

The First Conserved Mitochondrial Genome of *Polygraphus poligraphus* (Coleoptera: Curculionidae) and its Phylogenetic Implications

Xianmei Song¹, Yuchen Zhao¹, Peng Zhao¹, Yinxiang Ma¹, Ming Bai^{1,2} and Xinpu Wang^{1,*}

¹School of Agriculture, Ningxia University, Yinchuan, Ningxia 750021 P.R.China

²Key Laboratory of Zoological Systematics and Evolution, Institute of Zoology, Chinese Academy of Sciences, Beijing, 100101, P.R.China

ABSTRACT

Polygraphus poligraphus L., the four-eyed spruce bark beetle, belongs to the Curculionidae (Coleoptera), which mainly harms *Picea asperata* Mast and *Pinus armandii* Franch tree trunks. In this study, we sequenced and annotated the nearly complete mitogenome of *P. poligraphus* for the first time and predicted the secondary structures of its tRNAs. The results showed that the mitogenome of *P. poligraphus* was 15,302 bp (partial genome) in length with A + T content of 69.65% due to large-scale duplication. The nearly complete mitochondrial genome of *P. poligraphus* contained a set of 36 genes typical of the insect mitogenome, including 13 protein-coding genes (PCGs), 2 ribosomal RNA genes (rRNAs), 21 transfer RNA genes (tRNAs) but lacked tRNA-Ile, as for the typical insect mitogenome. The results of nucleotide skew statistics showed that the AT-skews and GC-skew of *P. poligraphus* were positive and negative, respectively, which were similar to other Scolytinae insects. All PCGs were initiated with the standard start codon ATN. All tRNA genes had the typical cloverleaf structure, except for the trnS1, which lacked a dihydroxyuridine (DHU) arm. Furthermore, we reconstructed phylogenetic trees of *P. poligraphus* based on the data set of the mitogenome's protein-coding gene sequences using the Bayesian inference (BI) method and maximum likelihood method (ML). In ML and BI analyses, the relationships of three genera of Scolytinae are *Polygraphus* + (*Gnathotrichus* + *Pityophthorus*). Our results presented the phylogenetic relationships and taxonomic status of *P. poligraphus* and highlight the need for further sequencing analyses and taxonomic revisions in additional bark beetle species.

Article Information

Received 29 April 2022

Revised 18 May 2022

Accepted 15 June 2022

Available online 13 October 2023

(early access)

Published 18 February 2025

Authors' Contribution

XMS assembled, finished, and annotated mitochondrial and plastid genomes and all data analyses, submitted sequences to NCBI, and wrote the first draft of all sections of the manuscript. YCZ and PZ assisted in collecting *Polygraphus poligraphus*. YXM participated in the experiments. MB and XPW supervised this study, contributed to the design of the study and drafting the manuscript.

Key words

Scolytinae, Bark beetle, Phylogeny

INTRODUCTION

The mitochondrion is a fundamental eukaryotic organelle, descended from an alphaproteobacterium that formed a permanent symbiosis with the ancestral eukaryote roughly two billion years ago. The mitochondrial genomes of arthropods have been studied extensively, and insects represent approximately 80% of the arthropod mt genomes that have been sequenced (Cameron, 2014). Insect mitochondrial genomes are small, typically a double-stranded circular molecular structure ranging from 14 to 19 kb in size. With few exceptions, all animal mitochondrial genomes contain a typical set of 37 genes:

13 protein-coding genes (PCGs) (*ATP6*, *ATP8*, *COI-III*, *ND1-6*, *ND4L*, and *CYTb*), 2 ribosomal RNA genes (rRNAs) (*rnl* and *rns*), 22 transfer RNA genes (tRNAs), and a putative control region (A+T-rich region) (Wolstenholme, 1992; Boore, 1999; Li *et al.*, 2009). Compared with partial mitochondrial genes the whole mitogenome can provide more meaningful information such as the arrangement of gene sequences, secondary structures of RNA, codon usage and structural features of the A+T-rich region (Song *et al.*, 2018; Hu and Wang, 2019; Wang *et al.*, 2019). This is because of the unique features of a complete mitochondrial genome including simple genetic structure, maternal inheritance, high rate of evolution and low rate of recombination (Curole and Kocher, 1999; Lin and Danforth, 2004; Ho and Gilbert, 2010). Over the past decade, mitochondrial genomes have become widely used for molecular evolution, population genetics, systematics and phylogenetics (Ribera *et al.*, 2004; Cameron, 2014; Zhang *et al.*, 2015; 2016; Sun *et al.*, 2020; Zeng *et al.*, 2021).

Coleoptera, the largest insect order, contains four suborders (Archostemata, Adephaga, Polyphaga and Myxophaga), 17 superfamilies, 168 families and over

* Corresponding author: wangxinpu@nxu.edu.cn
0030-9923/2025/0002-0651 \$ 9.00/00



Copyright 2025 by the authors. Licensee Zoological Society of Pakistan.

This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

380,000 described species. Of these, about 10,000 are known in China (Hatch, 1956; Hunt *et al.*, 2007). *Polygraphus poligraphus* L., four-eyed spruce bark beetle, belongs to Scolytinae of Curculionidae of Coleoptera (Wood, 1977). It is a harmful, wide-spread invasive insect and one of the 236 dangerous forest pests announced by the State Forestry and Grassland Administration of China. *Polygraphus poligraphus* is mainly distributed in Russia, Finland, Norway, Sweden, Denmark, Poland, Germany, Czechia, Austria, Turkey, Yugoslavia and Gansu, Jilin, Liaoning, Heilongjiang, Neimenggu, and Ningxia provinces in China (Kang, 2016). It mainly damages *Picea asperata* Mast and *Pinus armandii* Franch as adults and can cause the death of entire forests in severe cases. Nevertheless, *P. asperata* and *P. armandii* are important timber forests, ecological public welfare forests, water conservation forests and greening trees, and occupy an irreplaceable position in the forest resources of China (Kang, 2016; Duan, 2020). The morphology, biology and biological and chemical control of *P. poligraphus* have been studied (Yin *et al.*, 1984; Sun, 2005; Viklund *et al.*, 2019). However, it is imperative to integrate the sustainable development of forest ecosystems with sustainable control techniques for bark beetles.

