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КРАТКИЕ СООБЩЕНИЯ

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A MOLECULAR STUDY OF THE HOLOTYPE OF *MICROTUS OECONOMUS SHANTARICUS* OGNEV 1929 (RODENTIA, CRICETIDAE)

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The Shantar vole was described by Ognev (1929) as a subspecies of the root vole – *Microtus oeconomus shantaricus* Ognev 1929. It remained in this status for more than 80 years. However, the examination of the m1 paraconid of voles from the Bolshoy Shantar island including the type demonstrated that they can not be attributed to *M. oeconomus* (Dokuchaev, 2014). In the last decade it became clear that the vole first described as *M. maximowiczii gromovi* Vorontsov, Boeskorov, Lyapunova et Revin 1988 (Vorontsov et al., 1988) is a separate species *Microtus* (*Alexandromys) gromovi*, which is related more closely to *M. middendorffii* and *M. mongolicus* than to *M. maximowiczii* (Bannikova et al., 2010; Harring et al., 2011). The range of this species includes SE Yakutia a part of Dzhugdzhur mountain ridge and the Uda river basin. Based on similarity in pelage coloration, external traits and m1 pattern it was hypothesized that the Shantar vole and *M. gromovi* can be conspecific (Dokuchaev, 2014; Dokuchaev, Oleynikov, 2014). This supposition was supported by molecular data, which demonstrated that the mtDNA of *shantaricus* and *gromovi* are highly similar, thus suggesting the identity of the two taxa (Dokuchaev, Sheremetyeva, 2018. In this case, the valid species name should be *M. shantaricus* Ognev 1929. However, to validate this conclusion it is necessary to examine the molecular data on the holotype of *Microtus oeconomus shantaricus*.

MATERIALS AND METHODS

The holotype of *M. o. shantaricus* (dry skin and skull) is stored in the collection of Zoological Museum of Moscow State University (ZMMU, collection number S-31137, collected by Dulkeit G., 23.05.1925; Bolshoy Shantar island). DNA was extracted from dry tissue (small fragment of skin <1 mm^2) and purified using the QIAamp DNA MiniKit (Qiagen) including an overnight lysis step at 56°C and longer incubation with EB-buffer (5 min) at the purification step.

We amplified a portion of the mitochondrial cyt*b* (cytochrome *b*) gene. DNA was highly degraded, so only short fragments (100–200 bp) could be obtained. Three overlapping fragments were amplified using the combination of internal primers designed for this study:

PCR was set up in a total volume of 20 μL that contained 4.0 μL of ready to use 5X screen-mix HS, 1.0 μL each of 10 μM primers, and 2 μL of total genomic DNA. The PCR programme for amplification of short fragments included an initial denaturation at 95°C for 3 min, 45 cycles of 95°C for 30 s, annealing for 30 s and 72°C for 30 s, and a final extension of 72°C for 6 min. The annealing temperatures are given in Table 2. All stages of the extraction process included a negative control run in parallel. To avoid contamination, extraction and amplification of the DNA from the museum specimens were carried out in the ZMMU Laboratory of Historical DNA, exclusively equipped for work with museum DNA specimens, where no previous work on fresh tissues had been performed. All sequences were assembled in SeqMan (DNASTAR Inc., Madison, WI, USA). The total length of the obtained fragment of cyt*b* of the holotype is 392 bp.

In addition, we obtained the complete cyt*b* sequences of five newly collected specimens of the Shantar vole, which were trapped in 2016–2017 in the eastern part of the Bolshoy Shantar island (55°3′17.52″ N, 138°2′56.46″ E). All specimens were deposited in the collection of the Zoological Institute RAN, Saint-Petersburg (ZIN 105921, 105925, 105929, 105933, 105934). Amplification and sequencing was performed following Bannikova et al. (2010) with the use of the primer pair Lvole/Harvic. All procedures with the fresh tissues were conducted in a separate laboratory after the sequencing of the holotype had been com-

