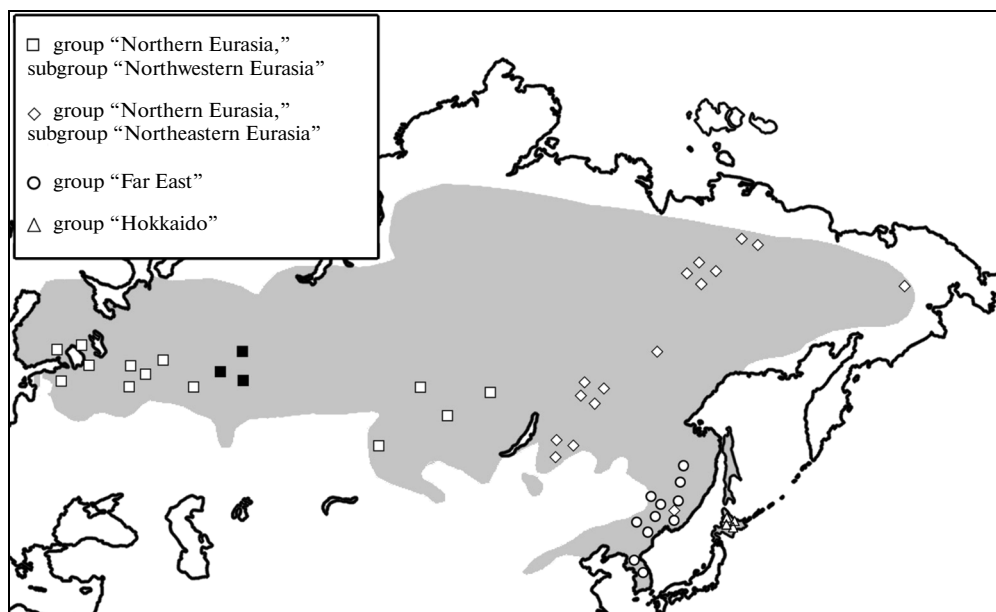
Fig. 1. Collection points of samples used in the analysis of *cyt b* gene sequences. The range of *P. volans* is shown in gray. The inset shows the affiliation of the samples to the phylogeographic groups. The filled and empty symbols designate our data and published data, respectively [3, 4].
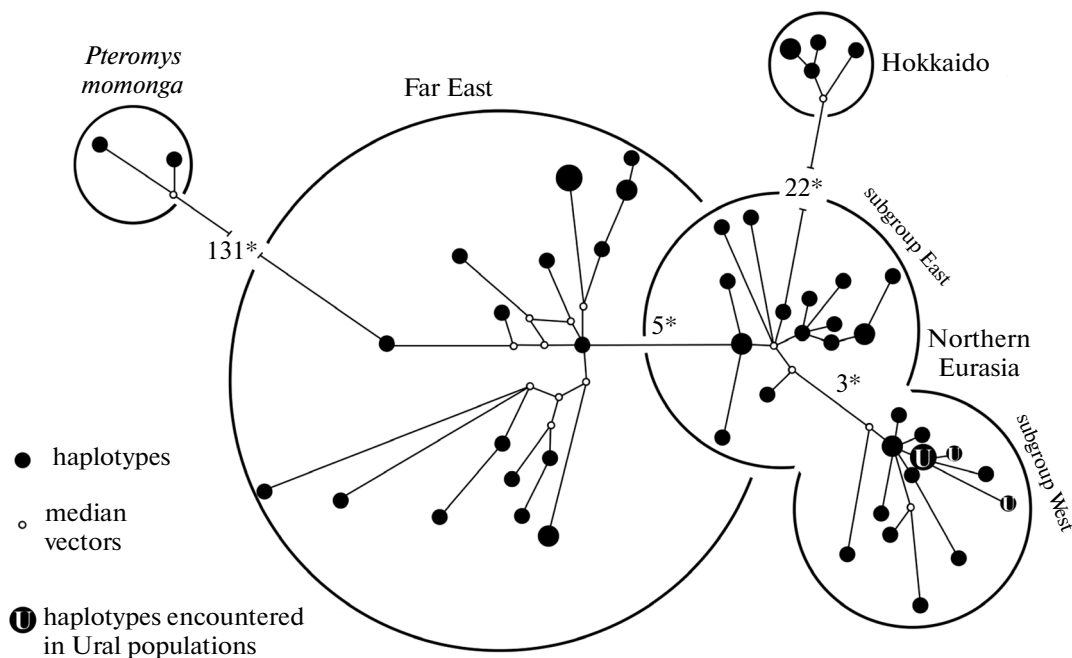
Fig. 2. Median joining network of *cyt b* gene haplotypes of *P. volans* (Network v. 4.6.1.2). The size of the black circles is proportional to the haplotype frequency, and the branches length is proportional to the mutational steps separating the hapotypes.

western and eastern slopes of the Urals to the same phylogroup confirms the conclusion that the Ural Mountains did not implement a barrier function in the postglacial period during the dispersal of arboreal *P. volans*, unlike the terrestrial rodents [3].

