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Short title
New records of A. middendorffii in Russia and Mongolia

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New records of *Alexandromys middendorffii* (Rodentia, Arvicolinae) in the Sayan Mountains in Russia and Mongolia

Abstract. New records of Middendorf's vole (*Alexandromys middendorffii*) in the Russian and Mongolian parts of the Eastern Sayan are reported. Species identification was based on genetic (nucleotide sequence of the cytochrome b) and morphological (configuration of the occlusal surfaces of the M3 and m1 molars) analyses. Middendorf's vole has persisted since the Pleistocene in a small area of the alpine tundra of the Eastern Sayan and Khamar-Daban, and possibly also in the adjacent ridges of the Altai–Sayan and Baikal mountain regions. The discovery of Middendorf's vole near Lake Khuvsgul adds a new species to the list of mammals in Mongolia and simultaneously removes this species from the list of animals endemic to the Russian Federation.

Keywords. Arvicolinae, grey voles, *Alexandromys middendorffii*, Eastern Sayan, Lake Khuvsgul.

Middendorff's vole, *Alexandromys middendorffii* Polyakov, 1881, is a species of gray vole belonging to the tribe Arvicolini, and is phylogenetically close to the Mongolian vole *A. mongolicus* Radde, 1861 and Gromov's vole *A. gromovi* Vorontsov, Boeskorov, Ljapunova and Revin, 1988 (Pavlinov and Lissovsky, 2012; Bannikova et al., 2010; Lissovsky et al., 2010). For a long time it was believed that the Middendorff's vole occurred only north of the Polar Circle (66°33' N) and this species is a typical representative of the subarctic fauna (Schwartz and Pyastolova, 1971). The range of this species covers the territory stretching from the Kanin Peninsula (between the White and Barents Seas) in the west and the Sea of Okhotsk, the Koryak Highlands and the Chukotka Peninsula in the east (Shenbrot and Krasnov, 2005; Krystufek and Shenbrot, 2022). In addition, there are isolated records of this species in the south of Eastern Siberia: Russia, Buryatia, Okinsky district (Lissovsky et al., 2017; Golenishchev et al., 2018).

There are currently three subspecies of Middendorff's vole (Shenbrot and Krasnov, 2005) separated along an east-west gradient. The geographical barriers between the subspecies are the large rivers of northern Asia (Ob, Lena). The nominative subspecies *A. m. middendorffii* Poljakov, 1881, described from the Taymyr Peninsula, inhabits Western and Central Siberia from the mouth of the Ob River and Taz Estuary in the west to Khatanga Bay in the east (Gromov and Polyakov, 1977). Along the coast of the Barents and Kara seas between the Kanin and Yamal peninsulas and the northern Ural Mountains is the range of *A. m. ryphaeus* Heptner, 1948 (Bobretsov et al., 2012). The range of *A. m. hyperboreus* Vinogradov, 1933 covers the territory of Sakha (Yakutia), the Chukotka Peninsula, and the Magadan region. Thus, until this study, Middendorff's vole was considered endemic to the Russian Federation.

We captured Middendorf's voles during a field study of the small mammal community in the tundra zone of the Eastern Sayan Mountains in the Okinsky district of the Republic of Buryatia (Russia) and Khuvsgul aimag (Mongolia). In August 2015, two voles were captured in larch woodland on the left (north) bank of the Sentsa River, and another individual was captured in mountain tundra at the top of Bural-Sardag Mt., both in Russian territory of the Eastern Sayan. In Mongolia, a single vole was captured on July, 2019 in a mixed-grass meadow near the small lake Khokh Nuur in the lower reaches of the Jargalant Gol, 1 km from its confluence with Lake Khuvsgul (Fig. 1).