Table 1. Primers used in the study

L358a	5'-CTATTCGCCGTGATAGCCACAGCAT
H506a	5'-CGTGTGAGGGTGGCTTTGTCTACTG
L407a	5'-GCCAAATATCATTCTGAGGAGCCAC
H629a	5'-GGGATTTTGTCTGTGTCTGAGTTTAGTC
L603a	5'-CGAAACAGGATCCAACAACCCAAC
H775a	5'-GGTGGGGTGTTGAGTGGGTTTGC

Table 2. Annealing temperatures and amplicon lengths

Primer pair	Annealing temperature, °C	Amplicon length, bp
L358/H506	56	173
L407/H629	55	250
L603/H775	55	195

pleted. The new sequences were submitted to Genbank (Accession numbers: MT990635–MT990640).

For comparison, thirty one sequences of *Microtus* (*Alexandromys* clade) were downloaded from Genbank (see Appendix) including five sequences attributed to *M. gromovi*. The latter specimens were collected in the lower Uda river and Dzhugdzur mountains. *M. oeconomus, M. mongolicus*, *M. alpinus* and *M. middendorffii* sensu lato were used as outgroups.

Following Bannikova et al. (2019), we treat *Alexandromys* not as a full genus but as a subgenus of *Microtus* based on relatively young (Pleistocene) ages of splits among the major lineages of grey voles (Bannikova et al., 2010).

To reconstruct the relationships among haplotypes the ML tree was generated in IQTREE version 1.6 (Nguen et al., 2015) using the set of models for the three codon positions inferred in Bannikova et al. (2019).

RESULTS AND DISCUSSION

The partial sequence of the holotype is identical to the haplotype found in four out of five specimens newly collected from the Bolshoy Shantar island. Thus, it is confirmed that *Microtus shantaricus* Ognev 1929 is the valid name for the species known previously as *Microtus gromovi*, which agrees with the results by Dokuchaev and Sheremetyeva (2018). The phylogenetic tree (Fig. 1) shows that sequences from the insular and mainland populations are placed into two moderately supported clades with the median p-distance of ~0.8%