With the rapid development of high-throughput sequencing technology, the number of insect mitochondrial genomes being studied is increasing. Over two years there were more than 100 whole sequenced mitochondrial genomes and more than 3000 partially sequenced mitogenomes placed in the GenBank database for Coleoptera (last visited on March 7, 2022) (Jeong *et al.*, 2020). Among these species, no information about the complete or nearly complete mitochondrial genome and phylogenetic position of *P. poligraphus* is mentioned, which impedes the application of biological control. In view of the large number of species of Scolytinae and the difficulty distinguishing them, accurate identification is essential to prevent the invasion of these species. Here we report for the first time the nearly complete mitogenome of *P. poligraphus* and clarify its phylogenetic position within the Scolytinae.

In the present study we analyzed the genome organization, nucleotide composition, composition biases, codon usage, constructed of tRNA secondary structures and phylogenetic relationships of the *P. poligraphus* mitogenome.

MATERIALS AND METHODS

Sample collection, identification and DNA extraction

Adult specimens of the *P. poligraphus* were collected at Luoshan (37°20'59"N, 106°18'9"E), 2108 m, Ningxia,

China on 15 Jun 2019. Currently the specimens are stored in the insect herbarium at the School of Agriculture, Ningxia University, China (SANXU, voucher number: YSSYXD201907). Fresh specimens were stored at -20 °C in 100% ethanol until used for DNA extraction. The specimens were identified by Dr. You Li (School of Forest Resources and Conservation, University of Florida, Gainesville, Florida 32611, USA). Total genomic DNA was extracted from the using the Biospin Insect Genomic DNA Extraction Kit (Qiagen, Hilden, Germany). according to the manufacturer's instructions. The DNA was stored at -20 °C for further analysis.

Mitogenome sequencing

Illumina sequencing was used to obtain the mitogenome sequence of *P. poligraphus*. Briefly, qualified DNA samples tested by electrophoresis were randomly interrupted with Covaris ultrasonic crusher with a length of about 350 bp. Then, the whole library was constructed using the NEBNext® Ultra™ DNA Library Prep Kit for Illumina (NEB, USA) to repair the end of the DNA fragments, add poly 'A', add sequencing joints, purify, PCR amplification and other steps. Subsequently, Qubit v2.0 was used for preliminary quantification, and the library was diluted to 2 ng/ µL. Lastly, Agilent 2100 was used to detect the inserted fragments of the library, the insert size was in line with the expectation, and the Q-PCR method was used to accurately quantify the effective concentration of the library to ensure the quality of the library.

Mitogenome annotation and analysis

The paired-end reads for mitochondrial genome sequences of *P. poligraphus* were assembled by MITObim v1.9 with the invertebrate genetic code employed (Hahn *et al.*, 2013). Subsequently, the mitochondrial genomes of *P. poligraphus* were annotated with Geneious 10.1.3 (<http://www.geneious.com/>) (Kearse *et al.*, 2012) with the mitogenomes of *Pityogenes bidentatus* (GenBank accession number KX035211) as references. Twenty-one tRNA gene annotations were re-identified and their secondary structures predicted by MITOS Web Server (<http://mitos.bioinf.uni-leipzig.de/index.py>) (Bernt *et al.*, 2013). Strand asymmetry was calculated according to the formulas: AT-skew = [A-T] / [A+T] and GC-skew = [G-C] / [G+C] (Perna and Kocher 1995). The A + T content, AT-skew, GC-skew, were graphically plotted by OriginPro 9.1 (Seifert, 2014). The base composition and the relative synonymous codon usage (RSCU) were calculated using MEGA version 7.0 (Kumar *et al.*, 2016).

Phylogenetic analyses

To reconstruct phylogenetic trees for the estimation

of *P. poligraphus* taxonomic status, the complete mitogenome sequences of 19 Scolytinae species and three outgroups (*Sitophilus zeamais*, *Cyrtotrachelus buqueti* and *Rhynchophorus ferrugineus*) were downloaded from GenBank (Table I). Phylogenetic trees were constructed using the Bayesian inference method (BI) and maximum likelihood method (ML) based on 13 mitochondrial protein-coding genes. PhyloSuite (Zhang *et al.*, 2020) was used to conduct, manage and streamline the analyses with the help of several plug-in programs. All 13 PCG nucleotide sequences were aligned in batches with MAFFT (Katoh and Standley, 2013) using codon alignment mode. The alignments were refined using the codon-aware program MACSE v. 2.03 (Ranwez *et al.*, 2018), which preserves reading frame and allows incorporation of sequencing errors or sequences with frameshifts. Ambiguously aligned fragments of 13 alignments were removed in batches using Gblocks (Talavera and Castresana, 2007). Model Finder (Kalyaanamoorthy *et al.*, 2017) was used to select the best-fit model using BIC criterion. BI phylogenies were inferred using MrBayes 3.2.6 (Ronquist *et al.*, 2012) under 'GTR+I+ F+G4' model and ML phylogenies were inferred using IQ-TREE (Nguyen *et al.*, 2015). The robustness of the ML tree topology was ascertained by 1000 bootstrap pseudoreplicates of the tree search.

