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

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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

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.

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 from14 to19 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

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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 O*rthotomicus 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	*Anisandrus dispar*	KX035217
		Xylosandrus crassiusculus	KX035196
		X. germanus	KX035202
		X. morigerus	KX035191
		Xyleborus sp.	KX035179
		Cyclorhipidion bodoanus	KX035219
		Dryocoetes autographus	KX035207
		D. villosus	KX035216
		Pityogenes bidentatus	KX035211
		Ips sexdentatus	KX035215
		Orthotomicus laricis	KX035213
		Gnathotrichus materiarius	KX035218
		Pityophthorus pubescens	KX035209
		Hypothenemus sp.	KX035224
		Trypophloeus asperatus	KX035204
		Trypodendron domesticum	KX035205
		T. signatum	KX035214
		Hylastes attenuatus	KX035212
		H. brunneus	KX035208
	Dryophthorinae	*Sitophilus zeamais*	KX373614
		Cyrtotrachelus buqueti	MG674390
		Rhynchophorus ferrugineus	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

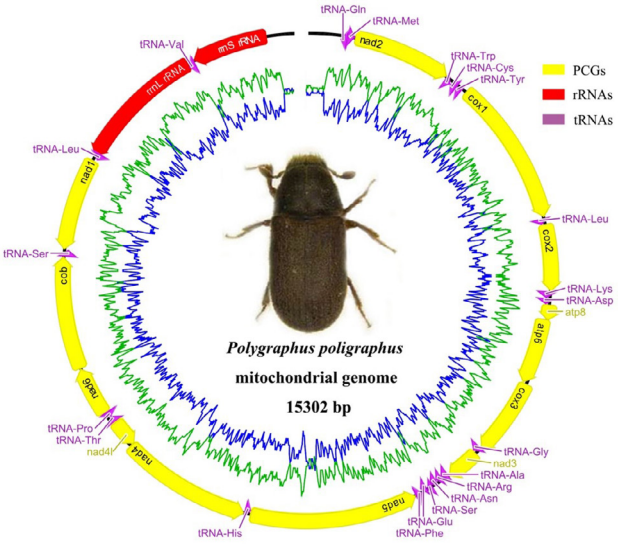

Fig. 1. Mitochondrial genome map of the *Polygraphus poligraphus*. Circular map was drawn with Geneious 10.1.3 (http://www.geneious.com/). The transcriptional direction is indicated with arrows.

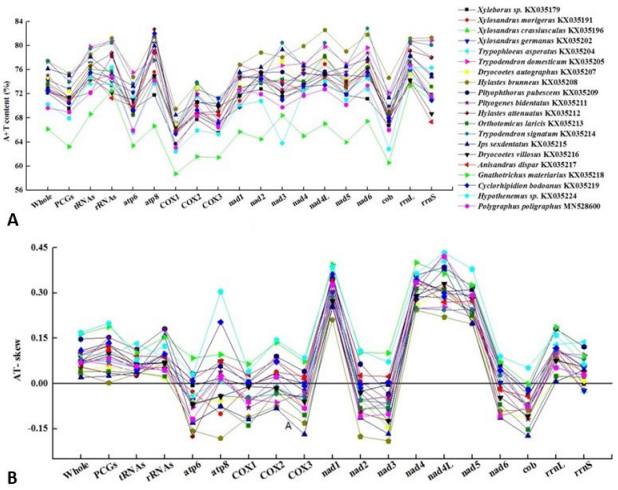

Fig. 2. Comparison of the A + T contents, nucleotide skewness of twenty speciesof Scolytinae. (A) A + T content; (B) AT-skew; (C) GC-skew.