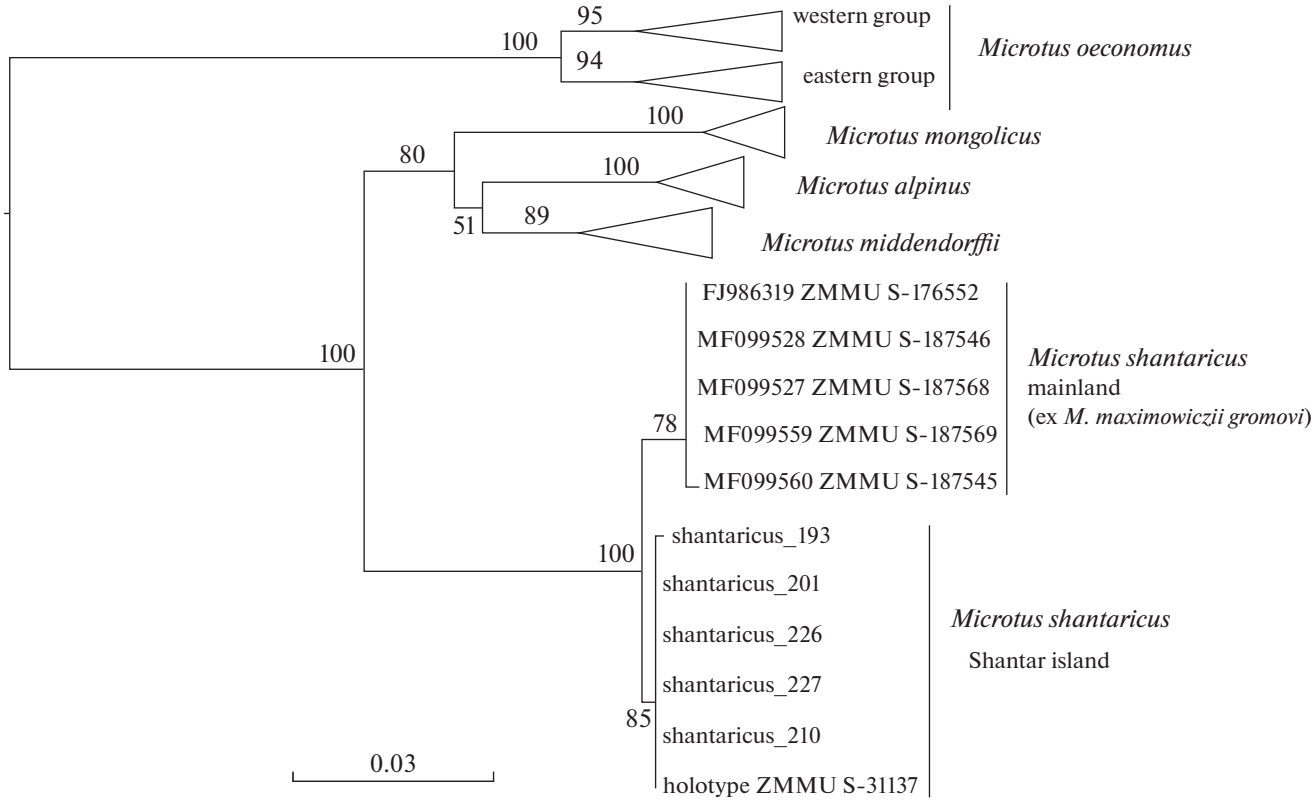

Fig. 1. The Maximum Likelihood tree inferred in IQTREE based on 41 cyt*b* sequences of *Microtus* (*Alexandromys* clade). Numbers above or below branches correspond to fast bootstrap support values estimated from 10000 replicates.

(nine substitutions per the complete gene). If we accept the cyt*b* substitution rate of 30% per Mya as estimated for population level events by Bannikova et al. (2010) then the two haplogroups could have separated in the second half of the Late Pleistocene around 25 Kya, the approximate 95% CI being 14–50 Kya. This timing is consistent with the fact that the Shantars were connected with the mainland during the regression of the Last Glacial (Ganeshin, 1956; Velizhanin, 1976), thus providing an opportunity for colonization. It should be noted that applying higher substitution rate of 40–45% per My as estimated for *Microtus agrestis* (Herman et al., 2014) would also produce the result compatible with the LGM divergence.

The location of the center of origin of the species and, hence, the direction of dispersal can not be established with certainty. It can be hypothesized that the primary distribution of *M. shantaricus* was coastal and that, during one of the interglacials, presumably in MIS 3 (Karga interstadial), when the Shantar islands were separated from the mainland by the transgression of the Sea of Okhotsk, the ancestral population became isolated in the Shantars. In the LGM time, the land bridge re-emerged and the insular relict population re-colonized the mainland. In this case, the relatively narrow species range and low level of population variation may be explained by the recent range expansion from the island refuge. To verify this scenario more phylogeographic data on mainland populations are needed.

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APPENDIX

Sequences downloaded from Genbank.

Microtus oeconomus: AY219999, AY220006, AY305099, AY305202;

Microtus middendorffii: AF163898, FJ986314, FJ986315, FJ986316, HM119493;

Microtus mongolicus: FJ986304, FJ986305, FJ986309, FJ986310, MF099539, MF099540, MF099562, MF099563, MF099565, MK750922, MK750923, MK750924, MK750925, MK750926, MK750927;

Microtus alpinus: MF099538, MK750917, MK750918, MK750919, MK750920, MK750921;

Microtus shantaricus: FJ986319, MF099527, MF099528, MF099559, MF099560.

МОЛЕКУЛЯРНОЕ ИССЛЕДОВАНИЕ ГОЛОТИПА *MICROTUS OECONOMUS SHANTARICUS* OGNEV 1929 (RODENTIA, CRICETIDAE)

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В результате секвенирования фрагмента гена цитохрома *b* голотипа *Microtus oeconomus shantaricus* подтверждено, что название *Microtus gromovi* должно быть заменено на *Microtus shantaricus*.

Ключевые слова: цитохром *b*, Дальний Восток России, молекулярные часы, последний ледниковый период