RESULTS AND DISCUSSION

Genome organization and base composition

A total of 26,528,032 Paired-End Reads with a reading length of 150 bp were obtained by Illumina HiSeq X Ten sequencing for mitochondrial genome assembly. Among the 20 Scolytinae species, *P. poligraphus* (GenBank accession number MN528600) had the smallest mitochondrial genome of 15,302 bp (partial genome) due to large-scale duplication, while *Orthotomicus laricis* had the largest of 18,887 bp (Fig. 1). The nearly complete mitochondrial genome of *P. poligraphus* contained the set of 36 genes typical of insect mitogenomes: 13 PCGs (ATP6, ATP8, COI-III, nad1-6, nad4L, and cob), 2 ribosomal RNA genes (rRNAs) (12S rRNA and 16S rRNA), 21 tRNAs (lack tRNA-Ile). Twenty-two genes are encoded on the majority strand (L-strand), and the remaining 14 genes are located on the minority strand (H-strand) in this mitogenome (Table II).

The nucleotide composition of the *P. poligraphus* mitochondrial genome was 37.26% of A, 32.39% of T, 18.46% of C, 11.89% of G and 69.65% of A+T content (Table III). Generally, the same region the mitochondrial genome as *P. poligraphus* of Scolytinae exhibited a strong base composition bias (65.16% (*Gnathotrichus materiarius*) -76.45% (*Hylastes brunneus*)) for A+T

content. The entire mitogenomes with a high A + T content benefit from the composition of PCGs, tRNAs and rRNAs. The A+T content in tRNAs was higher than that in PCGs in all 20 species. *Hylastes brunneus* had relatively weaker tRNA A+T content compared with other Scolytinae species (Fig. 2A). In addition to the A + T content, the skewness (AT-skew and GC-skew) of the base composition in nucleotide sequences was also used to describe the base composition of mitogenomes (Perna and Kocher, 1995; Kalyaanamoorthy *et al.*, 2017). The results of nucleotide skew statistics show that the AT-skews of *P. poligraphus* were slightly positive. The AT-skews of PCGs, tRNAs and rRNAs for whole mitogenomes in the Scolytinae are positive because the AT-skews value of nad1, nad4, nad4L, nad5 and rrnL are relatively greater and in other regions are slightly negative. Compared with other species, the AT-skews of *G. materiarius* were slightly lower (Fig. 2B). The GC-skew values are all negative in whole mitogenomes. The GC-skew of *P. poligraphus* is similar to other Scolytinae insects (Fig. 2C). The nucleotide skewness in Scolytinae mitochondrial genomes is consistent with that of most other insects (Wei *et al.*, 2010).

Table I. List of species used to construct the phylogenetic tree.

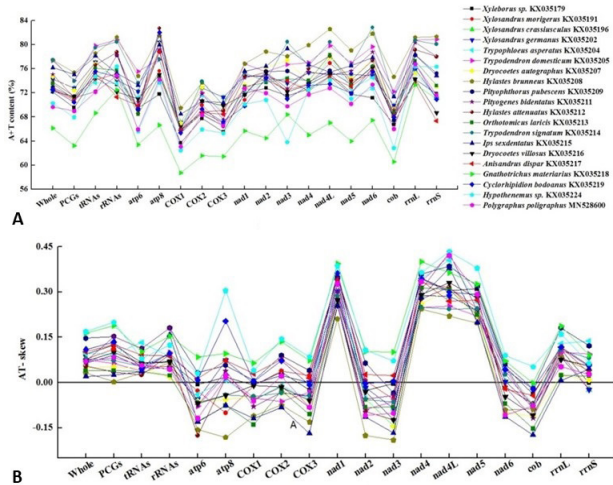
Family	Sub-family	Species	Accession number
Curculionidae	Scolytinae	<i>Anisandrus dispar</i>	KX035217
		<i>Xylosandrus crassiusculus</i>	KX035196
		<i>X. germanus</i>	KX035202
		<i>X. morigerus</i>	KX035191
		<i>Xyleborus</i> sp.	KX035179
		<i>Cyclorhipidion bodoanus</i>	KX035219
		<i>Dryocoetes autographus</i>	KX035207
		<i>D. villosus</i>	KX035216
		<i>Pityogenes bidentatus</i>	KX035211
		<i>Ips sexdentatus</i>	KX035215
		<i>Orthotomicus laricis</i>	KX035213
		<i>Gnathotrichus materiarius</i>	KX035218
		<i>Pityophthorus pubescens</i>	KX035209
		<i>Hypothenemus</i> sp.	KX035224
		<i>Trypophloeus asperatus</i>	KX035204
		<i>Trypodendron domesticum</i>	KX035205
		<i>T. signatum</i>	KX035214
		<i>Hylastes attenuatus</i>	KX035212
		<i>H. brunneus</i>	KX035208
	Dryophthorinae	<i>Sitophilus zeamais</i>	KX373614
		<i>Cyrtotrachelus buqueti</i>	MG674390
		<i>Rhynchophorus ferrugineus</i>	KT428893

Table II. Mitochondrial genome organization of *P. poligraphus*.

