Complete mitochondrial genome of the leaf-rolling-weevil, *Apoderus jekelii* Roelofs, 1874 (Coleoptera: Attelabidae)

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**ABSTRACT**

We sequenced 15,803 bp of the leaf-rolling-weevil, *Apoderus jekelii* (Coleoptera: Attelabidae) mitochondrial genome (mitogenome) that lacked \(~8000\) bp of the A + T-rich region for the completion of the genomic sequence. The *A. jekelii* mitogenome, which includes 1169 bp of A + T-rich region, possesses typical sets of genes [13 protein-coding genes (PCGs), 22 tRNA genes, and 2 rRNA genes] and gene arrangement of the species was identical to that commonly found in the majority of the insects. The start codon for ND1 was a rare TTG instead of typical ATN. Nearly, all PCGs ended with complete stop codons, TAA or TAG, except for ND5 which ended with an incomplete T. Phylogenetic analyses using the eight concatenated PCG sequences, which are commonly available for the mitogenome sequences of Curculionoidea, revealed Attelabidae as monophyletic, as well as the sister relationship between current *A. jekelii* and congeneric species *A. coryli* in Attelabidae, with the highest nodal supports both in Bayesian inference and maximum likelihood methods. In order to gain a more comprehensive picture of the phylogenetic relationships among the lineages of Attelabidae, an extended analysis with more taxonomic sampling will be necessary.

The leaf-rolling-weevil-weevil, *Apoderus jekelii* Roelofs, 1874 (Coleoptera: Attelabidae), is distributed in Korea, China, East Siberia, and Japan (Park et al. 2007). Adults occur twice per year from May to September (Park 2005). It is a forest pest feeding on several species of *Alnus* (*A. japonica*, *A. hirsuta*, *A. firma*, and *A. pendula*), heartleaf hornbeam (*Carpinus cordata*), and the Asian-beaked hazel (*Corylus sieboldiana*) (Park et al. 2007). One of the interesting features of the species includes different leaf-cutting patterns in adults such as a straight-cutting, single-cutting, and non-cutting types (Park et al. 2007, 2012; Park and Park 2014).

An *A. jekelii* adult was collected at Wangpi-gil, Geumgangsong-myeon, Ulijin-gun, Gyeongsangbuk-do, Republic of Korea (36°54′33″ N, 129°14′25″ E) on May 2017. This voucher specimen was deposited at the Chonnam National University, Gwangju, Korea, with the accession number CNU7498. DNA was extracted from the hind legs of the *A. jekelii* individual using a Wizard Genomic DNA Purification Kit (Promega, Madison, WI, USA) and three long overlapping fragments (LFS; *COI*-ND5, ND5-1rRNA, and 1rRNA-COI) were amplified. These LFs were subsequently used as templates to amplify 26 × 500–700 bp long short overlapping fragments. All primers were designed from the available mitogenome sequences of Attelabidae (data not shown). The *A. jekelii* sequence was deposited in GenBank with the accession number MK292540. Currently, only two mitogenomes are available for Attelabidae in which *A. jekelii* is included and even these are incomplete, lacking a substantial portion of the genome (*Apoderinae* sp. and *Apoderus coryli*). Thus, a phylogenetic analysis was conducted using the eight commonly available PCGs (5382 bp including gaps) after the well-aligned conserved blocks were selected using Gblocks 0.91b software (Castresana 2000), with the inclusion of representative sequences of other phylogenetically close families in Scarabaeoidea, the sequences of which also lack in some portions of their genomes. Bayesian inference (BI) and maximum likelihood (ML) methods were conducted using MrBayes ver. 3.2.2 (Ronquist et al. 2012) and RAxML-HPC2 ver. 8.0.24 (Stamatakis 2014), respectively, implemented on the CIPRES Portal ver. 3.1 (Miller et al. 2010). *Prosopocoilus astacoides Blanchardi* belonging to Lucanidae in Scarabaeoidea was used as an outgroup. Trees were visualized with FigTree ver. 1.42 (http://tree.bio.ed.ac.uk/software/figtree/) (Figure 1).

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The *A. jekelii* mitogenome, which includes an unfinished portion of the A + T-rich region, was 15,803 bp and possessed the typical sets of genes (13 PCGs, 22 tRNA genes, and 2 rRNA genes). The arrangement of this genome was identical to that typically observed in other insects (Cameron 2014). Previously, the coleopteran mitogenomes with substantially large A + T-rich regions have been sequenced in several species: 5654 bp in *Protaetia brevitarsis* (Kim et al. 2014), 1629 bp in *Cicindela anchoralis* (Wang et al. 2018), 3100 bp in *Prosopocoilus astacoides blanchardi* (Kim et al. 2015), 4469 bp in *C. coryli* in a sister group with both BI and ML analyses showing the same monophyletic group also with the highest nodal support. Twelve PCGs had the typical ATN start codon, whereas *ND1* had an atypical TTG codon. Nearly, all PCGs ended with complete stop codons such as TAA or TAG, but *ND5* ended with an incomplete T.

Phylogenetic analyses based on the concatenated sequences of eight PCGs placed *A. jekelii* and congenic species *A. coryli* in a sister group with both BI and ML analyses showing the highest nodal support. Three available species of Attelabidae, within which current *A. jekelii* belongs, formed a strong monophyletic group also with the highest nodal support in both analyses (Figure 1). Currently, mitogenome sequences are available only from three species including current *A. jekelii* and the two previously reported species have unfinished genes and regions. Therefore, we expect that our mitogenome sequences of *A. jekelii* will be used for primer design and further improved annotation of taxonomically close groups, along with a phylogenetic analysis of the Attelabidae, despite our sequence largely missing the A + T-rich region.

**Figure 1.** Phylogenetic tree of Curculionoidea. Maximum likelihood (ML) and Bayesian inference (BI) methods produced the same topology based on concatenated eight PCGs (ATP8, ATP6, COII, ND3, ND5, ND4, ND4L, and ND6). The numbers at each node specify bootstrap percentages from 1000 pseudoreplicates by ML analysis (first value) and Bayesian posterior probabilities in percent from the BI analysis (second value). The scale bar indicates the number of substitutions per site. Lucanidae (*Prosopocoilus astacoides blanchardi*) was utilized as an outgroup. GenBank accession numbers are as follows: *Platystomus albinus*, KX087337 (Linar et al. 2018); *Rhopalapion longirostre*, JN163967 (Haran et al. 2013); *Dydrirhynchus australicus*, JN163964 (Haran et al. 2013); *Hypera postica*, JN163953 (Haran et al. 2013); *Deporaus tristis*, KX087280 (Linar et al. 2018); *Deporaus betulae*, JN163945 (Haran et al. 2013); *Byctiscus populi*, JN163965 (Haran et al. 2013); *Apoderus coryli*, JN163966 (Haran et al. 2013); *Apoderinae*, MH473531 (Gillett et al. 2014), and; *Prosopocoilus astacoides blanchardi*, KF364622 (Kim et al. 2015).

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