

Sequencing and characterization of the complete mitochondrial genome of *Pseudoregma bambucicola* (Hemiptera: Hormaphidinae) from Guizhou, China

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ABSTRACT

The bamboo aphids, *Pseudoregma* spp., are the commonest insect pests found in ornamental bamboos throughout southeastern Asia. In this study, the mitochondrial genome of a representative of *Pseudoregma bambucicola* isolated from the bamboo *Bambusa multiplexcv* in Guizhou of China was determined through Illumina MiSeq platform. The entire genome was 16,705 bp in length and encoded 13 protein-coding genes, 22 tRNA genes, and 2 rRNA genes. The phylogenetic analysis showed that the *P. bambucicola* (Guizhou isolate) clustered together with another two isolates from Sichuan and Fujian of China, respectively, and together formed a monophyletic relationship with *Hormaphis betulae* in Hormaphidinae. The mitochondrial DNA data presented here should contribute to future molecular identification, population genetic, and evolutionary biological studies of *P. bambucicola*.

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The aphids of the genus *Pseudoregma* are the common insect pests of bamboos that are widely distributed throughout the warmer regions of southeastern Asia (Fukatsu et al. 2001). These aphid species, for instance, *Pseudoregma alexanderi*, *P. bambucicola*, *P. Carolinensis*, and *P. koshunensis* mainly infest ornamental bamboos and cause their growth stunting and even cause death (Fukatsu et al. 2001; Ijichi et al. 2004; Nong et al. 2017). Until now there has been significant advance of knowledge made in morphology, ecologies, behavior and chemical control as well as systematics of *Pseudoregma* spp., especially in *P. bambucicola* (Fukatsu et al. 2001; Nong et al. 2017, 2019a, 2019b; Zhang et al. 2019). Nevertheless, it is still existing gaps in the understanding *P. bambucicola* including its molecular epidemiology and population genetic diversity due to limited marker resources (Nong et al. 2017, 2019a, 2019b; Zhang et al. 2019). Mitochondrial (mt) DNA is a valuable marker resource and is being widely used for genetics and molecular identification of plant aphids (Cameron 2014; De Mandal et al., 2014; Marquina et al. 2019). In this study, we characterized the complete mitochondrial genome of a representative of *P. bambucicola* sampled from Guizhou of China and added novel mt DNA data to the this species.

In May 2020, about 200 aphids were sampled from the *Bambusa multiplexcv* which was planted in Guiyang city (27°07'N, 107°05'E), Guizhou province, China. These aphid specimens were identified as *P. bambucicola* according to the

taxonomic key of Stern (1997) and the molecular sequencing by amplification of the mt *cox2* and *cytb* genes (Nong et al. 2019a, 2019b). Twenty aphid specimens were pooled for mt DNA extraction and the remaining were fixed in 5% formalin solution and archived in the Insect Museum of Bamboo Diseases and Pest control and Resources Development Key Laboratory of Sichuan Province, China, under voucher number NX2019_14. The mt genome was sequenced using the Illumina MiSeq platform (Novogene, Beijing, China). The genome was assembled by MITObim (Hahn et al. 2013) and annotated using MITOS (Bernt et al. 2013). The complete genome sequence has been deposited in GenBank under accession number: MT916291.

The mitochondrial genome of *P. bambucicola* (Guizhou isolate) was 16,705 bp in length and encoded 13 protein-coding genes (PCGs), 22 tRNA genes (tRNAs), and 2 rRNA genes (rRNAs). Similar to the congeneric species (Zhang et al. 2019; Nong et al. 2020), nine PCGs and 15 tRNAs were found to be transcribed on the forward strand (J-strand) while the remaining genes were located on the reverse strand (N-strand). Across the 13 PCGs, except for the *nad4* deduced to use an incomplete stop codon 'T', the rest were predicted to use the typical TAG ($n = 5$) or TAA ($n = 7$) as the stop codon. Twenty-two tRNAs ranged from 51 bp (tRNA-Cys) to 73 bp (tRNA-Lys) in length and all can be folded into typical clover-leaf like secondary structures, with the exception of tRNA^(AGN)-Ser.

