

Mitochondrial genome of *Hormaphis betulae* and its comparative analysis with *Pseudoregma bambucicola* (Hemiptera: Hormaphidinae)

Xiang Nong^{a,b*}, Yunjian Liu^{c*}, Lidan Wang^{c*}, Shengnan Zhong^{a,b,d}, Xiaobo Yu^{a,b} and Yue Xie^c

^aBamboo Diseases and Pest control and Resources Development Key Laboratory of Sichuan Province, Leshan, China; ^bCollege of Life Science, Leshan Normal University, Leshan, China; ^cDepartment of Parasitology, College of Veterinary Medicine, Sichuan Agricultural University, Chengdu, China; ^dCollege of Food and Biological Engineering, Xihua University, Chengdu, China

ABSTRACT

Hormaphis betulae (Hemiptera: Hormaphidinae) is a common aphid of birch plants. Here, the complete mitochondrial genome sequence of a representative of this aphid from China was determined using next generation sequencing platform. The genome was 15,129 bp in length and encoded 13 protein-coding genes, 22 tRNA genes, and 2 rRNA genes. The phylogeny revealed that two Chinese isolates of *H. betulae* clustered together and formed a monophyletic relationship with *Pseudoregma bambucicola* in the subfamily Hormaphidinae, supporting their species validity in Aphididae. The cumulative mitochondrial DNA data provides a better understanding of the phylogenetic relationship of this species in plant aphids.

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Hormaphis betulae (Hemiptera: Hormaphidinae) is a common aphid found on birch plants and can cause conical pouch galls on leaves (Kurosu and Aoki 1991). Morphologically, these galls are pale green in color, flattened, circular and surrounded by a fringe of beam-like wax threads. Moreover, *H. betulae* was also reported on the witch hazel in Japan (Aoki and Kurosu 1991). Although there has been significant progress about the ecology and behavior of *H. betulae* made so far, still major gaps exist in the understanding of genetics and molecular epidemiology of this aphid because of few genetic markers available (Kurosu and Aoki 1991; Li et al. 2017). Mitochondrial DNA (mtDNA) has proven to be a valuable source of molecular markers and is being widely used for genetics and molecular identification of many insect pests including plant aphids (Cameron 2014; De Mandal et al. 2014; Marquina et al. 2019). Here, we reported the complete mitochondrial genome of a representative *H. betulae* from the Sichuan Province, China, and compared with the aphid *Pseudoregma bambucicola* from the same subfamily Hormaphidinae (Nong et al. 2019).

In April 2019, numerous aphids were sampled from the *Betula nana* in Leshan city (28°35'N, 103°48'E), Sichuan province, China. After morphological identification, the aphid specimens ($n = 200$) were identified as *H. betulae* according to the taxonomic key of Blackman and Eastop (2017) and the molecular confirmation by amplification and sequencing of the mitochondrial cytochrome c oxidase subunit 1 gene (Li et al. 2017). Twenty aphid specimens were used for mtDNA 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_13. The complete mtDNA was sequenced using the Illumina HiSeq platform (BGI, Shenzhen, China). The genome was assembled using MITObim (Hahn et al. 2013) and annotated with MITOS (Bernt et al. 2013). The sequence has been deposited in GenBank under the accession number MN832853.

The complete mitochondrial genome sequence of *H. betulae* was 15,129 bp in length, belonging to one of the shortest aphid mitochondrial genomes described so far. As with mtDNAs of *P. bambucicola* and other aphids sequenced thus far (Wang et al. 2013; Li et al. 2017; Nong et al. 2019; Zhang et al. 2019), this genome also encoded 13 protein-coding genes (PCGs), 22 tRNA genes (tRNAs) and two rRNA genes (rRNAs). Compared to those of *P. bambucicola*, however, most of these genes ($n = 23$) were transcribed on the forward strand (J-strand) and only 14 were located on the reverse strand (N-strand). Among the 13 PCGs, all genes started with a typical ATN codon and used the typical TAG or TAA as the stop codons, except for the *cox1* and *nad4* which were deduced to use an incomplete stop codon 'T'. 22 tRNAs ranged from 61 bp (tRNA-Val) to 73 bp (tRNA-Lys) in lengths and each formed a typical clover-leaf like secondary structure, except for tRNA^(GCT)-Ser. For two rRNAs, the *rrnL* (1,275 bp) was placed between tRNA^(TAG)-Leu and tRNA-Val

CONTACT Xiang Nong  nongx2008@163.com  Bamboo Diseases and Pest control and Resources Development Key Laboratory of Sichuan Province, Leshan, 614000, China; College of Life Science, Leshan Normal University, Leshan 614000, China; Yue Xie  xyue1985@gmail.com  Department of Parasitology, College of Veterinary Medicine, Sichuan Agricultural University, Chengdu 611130, China

*These authors have contributed equally to this work.

