

The Identity of Gray Voles (Cricetidae, Rodentia) from Bolshoi Shantar Island, Sea of Okhotsk, with Gromov's Vole (*Alexandromys gromovi* Vorontsov et al. 1988)

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Abstract—The identification of gray voles from Bolshoi Shantar Island with Gromov's vole from the mainland is confirmed using molecular data. The increased diversity of East Asian voles in the “Shantar corner” of the Sea of Okhotsk might be accounted for by counter range expansions of the “southern” and “northern” vole forms in different periods of the Late Pleistocene and Holocene. Their modern marginal populations in the territories studied can therefore be regarded as being relict in character.

Keywords: East Asian voles, *Alexandromys*, *Microtus oeconomus shantaricus*, *Alexandromys gromovi*, Bolshoi Shantar Island

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INTRODUCTION

In eastern Russia, East Asian voles (genus *Alexandromys* Ognev 1914) are represented by nine species. These are *A. oeconomus* Pallas 1776 (Root vole), *A. fortis* Büchner 1889 (Reed vole), *A. maximowiczii* Schrank 1859 (Maximowicz's vole), *A. middendorffii* Poljakov 1881 (Middendorf's vole), *A. evoronensis* Kovalskaya et Sokolov 1980 (Evoron vole), *A. gromovi* Vorontsov, Boeskorov, Lyapunova et Revin 1988 (Gromov's vole), *A. sachalinensis* Vasin 1955 (Sakhalin vole), *A. mongolicus* Radde 1861 (Mongolian vole), and *A. mujanensis* Orlov et Kovalskaya 1978 (Muya vole).

Moreover, in the Uda River basin alone four species of this genus were found. Seven species from the list above were found in the same area together with neighbor territories, including Sakhalin.

In recent years East Asian voles have undergone various analyses (Frisman et al., 2009; Sheremetyeva et al., 2009; Bannikova et al., 2010; Lissovsky et al., 2010; Haring et al., 2011; Voyta et al., 2013; Frisman et al., 2016). However, voles from the Bolshoi Shantar Island were not included in the area of interests of investigators, as it was believed that this island is populated by only one subspecies of root vole, *Microtus* (= *Alexandromys*) *oeconomus shantaricus* Ognev 1929 (Ognev, 1929). At the same time, analysis of their teeth showed that, according to the paraconide com-

plex pattern at m1, these voles cannot refer to the root vole (Dokuchaev, 2014).

The other variations of gray voles that populate the mainland near the Shantar Islands include Gromov's vole, the population area of which is restricted to southeastern Yakutia and the southern part of Western Priokhotye. The similarity of the “gromovi” form to the *terra typica* and voles from the Ayan suburbs was observed by Vorontsov et al. (1988) and was later confirmed by Sheremetyeva et al. (2009). Study of gray voles from Bolshoi Shantar Island revealed their similarity to *A. gromovi* by the fur color and the occlusal surface pattern of m1. Taking into account the specificity of the geographical distribution of the compared forms, it was suggested that they were identical (Dokuchaev, 2014; Dokuchaev and Oleynikov, 2014). The goal of this study was to confirm the identity of gray voles from Bolshoi Shantar Island and Gromov's vole by methods of molecular genetics.

MATERIALS AND METHODS

The collection of small mammals on Bolshoi Shantar Island was carried out in the period from August 6, 2016, to August 9, 2016. Only one specimen of the Shantar vole was obtained because of the low population number of rodents. Pieces of its muscular tissue were taken and preserved in 96% ethanol. The

sample used in the present study is deposited (no. 133-16) in the Genetic Mammalian Tissue Collection of the Federal Scientific Center of the East Asia Terrestrial Biodiversity, Far East Branch, Russian Academy of Sciences (Vladivostok, Russia). The nucleotide sequences of the control region of mtDNA of seven species of East Asian voles were analyzed previously (Haring et al., 2011). These were *A. oconomus* (GenBank/NCBI HM135932 and HM135943), *A. fortis* (GenBank/NCBI HM135835 and HM135837), *A. maximowiczii* (GenBank/NCBI HM135863, HM135865 and HM135868), *A. middendorffii* (GenBank/NCBI HM135899), *A. sachalinensis* (GenBank/NCBI HM135900 and HM135901), *A. gromovi* (GenBank/NCBI HM135891–HM135898), and *A. mongolicus* (Southeastern Transbaikalia, nos. 283-13, 284-13, and 285-13, Genetic Mammalian Tissues Collection of Federal Scientific Center of the East Asia Terrestrial Biodiversity, Far East Branch, Russian Academy of Sciences), which were collected in different places in different years. These sequences were used to analyze their relationships with the Shantar vole.