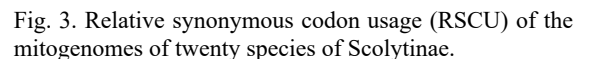
Feature	Strand	Location	Size(bp)	Start code	Stop codon	Anticodon	Intergenic nucleotides
trnQ	H	346–413	68			TTG	–1
trnM	L	413–481	69			CAT	0
nad2	L	482–1486	1,005	ATT	TAA		2
trnW	L	1489–1556	68			TCA	0
trnC	H	1557–1623	67			GCA	4
trnY	H	1628–1692	65			GCA	34
cox1	L	1727–3229	1,503	ATC	TAA		2
trnL2	L	3232–3294	63			TAA	0
cox2	L	3295–3973	679	ATT	T		0
trnK	L	3974–4043	70			CTT	0
trnD	L	4044–4107	64			GTC	0
atp8	L	4108–4266	159	ATT	TAG		–7
atp6	L	4260–4934	675	ATG	TAA		–1
cox3	L	4934–5719	786	ATG	TAA		6
trnG	L	5726–5789	64			TCC	0
nad3	L	5790–6143	354	ATA	TAA		8
trnA	L	6152–6215	64			TGC	0
trnR	L	6216–6279	64			TCG	1
trnN	L	6281–6345	65			GTT	–1
trnS1	L	6345–6404	60			TCT	1
trnE	L	6406–6466	61			TTC	–1
trnF	H	6466–6531	66			GAA	0
nad5	H	6532–8227	1,696	ATT	T		0
trnH	H	8228–8294	67			GTG	0
nad4	H	8295–9622	1,328	ATG	TA		–7
nad4l	H	9616–9909	294	ATG	TAG		3
trnT	L	9913–9977	65			TGT	0
trnP	H	9978–10041	64			TGG	2
nad6	L	10044–10550	507	ATG	TAA		0
cob	L	10551–11689	1,139	ATG	TA		0
trnS2	L	11690–11755	66			TGA	9
nad1	H	11765–12700	936	ATT	TAA		19
trnL1	H	12720–12786	67			TAG	–40
rrnL	H	12747–14080	1,334				–12
trnV	H	14069–14136	68			TAC	–1
rrnS	H	14136–14907	772				-

Table III. Composition and skewness in the *P. poligraphus* mitogenome.

Region	Size (bp)	A%	G%	C%	T%	A+T%	AT-Skew	GC-Skew
Mitogenome	15,302	37.26	11.89	18.46	32.39	69.65	0.070	-0.216
PCGs	11061	37.36	11.97	19.06	31.62	68.98	0.083	-0.228
tRNAs	1375	38.11	11.20	16.65	34.04	72.15	0.056	-0.196
rRNAs	2106	39.08	8.02	17.09	35.80	74.88	0.044	-0.361



The PCGs of the mitogenome were 11,061 bp long for *P. poligraphus* (Table III). Four PCGs (nad1, nad4, nad4L and nad5) were encoded on H-strand, and the other nine PCGs were located the L-strand. The sizes of 13 PCGs ranged from 159 bp (atp8) to 1696 bp (nad5) in *P. poligraphus* (Table II). All 20 mitogenomes had similar characteristics with the smallest sized PCG of atp8 and the largest that of nad5. All PCGs in the *P. poligraphus*



mitogenomes started with the standard ATN codon. The start codon ATG was shared with *cox3*, *atp6*, *nad4*, *nad4L*, *nad6* and *cob*; the start codon ATT was shared with *cox2*, *atp8*, *nad1*, *nad2* and *nad5*; *cox1* started with codon ATC; and the *nad3* started with codon ATA. The conservative stop codon TAA was shared with *cox1*, *cox3*, *atp6*, *nad1*, *nad2*, *nad3* and *nad6*; the stop codon TAG was shared with *atp8* and *nad4L*; *nad4* and *cob* stop with an incomplete codon TA–, and *cox2* and *nad5* end with the single nucleotide T–. TA– and T– denote that the TAA stop codon is presumed to be completed by the addition of 3' A residues to the mRNA. The incomplete termination codons are common across arthropod mitogenomes and are completed by post-transcriptional polyadenylation during the mRNA maturation process (Ojala *et al.*, 1981; Schuster and Stern, 2009).

The amino acid composition and the relative synonymous codon usage (RSCU) of mitogenomes of *P. poligraphus* and the other 19 Scolytinae species are summarized in Figure 3. The total number of codons in the PCGs ranged from 3060 (*Hylastes attenuatus*) to 3836 (*Dryocoetes villosus*). The pattern of codon usage was generally similar among Scolytinae mitogenomes such as the seven most frequently used codons: UUU, UUA, UAU, AUU, AAA, AAU and AUA, all composed wholly of A or U. In the *P. poligraphus* mitogenome, 3,542 amino acids were translated, of which 1,196 (33.77%) were encoded by the seven frequently used codons above. And, in the *H. brunneus* mitogenome, 1,672 (45.55%) amino acids were encoded by the seven frequently used codons; this was the greatest in the 20 Scolytinae mitogenomes. However, the codons absent in Scolytinae mitogenomes were different. In the *Xylosandrus crassiusculus*, *P. pubescens* and *Ips sexdentatus* mitogenomes, the GCG codon was absent, whereas the CCG and CGU codons were absent in *Trypodendron domesticum* and *Dryocoetes autographus* respectively. In general, the high C/G content in the absent codons effectively reflects nucleotide A + T bias in the mitochondrial PCGs among Scolytinae.

Transfer and ribosomal RNA genes

The 21 tRNAs of the *P. poligraphus* mitogenomes were scattered discontinuously over the partial mitogenome (due to large-scale duplication). The length of 21 tRNA genes ranged from 60 bp (*trnS1*) to 70 bp (*trnK*). The total length of tRNAs was 1,375 bp, accounting for approximately 9% of the mitogenome. Among them, eight tRNA genes were transcribed from the H-strand and 13 from the L-strand (Table II). As shown in Figure 4, most tRNAs sequences could fold into the typical clover-leaf secondary structure (including amino acid acceptor (AA) arm, dihydrouridine (DHU) arm, variable

(V) loop, anticodon (AC) arm and TΨC (T) arm), while *trnS1* (AGN) forms a simple loop due to lacking the stable DHU arm. The lack of a DHU stem in *trnS1* is generally present in Coleoptera insects and has been confirmed as a typical feature of metazoan mitogenomes (Garey and Wolstenholme, 1989; Wolstenholme, 1992; Lavrov *et al.*, 2000; Cameron, 2014; Chen and Du, 2017; Wang *et al.*, 2019; Jeong *et al.*, 2020). In tRNA genes of the *P. poligraphus* mitogenome, a great number of nucleotide substitutions are found in five different stems. Compared with variable TΨC and DHU loops, the anticodon stem and loop is highly conserved (Fig. 4). Except for the classic AU and CG pairs, we recognized 21 mismatched base pairs in the tRNA genes secondary structures of *P. poligraphus*. Among them, 19 were G-U mismatched base pairs, one was a U-U pair and two were G-G pairs. The overrepresented pattern of the non-canonical G-U pairs in tRNA genes of the mitogenome is commonly present in other insects (Yang *et al.*, 2018; Jeong *et al.*, 2020).