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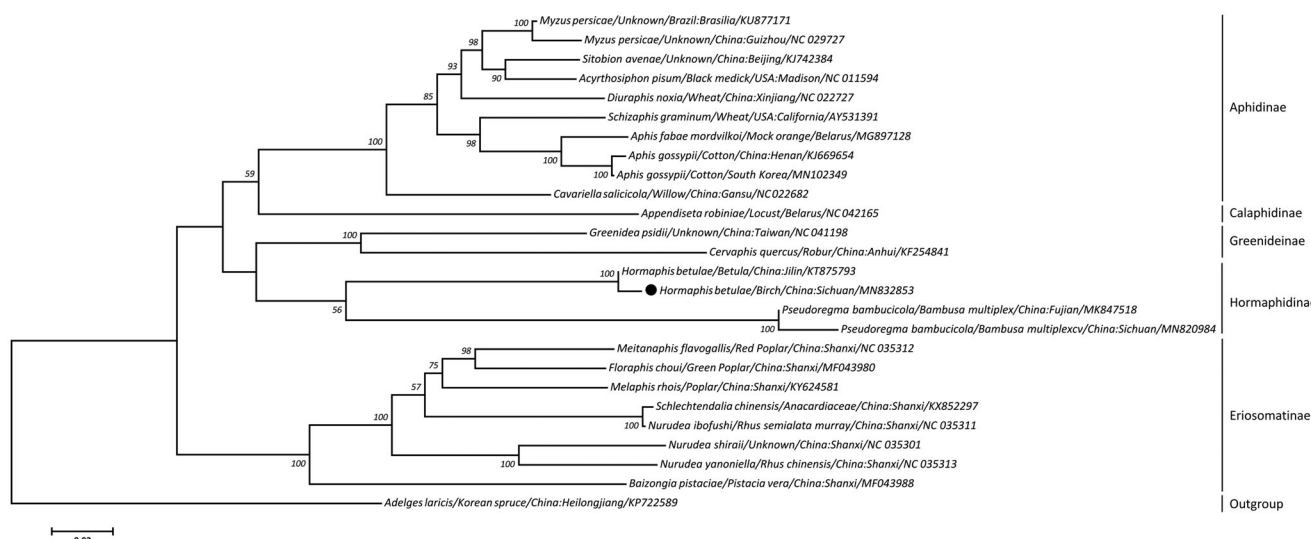


Figure 1. Inferred maximum likelihood (ML) tree based on concatenated amino-acid sequences of 13 mitochondrial protein-coding genes of *H. betulae* and other related aphid species, using MtArt + I + G model with 10,000 bootstrap replications (<50% support not shown). The black dot represents the species in this study.

and the *rnrS* (776 bp) between tRNA-Val and D-loop region, consistent with those of *P. bambucicola* (Nong et al. 2019). The D-loop region (551 bp) with 83.1% A + T content was located between *rnrS* and tRNA-Ile.

A maximum-likelihood (ML) phylogeny was reconstructed on the basis of a concatenated amino acid sequence of 13 protein-coding genes from 26 aphid species, using *Adelges laricis* as outgroup. This phylogenetic tree showed that two Chinese isolates of *H. betulae* clustered together and formed a branch that was monophyletic with *P. bambucicola* in the subfamily Hormaphidinae, with 100% bootstrap confidence, supporting their species validity among the family Aphididae (Figure 1). In addition, each subfamily of Aphididae, Calaphidinae, Greenideinae, Eriosomatinae or Hormaphidinae within this topology formed a monophyletic group in Aphididae, which were consistent with the results of recent molecular studies (Li et al. 2017; Nong et al. 2019; Zhang et al. 2019). Taken together, the complete mtDNA of *H. betulae* sequenced here added a novel marker resource for genetic and evolutionary biological studies of this birch pest.

Disclosure statement

No potential conflict of interest was reported by the authors.

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References

- Aoki S, Kurosu U. 1991. Host alternation of *Hormaphis betulae* (Homoptera, Aphidoidea). *Jpn J Entomol.* 59:164.
- Bernt M, Donath A, Jühling F, Externbrink F, Florentz C, Fritzsche G, Pütz J, Middendorf M, Stadler PF. 2013. MITOS: improved de novo metazoan mitochondrial genome annotation. *Mol Phylogenet Evol.* 69(2): 313–319.
- Blackman RL, Eastop VF. 2017. Aphids on the world's plants. An identification and information guide. (accessed 10 April 2017).
- Cameron SL. 2014. Insect mitochondrial genomics: implications for evolution and phylogeny. *Annu Rev Entomol.* 59(1):95–117.
- De Mandal S, Chhakhuak L, Gurusubramanian G, Kumar NS. 2014. Mitochondrial markers for identification and phylogenetic studies in insects – a review. *DNA Barcodes.* 2(1):19.
- Hahn C, Bachmann L, Chevreaux B. 2013. Reconstructing mitochondrial genomes directly from genomic next-generation sequencing reads—a baiting and iterative mapping approach. *Nucleic Acids Res.* 41(13): e129–e129.
- Kurosu U, Aoki S. 1991. Gall cleaning by the aphid *Hormaphis betulae*. *J Ethol.* 9(2):51–55.
- Li YQ, Chen J, Qiao GX. 2017. Complete mitochondrial genome of the aphid *Hormaphis betulae* (Mordvilko) (Hemiptera: Aphididae: Hormaphidinae). *Mitochondrial DNA B Resour.* 28(2):265–266.
- Marquina D, Andersson AF, Ronquist F. 2019. New mitochondrial primers for metabarcoding of insects, designed and evaluated using *in silico* methods. *Mol Ecol Resour.* 19(1):90–104.
- Nong X, Wang LD, Liu Y, Zhong S, Yu X, Xie Y. 2019. The complete mitochondrial genome of the bamboo aphid *Pseudoregma bambucicola* and its phylogenetic position. *Mitochondrial DNA B Resour.* doi:10.1080/23802359.2019.1711225.
- Wang Y, Huang XL, Qiao GX. 2013. Comparative analysis of mitochondrial genomes of five aphid species (Hemiptera: Aphididae) and phylogenetic implications. *PLoS One.* 8(10):e77511.
- Zhang H, Deng J, Liu Q, Huang X. 2019. The mitochondrial genome of a social aphid, *Pseudoregma bambucicola* (Hemiptera: Aphididae: Hormaphidinae). *Mitochondrial DNA B Resour.* 4(2):2100–2101.