The captured individuals were assigned to the Middendorf's vole based on the sequencing of the cytochrome b (cyt b) gene. Total DNA was isolated using a 5% Chelex-100 solution (Bio-Rad Laboratories, Hercules, CA, USA). The isolation protocol followed the manufacturer's recommendations for InstaGene Matrix (Bio-Rad Laboratories, Hercules, CA, USA). We used 2–5 μL of the obtained supernatant to perform PCR. Fragments of the cyt b gene were amplified using a pair of primers H15985 and L14734 (Ohdachi et al., 2001). The amplicons were purified and sequenced directly on an ABI 3130XL Genetic Analyser at the Genomics Core Facility ICBFM SB RAS (Novosibirsk, Russia). Sequences of the homologous mtDNA fragments from *A. middendorffii* from South Siberia (GenBank/NCBI #MF099536, MF099583), the Putorana Plateau (#FJ986314), Yakutia (#MF099535, FJ986316, AF163898) and the Yamal Peninsula (#MF099537, HM119493) as well as one sequence from *A. mongolicus* (#MF099538) were used for constructing the phylogenetic trees. The 1140 bp cyt b sequences of all the sampled animals (6) were deposited in GenBank under accession numbers PP816985 - PP816990. Sequence alignment by Clustal Omega (Sievers and Higgins, 2014), and phylogenetic analysis by Maximum Likelihood method was conducted in MEGA 11 (Tamura et al., 2021). The best substitution model for unpartitioned loci (cyt b: GTR+G+I) were identified in MEGA 11 according to the Akaike information criterion. Branch support was estimated with 1000 bootstrap replicates. All positions containing missing data were eliminated (complete deletion option).

The phylogenetic tree constructed based on the nucleotide sequences of the cytochrome b gene is consistent with the subspecies structure of *A. middendorffii* (Fig. 2), with the subspecies *ryphaeus*, *hyperboreus* and *middendorffii* grouped separately on the tree. In this case, the Eastern Sayan specimens also are clustered separately. Individuals from the Sentsa River and the northern shore of Lake Khuvsgul (caught by us) are united with a vole from Botogol, Okinsky district of Buryatia (GenBank #MF099536). This Eastern Sayan group is basally joined by an individual captured in 1936 by A.S. Fetisov on the Khamar-Daban ridge, originally described by him as *Microtus arvalis baicalensis* Fetisov, 1941 and already in our century subsequently reidentified as *A. middendorffii* (Lissovsky et al., 2017).

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The studied voles correspond to the description for Middendorff's vole (Ognev, 1950) in terms of pelage color and linear measurements of the body and skull. The dorsal part of the body is brownish-reddish, the ventral part is ash-gray. The tail is bicolored; its length does not exceed ¼ of the body length.

In the taxonomy of gray voles, great importance is attached to studying the variability in the pattern of the occlusal surface of M3 (third upper) and m1 (first lower) molars. All the studied voles are characterized by more elaborate shape of M3, which is typical of Middendorff's voles in other parts of the range (Litvinov and Pozdnyakov, 1993; Meyer et al., 1996; Pokrovsky and Bolshakov, 1979). According to the classification of the M3 structure proposed for *Microtus arvalis* (Rörig and Börner, 1905), the M3 morphotypes of voles from the Sentsa River (Fig. 3A: *a*, *b*) and Lake Khuvsgul (Fig. 3A: *d*) territories correspond to the *duplicata* structure type, and the specimen from Bural-Sardag Mt. (Fig. 3A: *c*) corresponds to *variabilis*. Moreover, bilateral asymmetry of morphotypes was noted in the latter case. The shape of the m1 occlusal surface in the Middendorff's vole is usually less variable compared to that of M3 (Meyer et al., 1996) and has the species specific structure found in all the studied individuals (Fig. 3A).

Previously, it was believed that the Middendorf's vole was distributed exclusively in the forest-tundra zone of the high latitudes of Eurasia, but recent studies have shown a wider distribution of this species in the mountain systems of North Asia (Lissovsky et al., 2017; Krystufek and Shenbrot, 2022). The results of our research, based on the study of skins, the skull structure, dental system and the nucleotide sequence of the cytochrome b gene, showed that the Middendorff's vole lives in the southern part of the Eastern Sayan (both on the Russian and Mongolian territory), inhabiting high-mountain steppe and tundra biotopes.

