



The complete mitochondrial genome of the millipede *Epanerchodus koreanus* Verhoeff, 1937 collected in limestone cave of Korea (Polydesmidae: Polydesmida)

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ABSTRACT

We have determined the second mitochondrial genome of *Epanerchodus koreanus* Verhoeff, 1937 collected in limestone cave of Korea. The circular mitochondrial genome of *E. koreanus* is 15,581 bp long. It includes 13 protein-coding genes, two ribosomal RNA genes, and 22 transfer RNA genes. Its gene order was different from the rest three Polydesmida mitochondrial genomes, resulted from relocation of tRNAs, rRNAs, and *ND1*. The base composition was AT-biased (75.1%). Phylogenetic trees displayed phylogenetic relationship, which is congruent to previous study, except Sphaerotheriidae sp. clustering with Helminthomorpha.

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The genus *Epanerchodus* Attems, 1901 is comprising more than 70 species in the eastern Palearctic region (Golovatch 2014). Total eight millipedes belonging to the genus *Epanerchodus* Attems, 1901 have been reported in Korea (Mikhailjova and Lim 2006). *Epanerchodus koreanus* Verhoeff, 1937 (Polydesmidae: Polydesmida) is presented in both Korea and Asian part of Russia (Nguyen et al. 2016). In ecological aspects, *E. koreanus* is detritivores, and the most typical habitats of *E. koreanus* are leaf litter, the litter/soil, and around the organic matter at both entrance and twilight zone in a cave (Golovatch and Kime 2009; Golovatch 2015; Choi et al. 2016).

We completed the first mitochondrial genome of *E. koreanus*, collected in limestone cave, Ssang Cave, located in Mitan-myeon, Pyeongchang-gun, Gangwon-do, Republic of Korea (37°18'25.1"N, 128°31'58.6"E; its DNA were deposited at InfoBoss Cyber Herbarium (IN), IBS-00016). DNA was extracted using DNeasy Blood & Tissue Kit (QIAGEN, Hilden, Germany). Raw sequences from Illumina NovaSeq6000 (Macrogen, Korea) were filtered by Trimmomatic v0.33 (Bolger et al. 2014) and *de novo* assembled by Velvet v1.2.10 (Zerbino and Birney 2008), SOAPGapCloser v1.12 (Zhao et al. 2011), BWA v0.7.17 (Li 2013), and SAMtools v1.9 (Li et al. 2009) under the environment of Genome Information System (GeIS; <http://geis.infoboss.co.kr/>). Geneious R11 v11.1.5 (Biomatters Ltd, Auckland, New Zealand) was used to annotate based on three mitochondrial genomes of *Appalachioria falcifera* (NC_021933; Brewer et al. 2013), *Asiomorpha coarctata* (KU721885; Dong et al. 2016), and *Xystodesmus* sp. (KU721886; Dong et al. 2016).

Epanerchodus koreanus mitochondrial genome (GenBank accession is MT898420) was 15,581 bp long. It contained 13 protein-coding genes (PCGs), 37 tRNAs, and two rRNAs and its AT ratio was 75.1%. Gene order of *E. koreanus* mitogenome was different from those of the three Polydesmida mitochondrial genomes, resulted from both tRNA relocations, commonly found in Myriapoda subphylum (Brewer et al. 2013), and rearrangement of 16S rRNA, *ND4L*, and 12S rRNA.

We inferred the phylogenetic relationship of eleven Diplopoda mitochondrial genomes including *E. koreanus* mitochondrial genome and one outgroup species, *Scutigera caudata* (NC_008453) based on concatenated alignments of 13 conserved PCGs by MAFFT v7.450 (Katoh and Standley 2013). Bootstrapped Bayesian inference, maximum-likelihood, and neighbor-joining phylogenetic trees were constructed with MrBayes v3.2.7a (Huelsenbeck and Ronquist 2001) and MEGA X (Kumar et al. 2018). Phylogenetic trees displayed that *E. koreanus* was clustered in Polydesmida clade with high supportive values (Figure 1). In addition, they also presented phylogenetic relationship which was overall congruent to the previous studies (Sierwald and Bond 2007; Blanke and Wesener 2014), except Sphaerotheriidae sp. (NC_018361; Hu et al. 2020). Sphaerotheriidae sp. (NC_018361; Hu et al. 2020) belongs to infraclass Pentazonia, however, it clustered with infraclass Heminthomorpha in our phylogenetic tree (Figure 1). We expected that out mitochondrial genome of *E. koreanus* which is a new species recently identified based on morphological characters in the eastern Palearctic region (Golovatch 2013, 2014) can be a fundamental genetic