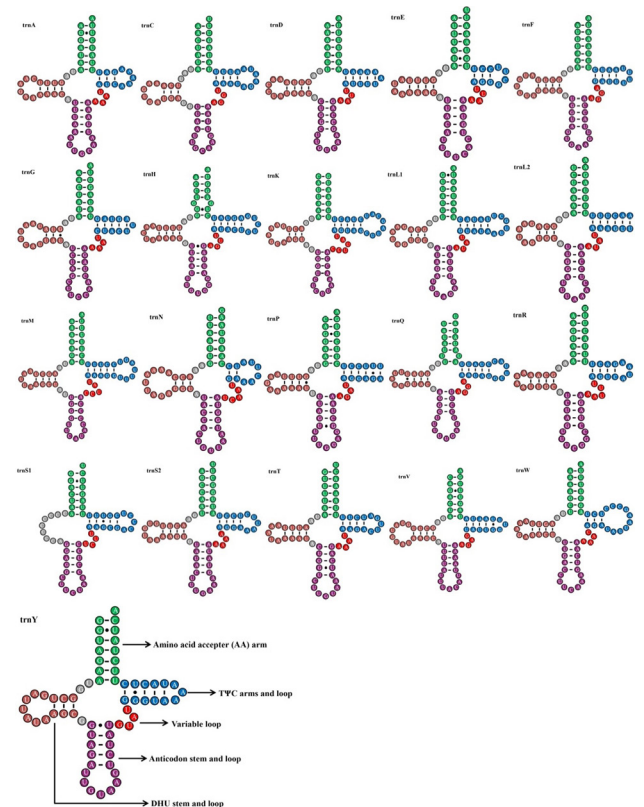


Fig. 4. Secondary structure for the tRNAs of *Polygraphus poligraphus*.

Two rRNA genes (*rrnL* and *rrnS*) were transcribed from the H-strand in *P. poligraphus*. The larger *rrnL* was 1,334 bp long, and located between the *trnL1* and *trnV*,

while the smaller *rrnS* was 772 bp in length and located behind *trnV* (Fig. 1, Table II). The rRNA genes presented a heavy AT nucleotide bias, with A + T content 74.88% in *P. poligraphus* (Table III). In the 20 mitogenomes of Scolytinae analyzed, the lengths of *rrnL* ranged from 1,239 (*Trypophloeus asperatus*) to 1,372 (*O. laricis*) bp, and of *rrnS* from 755 (*G. materiarius*) to 815 (*P. bidentatus*) bp.

Overlapping sequences and intergenic spacers

The mitogenome of *P. Poligraphus* have a total of 74 bp overlap sequences and 91 bp intergenic spacer sequences, which are all made up of 12 regions in the range from 1 to 40 bp and 1 to 34 bp, respectively. The longest overlap region is located between *trnL1* and *rrnL*, and the longest intergenic spacer region is located between *trnY* and *cox1*. However, in other Scolytinae species, the longest overlap region is located between tRNA-Leu1 and *rrnL* up to 66 bp (*G. materiarius*), and the longest intergenic spacer region is located between *rrnS* and tRNA-Ile up to 2,061 bp (*P. bidentatus*). All 19 Scolytinae species (except *O. laricis*) have identical overlap regions, *atp8-atp6* (7 bp); and all 16 Scolytinae species (except *T. asperatus*, *D. autographus*, *Pityophthorus pubescens* and *P. bidentatus*) also have identical overlap regions, *atp6-cox3* (1 bp). In 20 Scolytinae species, other regions (except tRNA-Asp-*atp8* and tRNA-Thr-tRNA-Pho regions) more and less present overlap or intergenic spacer sequences.

Phylogenetic analysis

We reconstructed phylogenetic trees based on 13 mitogenomes PCGs of the 20 Scolytinae species and three outgroups (*S. zeamais*, *C. buqueti* and *R. ferrugineus*) using MrBayes 3.2.6 and IQ-TREE (Fig. 5). In ML and BI analyses, the relationships of three genera of Scolytinae are *Polygraphus* + (*Gnathotrichus* + *Pityophthorus*). The result was consistent with previous results based on traditional classification analyses. The results presented the phylogenetic relationships and taxonomic status of *P. poligraphus*. On the one hand, since we did not sample all the genera of the Scolytinae, a more comprehensive sampling of the taxa is needed to fully resolve the genus relationships within the Scolytinae. On the other hand, our study adds to the limited data in existing databases. Most of the phylogenies of Scolytinae are reconstructed based on mitogenomes (Stauffer *et al.*, 1997; Cognato and Sperling, 2000), and we believe that more nuclear genes are needed to clarify the genus relationship of Scolytinae.