The alpine tundra of the Altai and Sayan regions is the remnant of a vast territory of "mammoth steppes", which were widespread in the Pleistocene in Eurasia and North America. Mammoth steppes occupied vast areas of the northern part of the globe for about one hundred thousand years, and about 12 kya there was significant reduction in area (Guthrie, 2001), along with extinction or significant reduction and fragmentation of the ranges of numerous species adapted to cold, and arid conditions (Pavelkova Ricankova et al., 2018).

Natural ecosystems similar to the last ice age are found in modern times in the Altai–Sayan mountainous region in Central Asia, where there were no significant changes between the cold phase of the Pleistocene and the Holocene (Pavelkova Ricankova et al., 2014). Reconstruction of the paleobiome (Tarasov et al., 2000) and pollen analysis (Pelankova, Chytru, 2009) suggested that the mountain steppes and tundras of Altai and Sayan are an analogue of the

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Pleistocene tundra-steppes. Most species of the Late Pleistocene biota of the Palaearctic became extinct but were preserved in these mountain systems (Chytry et al., 2019).

Middendorff's vole under the species name *Alexandromys hyperboreus* (North Siberian vole) is found in Pleistocene deposits of Altai, 238-38 kya (Agadzhanyan, Serdyuk, 2005; Agadzhanyan et al., 2021). In the Baikal region, remains of this species were found in sediments from the Last Glacial Period, 36-31 kya (Khenzykhenova et al., 2016). In the late Pleistocene, in the mammal fauna of the Baikal region and northern Mongolia, tundra species, including the Arctic lemming (*Dicrostonyx torquatus*) and Middendorff's vole, became extinct because of climate warming (Dupal et al., 2013; Khenzykhenova et al., 2021).

Studies of the modern fauna of small mammals inhabiting the Eastern Sayan and Khuvsgul regions showed that the Mongolian vole *Alexandromys mongolicus* lives in this region (Clark et al., 2006; Batsaikhan et al., 2022); meanwhile, the morphological and ecological uniqueness of the "Mongolian" voles from this territory was always noted. Publications devoted to the fauna and population of small mammals of the Khuvsgul region indicate that the "Mongolian" vole reaches high numbers not only in the mountain-steppe and mountain-forest communities of Munku-Sardyk mountain, but it also penetrates into the alpine tundra (1900–2300 m above sea level), where it occupies a subdominant position in numbers after the large-eared vole *Alticola macrotis* (Litvinov and Bazardorzh, 1992; Danilov and Prelovskiy, 2021; Litvinov et al., 2021). Litvinov and Bazardorzh (1992) reported the discovery of a "Mongolian" vole in 1987 in the southern coast of Lake Khuvsgul, in the valley of the Ongolog Gol River.

Taking into account the modern views on the range of *A. mongolicus*, as well as the high similarity of the morphometric, craniological and biotopic characteristics of *A. mongolicus* and *A. middendorffii*, some of the voles listed from the Khuvsgul region from the literature in the previous paragraph may belong to *A. middendorffii*. Of course, further specimen-based research in this region is necessary to fully resolve the distribution of this species in northern Mongolia.