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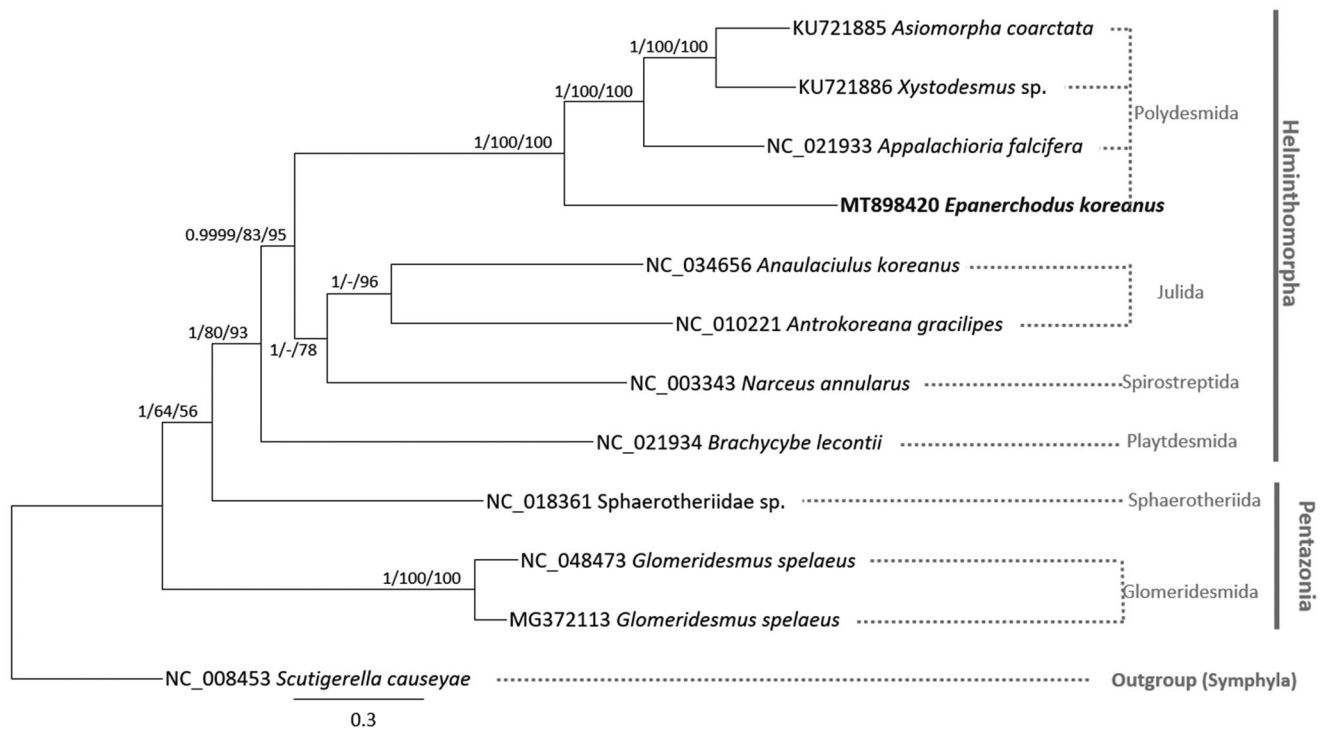


Figure 1. Bayesian inference (1,000,000 generations), maximum-likelihood (10,000 bootstrap repeats), and neighbor-joining (10,000 bootstrap repeats) phylogenetic trees of eleven Diplopoda, and one Symphyla mitochondrial genomes as an out group: *Epanerchodus koreanus* (MT898420; This study), *Asiomorpha coarctata* (KU721885; Dong et al. 2016), *Xystodesmus* sp. (KU721886; Dong et al. 2016), *Appalachioria falcifera* (NC_021933; Brewer et al. 2013), *Anaulacius koreanus* (NC_034656; Woo HJ et al. 2017), *Antrokoreana gracilipes* (NC_010221; Woo H-J et al. 2007), *Narceus annularus* (NC_003343; Lavrov et al. 2002), *Brachycybe lecontii* (NC_021934; Brewer et al. 2013), *Sphaerotheriidae* sp. (NC_018361; Dong et al. 2012), *Glomeridesmus spelaeus* (NC_048473; unpublished), *Glomeridesmus spelaeus* (MG372113; Nunes et al. 2017), and one Symphyla species, *Scutigera causeyae* (NC_008453; Podsiadlowski et al. 2007). Phylogenetic tree was drawn based on bayesian inference phylogenetic tree. The numbers above branches indicated posterior probability value of Bayesian inference, and bootstrap support values of maximum-likelihood and neighbor-joining phylogenetic trees, respectively. Order names were displayed as light gray color and infraclass names were written as gray color. In outgroup, class name was displayed as gray color.

information for understanding species diversity and distribution of millipedes in the genus *Epanerchodus*.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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Data availability statement

Mitochondrial genome sequence can be accessed via accession number MT898420 in NCBI GenBank.

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