The greatest mean intragroup distances (0.009; $SD = 0.001$) were revealed in the group "Far East," $SD = 0.001$); this finding, given the fact that this group occupies a relatively small area (Fig. 1), indicates that it occurred long ago. The mean intragroup distance in

Fig. 3. Chronogram built using the BEAST software package v. 1.8.0. Designations: Pv—previously described haplotypes [3, 4]; Pt—our data; Pm—*P. momonga* haplotypes.

the group "Northern Eurasia" was 0.007 ($SD = 0.001$); however, for the subgroups into which it is divided the level of intragroup differences was almost twice lower than the mean value for the group (0.004 for each subgroup, $SD = 0.001$). This finding, given the large area of the territories occupied by these groups, indicates their relatively recent and rapid dispersal. According to the available published data, groups "Hokkaido" and "Northern Eurasia" diverged 0.4−0.2 and 0.14−0.07 million years ago, respectively [3].

Data on the paleontological findings of fossil *P. volans* are scarce. According to radiocarbon dating, the flying squirrel appeared in large areas of Eurasia (Belarus, the Urals, Western Siberia, and Yakutia [8−10]) in the second half of the Holocene—the period of for-mation of the modern forest−taiga zone. The flying squirrel was not found in the numerous localities of the Quaternary faunas of the mammoth biota of the Urals, the oldest of which can be attributed to Mikulino interglacial period (Eemian); i.e., it emerged more than 100 thousand years ago. In the South and Middle Urals, *P. volans* was recorded as a rare species only in the late Holocene, when the fauna acquired the typical forest−taiga characteristics.

Older records of *Pteromys* are known from the Far East (24−30 Kyr and 13.8−14.2 Kyr [11] and Cis-baikalia (33 Kyr) [12]). Here, fossil flying squirrels in the Late Pleistocene sediments were found together with the species that disappeared in these regions in the Holocene.

The contradiction of phylogeographic and paleontological data is that the age of the most ancient findings of *P. volans* in the caves of Altai, according to the dating, is 29.2−69 Kyr or more [13]. Nevertheless, the modern flying squirrels of Altai fall into one subgroup with the flying squirrels from the Urals—"Northwestern Eurasia," which is genetically homogeneous and is, in fact, one of the youngest groups.

To calculate the divergence time of the intraspecific groups of *P. volans*, we considered three calibration date ranges—the presumable time of existence of a common ancestor of all *P. volans* (mainland and island populations): 250 ± 50, 70 ± 15, and 16 ± 5 Kyr. Each of the ranges coincides with periods of low sea level in the area of the Japanese islands [14], which is usually associated with the climate cooling. To verify the obtained results, we also used the known data on the findings of fossil *P. volans* and data on the findings of *P. momonga* on the Japanese islands. [15]

The obtained results are inconsistent with the previous assumptions [3] regarding the sequence and divergence time of phylogroups. Calculations with the use of the three selected date ranges showed that only the results of the analysis including the earliest range (250 ± 50 Kyr) do not contradict the known paleontological and paleoclimatic data (Fig. 3). According to them, the divergence time of *P. volans* and *P. momonga* is approximately 450 Kyr; the first divergence time of *P. volans* to the groups "Far East" and the group comprising the later diverged "Northern Eurasia" and "Hokkaido" is approximately 240 Kyr; the time of isolation of the island group "Hokkaido" is approximately 200 Kyr; and the beginning of internal differentiation of groups "Far East" and "Northern Eurasia" dates 200−120 Kyr.

The results obtained in this study rule out the possibility of existence of the flying squirrels in the Pleistocene faunas from the Far East to the Altai but do not answer the question as to why the expansion of this species to the northern and western regions was realized only in the Holocene. For a final conclusion about the time of origin of modern *P. volans* and the history of the formation of the species range, data of molecular genetic analysis of fossil findings are required.

ACKNOWLEDGMENTS

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Translated by M. Batrukova