CONCLUSIONS

In this present study, we sequenced and annotated the nearly complete mitogenome of *P. poligraphus* and predicted

the secondary structures of its tRNAs. The results showed that our newly-determined mitogenome of *P. poligraphus* had a similar composition to the typical insect mitogenome. In the secondary structure of tRNA, the lack of a DHU stem in *trnS1* is consistent with all Coleoptera insects and has been confirmed as a typical feature of metazoan mitogenomes. Our *P. poligraphus* mitogenome provides an important data resource for further studies and contributes to our understanding of the phylogeny. However, additional mitogenome samples are still needed to more satisfactorily resolve the phylogeny of the Scolytinae.

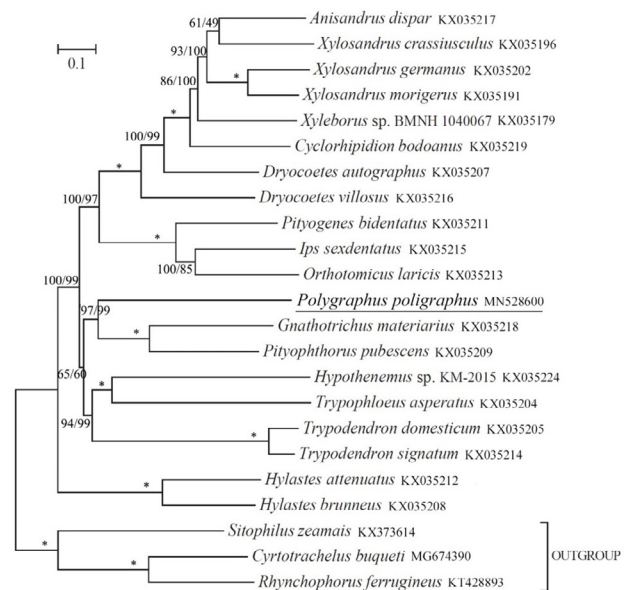


Fig. 5. Phylogenetic tree obtained from BI and ML analysis based on 13 mitochondrial protein-coding genes of 20 species within the Scolytinae. Three species within the subfamily Dryophthorinae were included as the outgroup taxa. Values at nodes indicate the support values and bootstrap values for the BI and ML trees, respectively. *, full support, bootstrap value = 100.

ACKNOWLEDGEMENTS

We are grateful to Dr. You Li (School of Forest Resources and Conservation, University of Florida, Gainesville, Florida 32611, USA) for identifying specimens.

Funding

This research was supported by the Key Research and Development Project of Ningxia (2021BEG02009), the First-class discipline of Prataculture Science of Ningxia University (No. NXYLXK2017A01) and the National Natural Science Foundation of China (No. 31961143002).

Data availability statement

Mitochondrial genome sequence can be accessed via accession number MN528600 in GenBank of NCBI at <https://www.ncbi.nlm.nih.gov/>. The associated BioProject, SRA, and BioSample numbers are PRJNA713518, SRR13972118 and SAMN18253525, respectively.

Statement of conflict of interest

The authors have declared no conflict of interests.

REFERENCES

- Bernt, M., Donath, A., Jühling, F., Externbrink, F., Florentz, C., Fritzsche, G., Pütz, J., Middendorf, M., and Stadler, P.F., 2013. MITOS improved de novo metazoan mitochondrial genome annotation. *Mol. Phylogenet. Evol.*, **69**: 313-319. <https://doi.org/10.1016/j.ympev.2012.08.023>
- Boore, J.L., 1999. Animal mitochondrial genomes. *Nucl. Acids Res.*, **27**: 1767-1780. <https://doi.org/10.1093/nar/27.8.1767>
- Cameron, S.L., 2014. Insect mitochondrial genomics: Implications for evolution and phylogeny. *Annu. Rev. Ent.*, **59**: 95-117. <https://doi.org/10.1146/annurev-ento-011613-162007>
- Chen, Z.T., and Du, Y.Z., 2017. The first two mitochondrial genomes from Taeniopterygidae (Insecta: Plecoptera): Structural features and phylogenetic implications. *Int. J. Biol. Macromol.*, **111**: 70-76. <https://doi.org/10.1016/j.ijbiomac.2017.12.150>
- Cognato, A.I., and Sperling, F.A.H., 2000. Phylogeny of *Ips* DeGeer species (Coleoptera: Scolytidae) inferred from mitochondrial cytochrome oxidase I DNA sequence. *Mol. Phylogenet. Evol.*, **14**: 445-460. <https://doi.org/10.1006/mpev.1999.0705>
- Curole, J.P., and Kocher, T.D., 1999. Mitogenomics: Digging deeper with complete mitochondrial genomes. *Trends Ecol. Evol.*, **14**: 394-398. [https://doi.org/10.1016/S0169-5347\(99\)01660-2](https://doi.org/10.1016/S0169-5347(99)01660-2)
- Duan, X.J., 2020. *Polygraphus poligraphus* special investigation and its potential hazards. *Rural Sci. Technol.*, **3**: 34-36.
- Garey, J.R., and Wolstenholme, D.R., 1989. Platyhelminth mitochondrial DNA: Evidence for early evolutionary origin of a tRNA^{ser}AGN that contains a dihydrouridine arm replacement loop, and of serine-specifying AGA and AGG codons. *J. mol. Evol.*, **28**: 374-387. <https://doi.org/10.1007/BF02603072>
- Hahn, C., Bachmann, L., and Chevreux, B., 2013. Reconstructing mitochondrial genomes directly from genomic next-generation sequencing reads—a baiting and iterative mapping approach. *Nucl. Acids Res.*, **41**: e129. <https://doi.org/10.1093/nar/gkt371>
- Hatch, M.H., 1956. The natural classification of the families of Coleoptera. *Annls entomol. Soc. Am.*, **49**: 102. <https://doi.org/10.1093/aesa/49.1.102>
- Ho, S.Y.W., and Gilbert, M.T.P., 2010. Ancient mitogenomics. *Mitochondrion*, **10**: 1-11. <https://doi.org/10.1016/j.mito.2009.09.005>
- Hu, P., and Wang, R., 2019. The complete mitochondrial genome of *Parantica sita sita* (Lepidoptera: Nymphalidae: Danainae) revealing substantial genetic divergence from its sibling subspecies *P. s. nipponica*. *Gene*, **686**: 76-84. <https://doi.org/10.1016/j.gene.2018.10.088>
- Hunt, T., Bergsten, J., Levkanicova, Z., Papadopoulou, A., John, O.S., Wild, R., Hammond, P.M., Ahrens, D., Balke, M., Caterino, M.S., Gómez-Zurita, J., Ribera, I., Barraclough, T.G., Bocakova, M., Bocak, L., Vogler, A.P., 2007. A comprehensive phylogeny of beetles reveals the evolutionary origins of a superradiation. *Science*, **318**: 1913-1916. <https://doi.org/10.1126/science.1146954>
- Jeong, J.S., Kim, M.J., and Kim, I., 2020. The mitochondrial genome of the dung beetle, *Copris tripartitus*, with mitogenomic comparisons within Scarabaeidae (Coleoptera). *Int. J. Biol. Macromol.*, **144**: 874-891. <https://doi.org/10.1016/j.ijbiomac.2019.09.165>
- Kalyaanamoorthy, S., Minh, B.Q., Wong, T.K.F., von Haeseler, A., and Jermin, L.S., 2017. Model finder: fast model selection for accurate phylogenetic estimates. *Nat. Methods*, **14**: 587-589. <https://doi.org/10.1038/nmeth.4285>
- Kang, X.M., 2016. Risk analysis of *Polygraphus poligraphus*, a dangerous pest. *For. Shanxi*, **3**: 44-45. (in chinese).
- Katoh, K., and Standley, D.M., 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Mol. Biol. Evol.*, **30**: 772-780. <https://doi.org/10.1093/molbev/mst010>
- Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Meintjes, P., and Drummond, A., 2012. Geneious basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics*, **28**: 1647-1649. <https://doi.org/10.1093/bioinformatics/bts199>
- Kumar, S., Stecher, G., and Tamura, K., 2016. MEGA7: Molecular evolutionary genetics analysis version

