The first complete mitochondrial genomes of snow sheep (Ovis nivicola) and thinhorn sheep (Ovis dalli) and their phylogenetic implications for the genus Ovis

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The genus Ovis is divided into two subgenus – Ovis, which includes argali (O. ammon), urial (O. vignei), Asiatic mouflon (O. orientalis), European mouflon (O. orientalis musimon), Cyprus mouflon (O. orientalis ophion), and domestic sheep (O. aries), and Pachyceros, which includes snow sheep (O. nivicola), thinhorn (O. dalli), and bighorn sheep (O. canadensis). To date, complete mitogenomes of the Pachyceros sheep were represented only by two sequences of O. canadensis. Here, we present the first complete mitochondrial genomes of O. nivicola and O. dalli. Both sequences were deposited in GenBank under accession numbers: MH779626 and MH779627, respectively.

For this study, a specimen of O. nivicola was collected from the Chersky Range, Russia (64°24'35"N; 143°17'08"E) and a specimen of O. dalli was collected from the Alaska Range, USA (63°48'6"N; 147°33'24"W). The samples were deposited in the biobank at the L.K. Ernst Federal Science Center for Animal Husbandry, Russia (Biobank State Registration No. 498808). Genomic DNA was extracted from muscle tissue using a Nexttec column (Nexttec Biotechnology GmbH, Germany). For next-generation sequencing of mtDNA, three primer pairs were placed in well-conserved regions and three overlapping (overlap > 290 bp) PCR products were produced. These products (6.5, 5.7, and 6.7 kb) were separately amplified, purified, and used for the preparation of sequencing libraries, which were then sequenced by 100-bp paired-end procedure on a HiSeq 1500 (Illumina). The hypervariable part of the control region was sequenced by Sanger technology using primers designed according to the flanking sequences assembled from the NGS data. Mitos WebServer (Bernt et al. 2013) was used to annotate the mitochondrial genomes. The number of tandem repeats was estimated with the Tandem Repeats Finder 4.09 (Benson 1999). The DNA sequences were aligned using the MUSCLE algorithm (Edgar 2004), as implemented in MEGA 7.0.26 (Kumar et al. 2016). PartitionFinder 2 (Lanfear et al. 2017) was used to select the best partitioning scheme and evolutionary models.

The whole mitochondrial genomes of O. nivicola and O. dalli are 16,471 bp and 16,464 bp in size, respectively. The gene arrangement pattern in both species is similar to the typical vertebrate mitochondrial genome, containing 13 protein-coding genes (PCGs), 22 tRNA genes, 2 rRNA genes, and a non-coding region. We found two 75 bp tandem repeats in the control regions of the newly assembled mitogenomes, which shows that all species of the Pachyceros subgenus are characterized by the same number of tandem repeats.

Phylogenetic analysis (Figure 1) using the Bayesian inference method in MrBayes 3.2.6 (Ronquist et al. 2012) was performed on the concatenated datasets of 13 PCGs and 2 rRNAs of the newly sequenced mitogenomes, five other Ovis species downloaded from GenBank (Hiendleder et al. 1998; Miller et al. 2012; Lv et al. 2015; Sanna et al. 2015; Gan 2016)
and the barbary sheep (Ammotragus lervia) sequence (Hassanin et al. 2009) taken as an outgroup. It was shown that O. nivicola and O. dalli clustered in the Pachyceros clade. Our results are consistent with the previous morphological classification.

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