The genetic analysis was performed in the Laboratory of Evolutionary Zoology and Genetics, Federal Scientific Center of the East Asia Terrestrial Biodiversity, Far East Branch, Russian Academy of Sciences (Vladivostok, Russia). DNA was isolated by the method of saline extraction as described in (Aljanabi and Martinez, 1997). The control region fragment was amplified by polymerase chain reaction (PCR) using the forward Pro+ (5'-ACC ATC AGC ACC CAA AGC TG-3') and reverse Phe- (5'-AAG CAT TTT CAG TGC TTT GCT T-3') primers. Amplification was carried out with the UNOII Thermoblock amplifier (Biometra, Germany) in a 25 µL reaction mixture, which included 1–2 µg of total DNA, 2.5 µL 10× buffer (Sibenzim, Russia), 1 µL of 20 mM dNTP mixture, 0.5 µL of each primer, 3 units *Taq*-polymerase (Sibenzim, Russia), and deionized water. PCR was carried out using the following program: 120 s DNA denaturation at 94°C, 40 cycles of amplification (94°C, 10 s, 52°C, 10 s and 72°C, 60 s) and 120 s chain completion at 72°C. The amplification products were sequenced in a cyclic manner using the Big Dye Terminator series 3.1 kit (Applied Biosystems, United States) with the forward and reverse primer under the following conditions: 60 s DNA denaturation at 96°C and 25 cycles of amplification (96°C for 30 s, 50°C for 10 s, and 60°C for 240 s).

The nucleotide sequences were analyzed with the ABI Prizm 3130 sequencer (Applied Biosystems, United States) in the Federal Scientific Center of the East Asia Terrestrial Biodiversity, Far East Branch, Russian Academy of Sciences (Vladivostok, Russia). The obtained sequences were edited and aligned with the BioEdit 7.0.9.0 program (Hall, 1999). All sequences were deposited in the GenBank/NCBI database, number MF345836–MF345839. The NJ phylogenetic tree was composed with the MEGA 6.0 program (Tamura et al., 2011).

RESULTS AND DISCUSSION

According to the data of molecular-phylogenetic analysis, the Shantar vole occupied a position in one cluster together with Gromov's vole, which points to a common origination of the island and continental forms (Fig. 1). Middendorff's vole and the Mongolian vole were shown to be the closest relations to the complex of the Shantar vole and Gromov's vole ("shantaricus/gromovi"), whereas root voles demonstrated the poorest relationships with all species compared (Fig. 1). These data are consistent with other authors (Bannikova et al., 2010). By the complex of morphological characteristics, such as fur color, conformational features, and loop pattern at the m1, the studied specimen of vole from Bolshoi Shantar Island also corresponded to the description of type specimens of *Alexandromys oconomus shantaricus* and *A. gromovi*. Taking into account these arguments (Dokuchaev, 2014; Dokuchaev and Oleynikov, 2014), the obtained data doubtlessly confirm the identity of the forms considered.

In considering the identity of the Shantar vole and Gromov's vole, one cannot evade the history of occurrence and development of these forms. Both the molecular and morphological data showed that Gromov's vole, together with the Mongolian vole and Middendorff's vole, should be combined into a clearly delineated group of closely related "mongolicus" species (Abramson and Lisovsky, 2012). The period of differentiation between *A. mongolicus*, *A. middendorffii*, and *A. gromovi* refers to the middle Pleistocene, i.e., 320 000–380 000 years ago (Bannikova et al., 2010). Presently, these species are well differentiated by the number and morphology of chromosomes. However, their allozyme and nucleotide similarity points to their relationships with one another (Bannikova et al., 2010; Lisovsky et al., 2010; Frisman et al., 2016).

It is scarcely possible to retrace the history of the Shantar vole from the Middle Pleistocene. Nevertheless, we can try to reconstruct the final stages of this process. Both the Middle and Late Pleistocene are known to include several glaciations, which were divided by periods of warming (*Stratigrafiya* ..., 1984). In the periods of decreasing temperature, the borders between natural zones drifted southwards, the sea level decreased, and the Shantar Islands became part of the mainland. An ancestor form of the Shantar vole could have populate this territory in one such period and, later, have become an isolated island form after the increase in the sea level. With the lapse of time, these voles could have accumulated the genetic and morphological differences that resulted in the occurrence of a new species. The formation of this species in any part of Western Priokhotye is unlikely.