- 7.0 for bigger datasets. *Mol. Biol. Evol.*, **33**: 1870-1874. <https://doi.org/10.1093/molbev/msw054>
- Lavrov, D.V., Brown, W.M., and Boore, J.L., 2000. A novel type of RNA editing occurs in the mitochondrial tRNAs of the centipede *Lithobius forficatus*. *Proc. natl. Acad. Sci.*, **97**: 13738-13742. <https://doi.org/10.1073/pnas.250402997>
- Li, H., Gao, J.Y., Liu, H.Y., and Cai, W.Z., 2009. Progress in the researches on insect mitochondrial genome and analysis of gene order. *Sci. Found. China*, **17**: 39-47. <https://doi.org/10.1088/1005-0841/17/2/004>
- Lin, C.P., and Danforth, B.N., 2004. How do insect nuclear and mitochondrial gene substitution patterns differ? Insights from Bayesian analyses of combined datasets. *Mol. Phylogenet. Evol.*, **30**: 686-702. [https://doi.org/10.1016/S1055-7903\(03\)00241-0](https://doi.org/10.1016/S1055-7903(03)00241-0)
- Nguyen, L.T., Schmidt, H.A., von Haeseler, A., and Minh, B.Q., 2015. IQ-TREE: A fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Mol. Biol. Evol.*, **32**: 268-274. <https://doi.org/10.1093/molbev/msu300>
- Ojala, D., Montoya, J., and Attardi, G., 1981. tRNA punctuation model of RNA processing in human mitochondria. *Nature*, **290**: 470-474. <https://doi.org/10.1038/290470a0>
- Perna, N.T., and Kocher, T.D., 1995. Patterns of nucleotide composition at fourfold degenerate sites of animal mitochondrial genomes. *J. mol. Evol.*, **41**: 353-358. <https://doi.org/10.1007/BF01215182>
- Ranwez, V., Douzery, E.J.P., Cambon, C., Chantret, N., and Delsuc, F., 2018. MACSE v2: Toolkit for the alignment of coding sequences accounting for frameshifts and stop codons. *Mol. Biol. Evol.*, **35**: 2582-2584. <https://doi.org/10.1093/molbev/msy159>
- Ribera, I., Nilsson, A.N., and Vogler, A.P., 2004. Phylogeny and historical biogeography of Agabinae diving beetles (Coleoptera) inferred from mitochondrial DNA sequences. *Mol. Phylogenet. Evol.*, **30**: 545-562. [https://doi.org/10.1016/S1055-7903\(03\)00224-0](https://doi.org/10.1016/S1055-7903(03)00224-0)
- Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D.L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M.A., and Huelsenbeck, J.P., 2012. MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Syst. Biol.*, **61**: 539-542. <https://doi.org/10.1093/sysbio/sys029>
- Schuster, G., and Stern, D., 2009. RNA polyadenylation and decay in mitochondria and chloroplasts. *Prog. Mol. Biol. Transl. Sci.*, **85**: 393-422. [https://doi.org/10.1016/S0079-6603\(08\)00810-6](https://doi.org/10.1016/S0079-6603(08)00810-6)
- Seifert, E., 2014. OriginPro 9.1: scientific data analysis and graphing software software review. *J. Chem. Inf. Model.*, **54**: 1552. <https://doi.org/10.1021/ci500161d>
- Song, N., Liu, H.Y., Yang, X.J., Zhao, X.C., and Lin, A.L., 2018. Complete mitochondrial genome of the darkling beetle *Gonocephalum outreyi* (Coleoptera: Tenebrionidae) with phylogenetic implications. *J. Asia Pac. Ent.*, **21**: 721-730. <https://doi.org/10.1016/j.aspen.2018.05.001>
- Stauffer, C., Lakatos, F., and Hewitt, G.M., 1997. The phylogenetic relationships of seven European *Ips* (Scolytidae, Ipinæ) species. *Insect Mol. Biol.*, **6**: 233-240. <https://doi.org/10.1046/j.1365-2583.1997.00177.x>
- Sun, H.Q., Zhao, W.X., Lin, R.Z., Zhou, Z.F., Huai, W.X., and Yao, Y.X., 2020. The conserved mitochondrial genome of the jewel beetle (Coleoptera: Buprestidae) and its phylogenetic implications for the suborder Polyphaga. *Genomics*, **112**: 3713-3721. <https://doi.org/10.1016/j.ygeno.2020.04.026>
- Sun, X.L., 2005. *Studies on species and genera of Scolytidae (Insecta: Coleoptera) with reference to invasive to China*. Beijing Forestry University, Beijing, China.
- Talavera, G., and Castresana, J., 2007. Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. *Syst. Biol.*, **56**: 564-577. <https://doi.org/10.1080/10635150701472164>
- Viklund, L., Rahmani, R., Bång, J., Schroeder, M., and Hedenström, E., 2019. Optimizing the attractiveness of pheromone baits used for trapping the four-eyed spruce bark beetle *Polygraphus poligraphus*. *J. appl. Ent.*, **143**: 721-730. <https://doi.org/10.1111/jen.12641>
- Wang, W.Q., Huang, Y.X., Bartlett, C.R., Zhou, F.M., Meng, R., and Qin, D.Z., 2019. Characterization of the complete mitochondrial genomes of two species of the genus *Aphaena* Guérin-Ménéville (Hemiptera: Fulgoridae) and its phylogenetic implications. *Int. J. Biol. Macromol.*, **141**: 29-40. <https://doi.org/10.1016/j.ijbiomac.2019.08.222>
- Wang, Y., Cao, J.J., Li, N., Ma, G.Y., and Li, W.H., 2019. The first mitochondrial genome from Scopuridae (Insecta: Plecoptera) reveals structural features and phylogenetic implications. *Int. J. Biol. Macromol.*, **122**: 893-902. <https://doi.org/10.1016/j.ijbiomac.2018.11.019>
- Wei, S.J., Shi, M., Chen, X.X., Sharkey, M.J.,

