Complete mitochondrial genome of *Stichaeus nozawae* Jordan & Snyder 1902 (Zoarcales: Stichaeidae)

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**ABSTRACT**

We report on the structure and composition of the first complete mitochondrial genome of *Stichaeus nozawae* (typical prickleback fish (Zoarcales, Stichaeidae)), obtained by dideoxy (Sanger) sequencing technique. Genome has a classic organization with 13 genes, 2 rRNAs, control region, and 22 tRNAs, including additional tRNAs with atypical codons previously found in many eelpouts. Phylogenetic analysis confirms the species identity of *S. nozawae* and validates its recent divergence from *S. grigorjewi* with unusually low interspecific genetic distance.

Fishes of the genus *Stichaeus* are known to maintain the type characters of the pricklebacks (Zoarcales: Stichaeidae) which taxonomy is the issue of concern (Mecklenburg and Sheiko 2004; Pitruk et al. 2011; Parin et al. 2014; Radchenko 2015; Moreva et al. 2016). Two of the species, *S. grigorjewi* (SG) and *S. nozawae* (SN), distributed along the Sea of Japan and southern part of the Sea of Okhotsk (Mecklenburg and Sheiko 2004; Parin et al. 2014) have sharp discordance between morphological and molecular genetic species attributes. While SG and SN notably differ in external morphology and ecological features (Kolpakov and Klimkin 2004; Pitruk et al. 2011), their molecular genetic data show a little interspecific divergence which is comparable with the intraspecific variation of eelpouts and other teleost fishes (Ward 2009; Kwon and Kim 2013; Moreva et al. 2016; Turanov et al. 2016). This may indicate recent splitting of two lineages, where incipient species have not accumulated enough nucleotide substitutions to delineate (e.g. Shedko 2017). On the other hand, the mitochondrial control region (CR) sequences of eelpouts demonstrate the lack of saturation compared to protein-coding genes (Turanov, Lee, et al. 2019) and, therefore, it might express the natural patterns of evolutionary constrains of mitochondrial genome (mitogenome). To approach this problem from the genomic point of view, we obtained the first sequence of mitogenome for SN and compared with the mitogenome of SG (Turanov, Rutenko, et al. 2019).

Total DNA was isolated from the white muscle tissue of the specimen (MIMB 36617, female) collected in Vostok Bay of the Sea of Japan (42.89° N, 132.73° E) by gill nets in 2 May 2017. The mitogenome was obtained by dideoxy (Sanger) sequencing technique based on the set of newly designed primers with amplification of 27 overlapping regions. Primers were designed by Sliding Window-Based PSO Algorithm (Yang et al. 2011) on the basis of the mitogenome sequences available in GenBank for eelpouts. The resulted chromatograms were assembled into mitochondrial genome fragments using Geneious Trial (Kearse et al. 2012). The produced consensus sequence of complete genome was annotated using MitoAnnotator (Iwasaki et al. 2013) and MITOS Web Server (Bernt et al. 2013). To analyze the genetic polymorphism and divergence between *S. grigorjewi* and *S. nozawae* sequences, we used DnaSP v5 (Librado and Rozas 2009) and MEGA 7.0 (Kumar et al. 2016). The details of molecular phylogenetic analysis implemented to confirm the taxonomic identity and relative position of the obtained genome can be found in the previous mito communication (Turanov, Rutenko, et al. 2019).

The mitogenome of *S. nozawae* (MK561854) is 16,533 bp long, with following nucleotide base composition: T (27.5%), C (28.1%), A (26.6%), G (17.8%). Genome annotation revealed 13 coding fragments with most of the genes residing on the heavy strand while ND6 as well as eight tRNAs were encoded on the light-strand. It contained 22 tRNAs along with additional tRNA-Leu and tRNA-Ser, both having atypical codons as it was also found in many eelpouts (see Ayala et al. 2017; Rutenko et al. 2019). The annotation also revealed 16S and 12S rRNAs and CR, 862 pb in length. Uncorrected genetic distance between SG and SN was 0.4%, while between species of *Anarhichas* ranged from 1.6 to 2%, interspecific distances among *Lycodes* were 7.2–11%, and species divergence within *Pholis* was 5.2%. There were...
Figure 1. ML-phylogenetic tree showing the relationships among zoarcoid fishes built on complete sequences of mitogenome. Numbers in nodes indicate the values of support based on 1000 replicates of non-parametric bootstrap test in order ML/NJ.

71 variable sites observed and 3 indels (residing in CR) between SG and SN. Most of the nucleotide substitutions between them were distributed along ND1 (13), ND4 (9), Cyt-b (8), ND2 (8), ATPase 6 (5), ND6 (6) and COI (4). Molecular phylogeny (Figure 1) on the basis of 14 eelpouts species together with *H. dybowskii* (outgroup) demonstrates the closest position of SG and SN and the topological consistence with previous results (Rutenko et al. 2019; Turanov, Rutenko, et al. 2019). Thus, mitochondrial genomic data confirm the recent speciation between SG and SN and stimulate further research on the mechanisms of their origin.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

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