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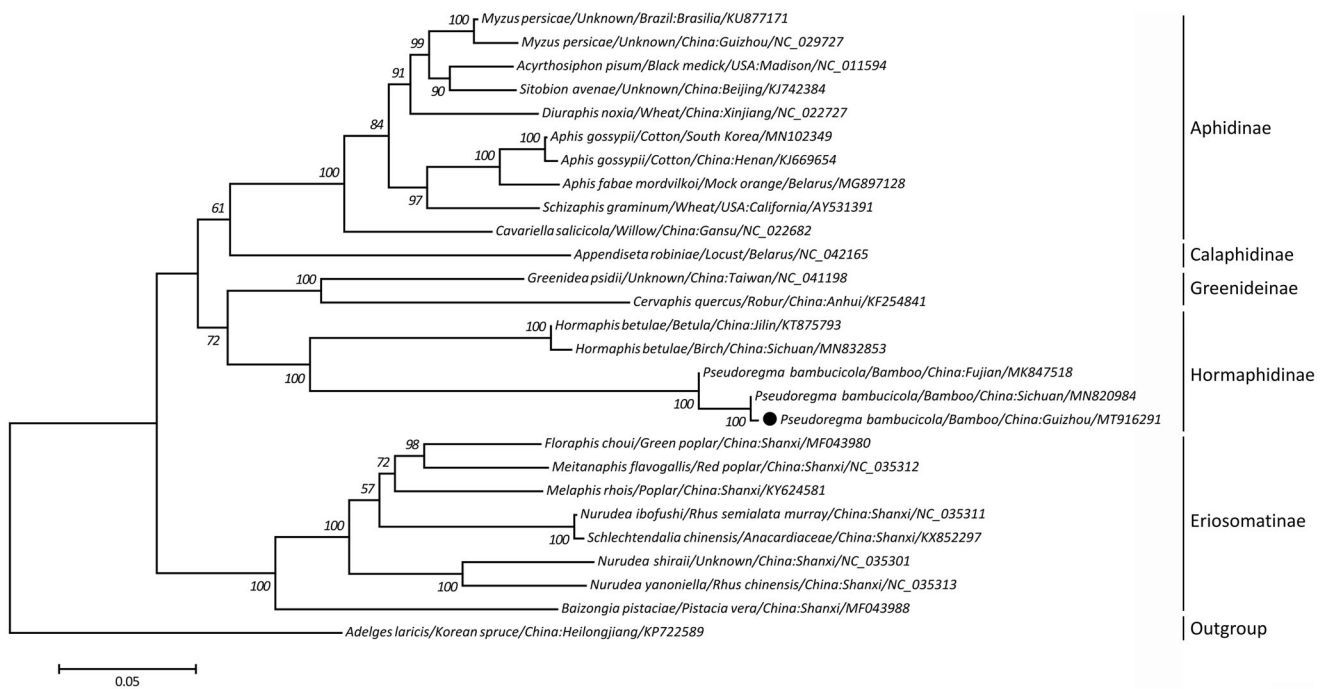


Figure 1. A maximum likelihood (ML) tree inferred from concatenated amino-acid sequences of 13 mitochondrial protein-coding genes of *P. bambucicola* and other related aphids, utilizing MtArt model with 1,000,000 bootstrap replications (<50% support not shown). The solid black cycle represents the species in this study.

Within two rRNAs, the large (*rrnL*; 1,284 bp) and small rRNA (*rrnS*; 758 bp) subunits were located between tRNA^(CUN)-Leu and tRNA-Val and between tRNA-Val and D-loop region, respectively. The D-loop region (849 bp) with 95.9% A + T content was present between *rrnS* and tRNA-Ile. In addition, a total of 207 bp intergenic spacers were present at 17 positions and the lengths of the spacers were 1–27 bp.

Based on a concatenated amino acid sequence of 13 protein-coding genes from 27 aphids, a maximum-likelihood (ML) phylogeny was reconstructed using *Adelges laricis* as the outgroup. The phylogenetic analysis showed that *P. bambucicola* (Guizhou isolate) clustered with two isolates from Sichuan and Fujian of China, respectively, and together formed a monophyletic relationship with *Hormaphis betulae* in the subfamily Hormaphidinae, with 100% bootstrapping confidences (Figure 1). In addition, within this tree topology, Aphidinae, Calaphidinae, Greenideinae, Eriosomatinae, and Hormaphidinae were treated as monophyletic groups, consistent with results of recent molecular studies (Wang et al. 2013; Li 2017; Zhang et al. 2019), demonstrating the phylogenetic stability of these subfamily in Aphididae. Taken together, the complete mt genome of *P. bambucicola* (Guizhou isolate) characterized here should contribute to a better understanding of phylogenetic relationships among Aphididae species and also serve molecular identification, population genetic and evolutionary biological studies of *P. bambucicola*.

Disclosure statement

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

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Data availability statement

The data that support the findings of this study are openly available in GenBank of NCBI at <https://www.ncbi.nlm.nih.gov>, reference number MT916291.

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