- Achterberg, C., Ye, G.Y., and He, J.H., 2010. New views on strand asymmetry in insect mitochondrial genomes. *PLoS One*, **5**: e12708. <https://doi.org/10.1371/journal.pone.0012708>
- Wolstenholme, D.R., 1992. Genetic novelties in mitochondrial genomes of multicellular animals. *Curr. Opin. Genet. Dev.*, **2**: 918-925. [https://doi.org/10.1016/S0959-437X\(05\)80116-9](https://doi.org/10.1016/S0959-437X(05)80116-9)
- Wolstenholme, D.R., 1992. Animal mitochondrial DNA: structure and evolution. *Int. Rev. Cytol.*, **141**: 173-216. [https://doi.org/10.1016/S0074-7696\(08\)62066-5](https://doi.org/10.1016/S0074-7696(08)62066-5)
- Wood, S.L., 1977. Introduced and exported American Scolytidae (Coleoptera). *Great Basin Nat.*, **37**: 67-74. <https://www.jstor.org/stable/41711552>
- Yang, W.Z., Zhang, Y., Feng, S.Q., Liu, L.J., and Li, Z.H., 2018. The first complete mitochondrial genome of the Japanese beetle *Popillia japonica* (Coleoptera: Scarabaeidae) and its phylogenetic implications for the superfamily Scarabaeoidea. *Int. J. Biol. Macromol.*, **118**: 1406-1413. <https://doi.org/10.1016/j.ijbiomac.2018.06.131>
- Yin, H.F., Huang, F.S., and Li, Z.L., 1984. *Economic entomology of China (Section 29: Coleoptera: Scolytidae)*. Science Press, Beijing. pp. 56-183.
- Zeng, L.Y., Pang, Y.T., Feng, S.Q., Wang, Y.N., Stejskal, V., Aulicky, R., Zhang, S.F., and Li, Z.H., 2021. Comparative mitochondrial genomics of five *Dermestid* beetles (Coleoptera: Dermestidae) and its implications for phylogeny. *Genomics*, **113**: 927-934. <https://doi.org/10.1016/j.ygeno.2020.10.026>
- Zhang, D., Gao, F., Jakovlić, I., Zou, H., Zhang, J., Li, W.X., and Wang, G.T., 2020. PhyloSuite: An integrated and scalable desktop platform for streamlined molecular sequence data management and evolutionary phylogenetics studies. *Mol. Ecol. Resour.*, **20**: 348-355. <https://doi.org/10.1111/1755-0998.13096>
- Zhang, H.L., Liu, N., Han, Z.P., and Liu, J.X., 2016. Phylogenetic analyses and evolutionary timescale of Coleoptera based on mitochondrial sequence. *Biochem. Syst. Ecol.*, **66**: 229-238. <https://doi.org/10.1016/j.bse.2016.04.014>
- Zhang, Z.Q., Wang, X.J., Li, R.Z., Guo, R.J., Zhang, W., Song, W., Hao, C.F., Wang, H.P., and Li, M.L., 2015. The mitochondrial genome of *Dastarcus helophoroides* (Coleoptera: Bothrideridae) and related phylogenetic analyses. *Gene*, **560**: 1-9. <https://doi.org/10.1016/j.gene.2014.12.026>