Protein-coding genes and codon usage

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*

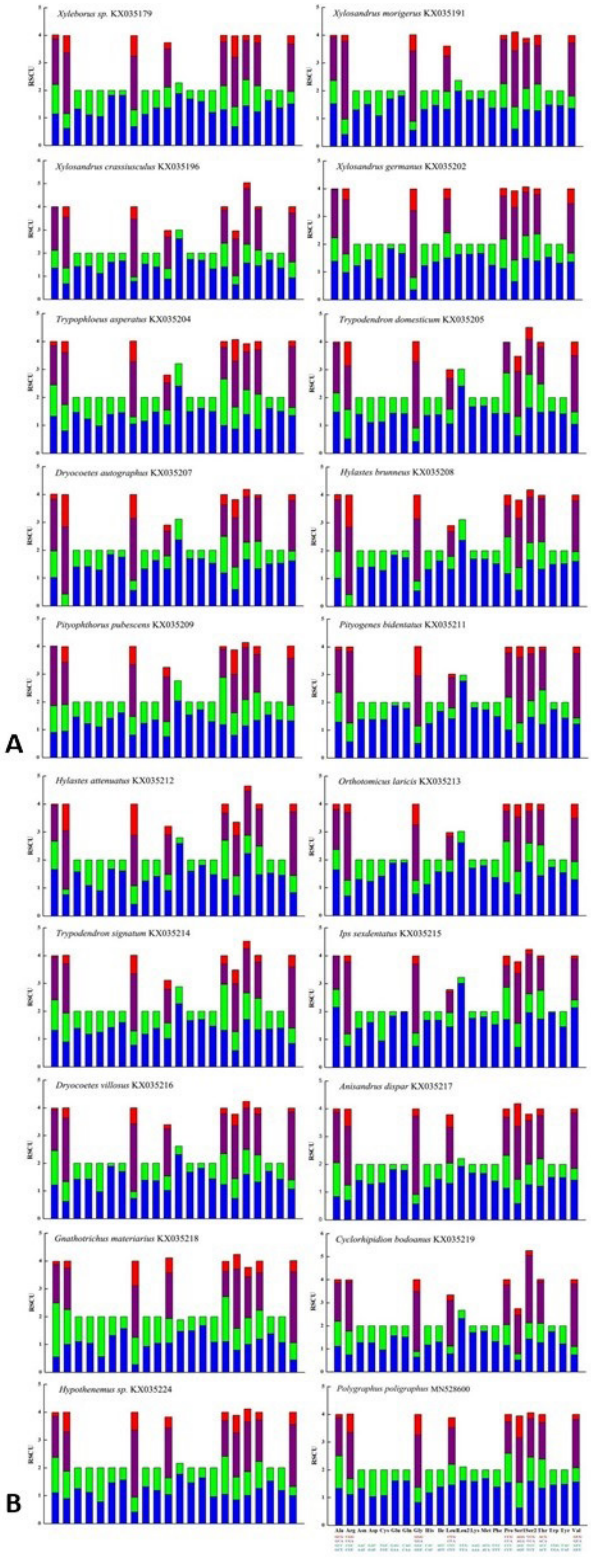

Fig. 3. Relative synonymous codon usage (RSCU) of the mitogenomes of twenty species of Scolytinae.

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).

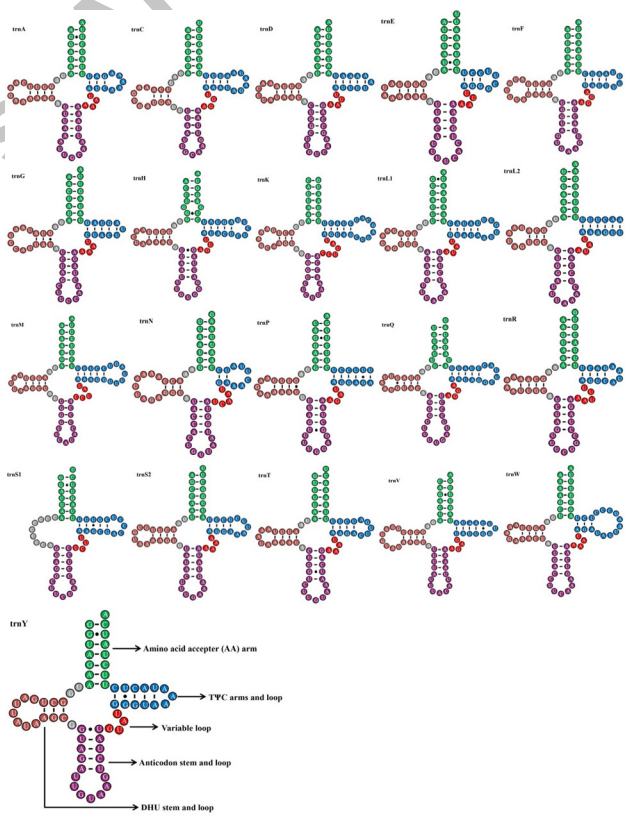

Fig. 4. Secondary structure for the tRNAs of *Polygraphus poligraphus*.

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-Leu1and 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 indentical overlap regions, atp8-atp6 (7 bp); and all 16 Scolytinae species (except *T. asperatus*, *D. autographus*, *Pityophthorus pubescens* and *P. bidentatus*) also have indentical 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 phylogenys 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.

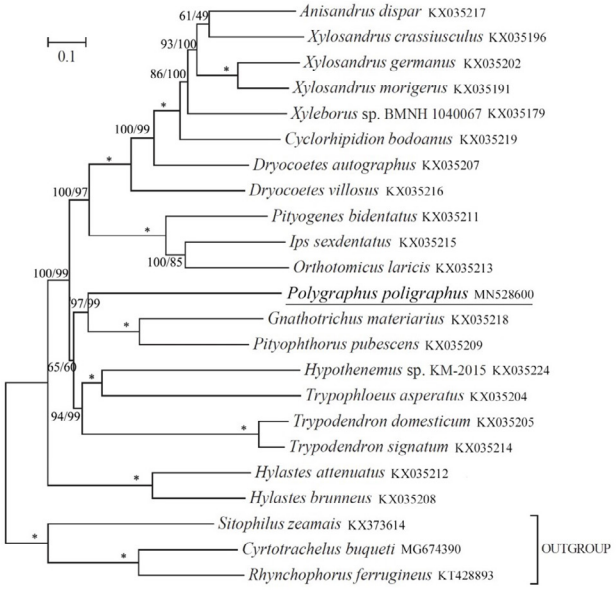

Fig. 5. Phylogenetic tree obtained from BI and ML analysis based on 13 mitochondrial protein-coding genes of 20 species within the Scolytinae. Three species within the subfamily Dryophthorinae were included as the outgroup taxa. Values at nodes indicate the support values and bootstrap values for the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

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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.

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