In addition to the Eastern Sayan, there is a known discovery of a Middendorff's vole on the Khamar-Daban ridge (Southern Baikal region), confirmed by genetic analysis. This vole was described by A.S. Fetisov as a subspecies of the common vole *Microtus arvalis baicalensis* (Fetisov, 1941), but seventy years later it was identified as *Alexandromys middendorffii* (Lissovsky et al., 2017). The place where the specimen was captured and included in genetic analysis was "Russia, Buryatia, Dzhidinskiy District, Snezhnaya River." The distance from this point on the Khamar-Daban ridge to the new records of this species in the Eastern Sayan is about 300 km (as the crow flies). However, the same work (Fetisov, 1941) also mentioned the place where voles of the same form were captured in 1936 in the area of the Munku-Sardyk mountain range, in the vicinity of the village of Mondy, Tunkinsky district of Buryatia. Unfortunately, the type series, stored in the museum of Irkutsk State University (Irkutsk, Russia), has been lost, which prevents verification of the taxonomic affiliation of those specimens. We can only hypothesize that A.S. Fetisov also may have caught Middendorf's vole in the vicinity of the Mondy, mistakenly identifying it as *M. arvalis baicalensis* Fetisov, 1941.

Hence, our data confirm *A. middendorffii* wider distribution than previously recognized, which is not confined to the high latitudes of Eurasia, but is fragmented at its southern extent. Middendorf's vole has been preserved since Pleistocene in a small area of the mountain tundra of the Eastern Sayan and Khamar-Daban, and possibly also in the adjacent ridges of the Altai-Sayan and Baikal regions. Together with gradual warming and humidification of the climate as well as northward retreat of the "mammoth steppe", this species probably has not become extinct throughout the Palaearctic, like many other representatives of the Pleistocene tundra-steppe fauna, but survived in the unique refugia of the mountains of Southern Siberia.

Our discovery of the Middendorff's vole at Lake Khuvsgul adds a new species to the list of mammals in Mongolia and simultaneously removes Middendorff's vole from the list of animal species endemic to the territory of the Russian Federation.

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Figure 1. Map of Middendorff's vole records in the Eastern Sayan. Circles indicate our data, and squares indicate literature data: (1) Sentsa River; (2) Bural-Sardag Mt.; (3) Khuvsgul Lake; (4) Botogol settlement (Golenishchev et al., 2018); and (5) Khamar-Daban Mt. range (Lissovsky et al., 2017). The main range of the species is shown by red diagonal stripes. Photos of the biotopes where specimens were captured are provided on the right side of the figure.

Figure 2. Phylogenetic tree of *A. middendorffii* based on the nucleotide sequences of the mitochondrial cyt b (1140 bp) using maximum likelihood with GRT + G + I evolution model. Support indices were calculated for 1000 repetitions; support indices (>70%) are displayed in the respective nodes. The scale bar indicates the number of nucleotide substitutions per site. New isolates are shown in bold.

Figure 3. Configuration of the occlusal surfaces of the M3 and m1 molars of the studied Middendorff's voles. **(A):** (a, b) Sentsa River, (c) Bural-Sardag Mt., (d) Lake Khuvsgul; **(B):** skin of the individual (№62561) from Bural-Sardag Mt. from the collection of the Zoological Museum of the Institute of Systematics and Ecology of Animals SB RAS, Novosibirsk, Russia.