Taking into account the limited distribution of voles of the "shantaricus/gromovi" complex, one may suggest that they populated the Shantar Islands only during the Kargin interglacial period. Later, these islands were united with the mainland to form the Sar-

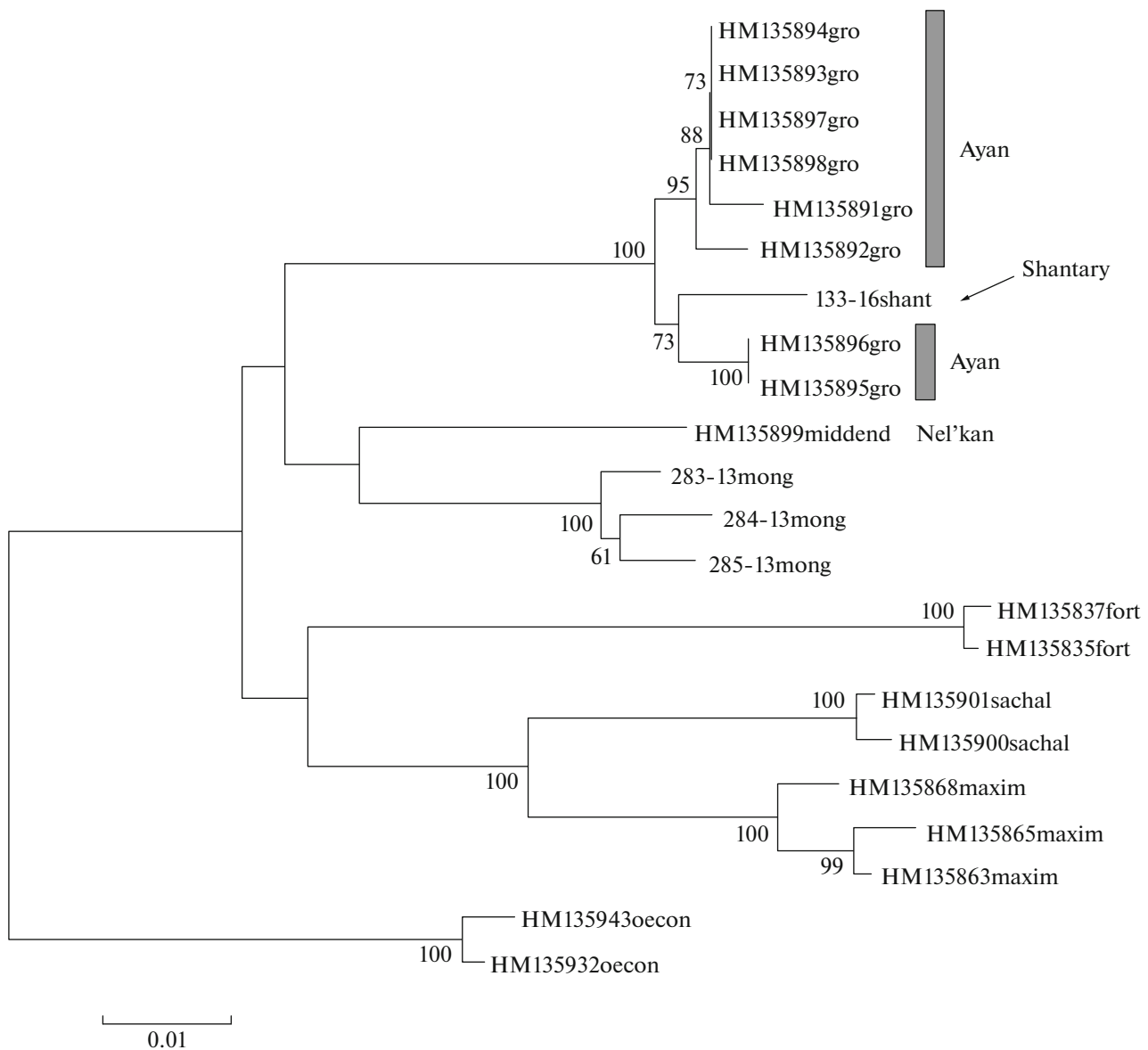


Fig. 1. The NJ phylogenetic tree of seven species of East Asian voles and the Shantar vole (indicated by an arrow) composed from the mtDNA control region sequencing data: gro, *A. gromovi*; shant, Shantar vole; middend, *A. middendorffii*; mong, *A. mongolicus*; fort, *A. fortis*; sachal, *A. sachalinensis*; maxim, *A. maximowiczii*; oecon, *A. oeconomus*.

tan glaciation, and the Shantar vole could have had the chance to populate the neighboring continental territories. The next increase in the sea level at the end of the Late Pleistocene divided the island and continental parts of the formerly common population.

It was found that seven species of voles that belong to the *Alexandromys* genus inhabit the southern parts of Western Priokhotye on a relatively small continental territory and neighboring islands (Fig. 2). In contrast to, for example, the shrews of the genus *Sorex*, such a multispecies community of representatives of one genus is not typical for rodents.

The Sea of Okhotsk coast is known to serve periodically as a kind of “ecological passage,” though which,

in favorable periods of the past, many species of plants and animals were distributed far to the North (Dokuchaev, 2012). Indeed, the Slender shrew (*Sorex gracillimus* Thomas 1907), Eurasian water shrew (*Neomys fodiens* Pennant 1771), and Korean field mouse (*Apodemus peninsulae* Thomas 1907) moved northwards to 60° N in this way.

The advancement of these species northwards occurred, most probably, during the Holocene climatic optimum. However, neither the long-clawed shrew (*Sorex unguiculatus* Dobson 1890) nor the Reed vole or Maximowicz’s vole went northwards beyond the Uda River basin. Conversely, during the periods of temperature decrease, northern forms got the oppor-

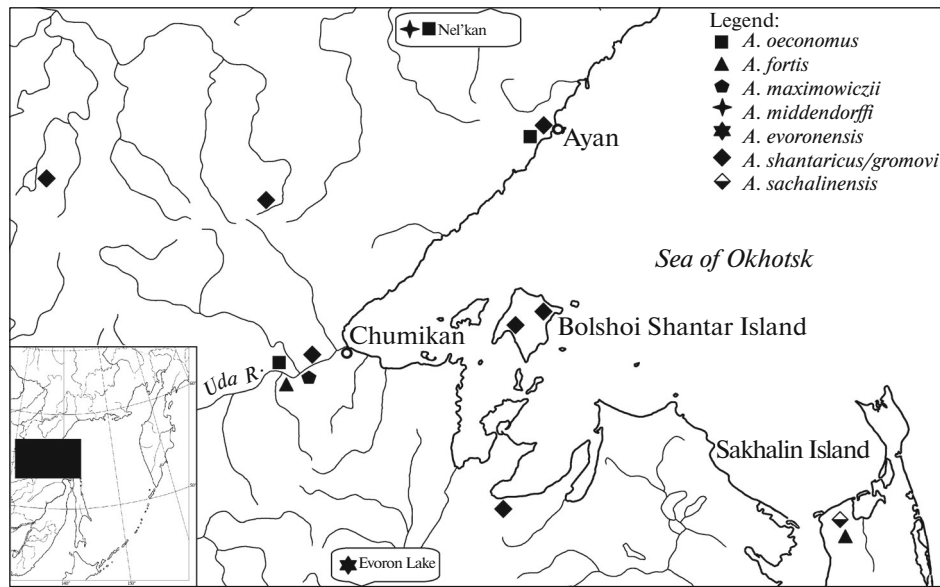


Fig. 2. East Asian vole species inhabiting the “Shantar corner” of the Sea of Okhotsk and neighboring territories. Resources: Alina and Reimers, 1975; Vorontsov et al., 1988; Voronov, 1992; Tiunov, 2003, 2003a; Tiunov et al., 2009; Sheremetyeva et al., 2010; Haring et al., 2011; Dokuchaev, 2012; Dokuchaev and Oleynikov, 2014; depositaries of the Zoological Museum of Moscow State University (Moscow, Russia), collections of N.E. Dokuchaev, 2016.

tunity to widen the southern borders of their areal. For example, Sakhalin Island is inhabited by such exotic northern representatives of quaternary fauna as the musk ox (*Ovibos moschatus* Zimmerman 1780) and Palearctic collared lemming (*Dicrostonyx torquatus* Pallas 1778) (Alekseeva and Gorbunov, 2007).

The concentration of East Asian vole species in the area of “Shantar corner” of the Sea of Okhotsk may be due to the widening of the “southern” and “northern” vole population areas in the opposite direction during the periods of the Late Pleistocene and Holocene. Therefore, their modern border-populations on the considered territory demonstrate relict characteristics. “Southern” forms include species such as *A. fortis*, *A. maximowiczii*, and *A. evoronensis*, whereas *A. oecconomus* and *A. middendorffi* belong to the “northern” group. Island forms, such as the Shantar vole and *A. sachalinensis*, which were formed under the conditions of isolation, also made their impact into the increase in biodiversity in this region. Moreover, the Shantar vole, in contrast to the Sakhalin vole, populated the neighboring continental territories as well during the periods of a decrease in the sea level.

To solve finally the question of the nomenclature of the Shantar vole and *A. gromovi*, it is necessary to study the type specimen, which was determined by Ognev (1929) as *Microtus oecconomus shantaricus* Ognev 1929, by the methods of molecular genetics.

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