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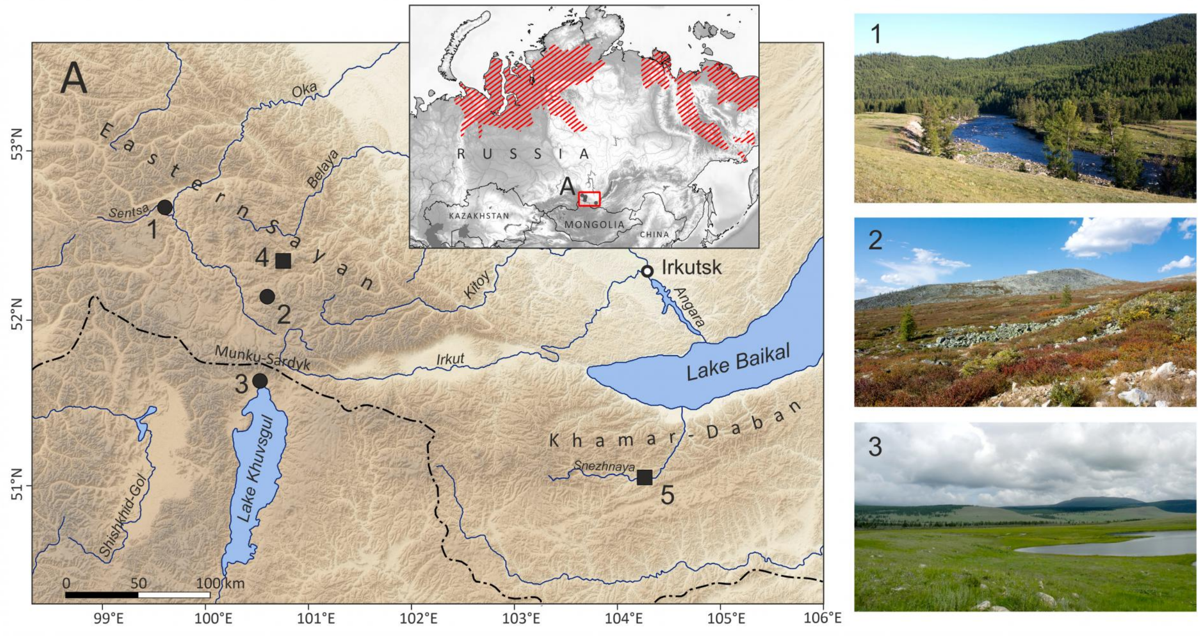

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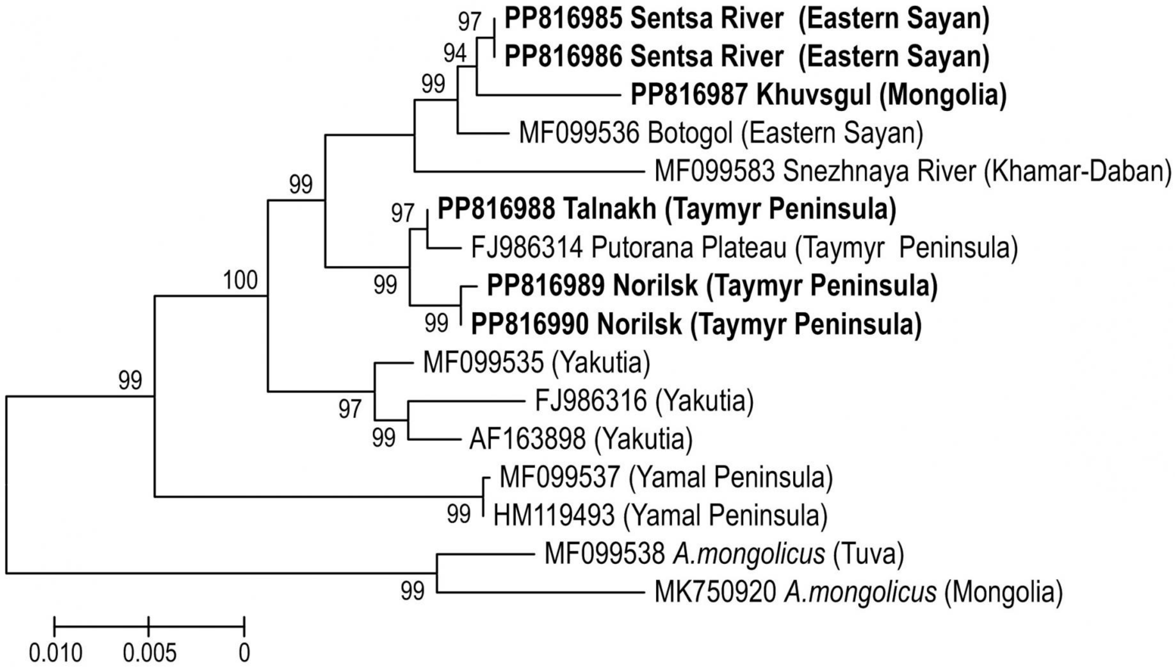

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Figure 3

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