The First Conserved Mitochondrial Genome of Polygraphus Poligraphus (Coleoptera: Curculionidae) and Its Phylogenetic Implications

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Abstract

**Background:** *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. So far, there is no mitochondrial genome reported for *P. poligraphus*.

**Results:** 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. Phylogenetic analysis indicated that the *P. poligraphus* mitogenome clustered with *Gnathotrichus materiarius* and *Pityophthorus pubescens* mitogenomes in a monophyletic manner. The phylogeny of these three genera of Scolytinae is presented as Polygraphus + (Gnathotrichus + Pityophthorus).

**Conclusions:** The results presented herein will provide a reference for further molecular taxonomy, evolution and phylogenetic research of *P. poligraphus*. However, additional mitogenome samples are still needed to more satisfactorily resolve the phylogeny of the Scolytinae.

**Background**

The mitochondrion is a fundamental eukaryotic organelle, descended from an alphaproteobacterium that formed a permanent symbiosis with the ancestral eukaryote roughly 2 billion years ago [1]. The mitochondrial genomes of arthropods have been studied extensively, and insects represent approximately 80% of the arthropod mt genomes that have been sequenced [1]. 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) [2–4]. 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 [5–7]. 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 [8–10]. Over the past decade, mitochondrial genomes have become widely used for molecular evolution, population genetics, systematics and phylogenetics [1, 11–15].

Coleoptera, the largest insect order, contains 4 suborders (Archostemata, Adephaga, Polyphaga and Myxophaga), 17 superfamilies, 168 families and over 380,000 described species. Of these, about 10,000 are known in China [16, 17]. Polygraphus poligraphus L., four-eyed spruce bark beetle, belongs to Scolytinae of Curculionidae of Coleoptera [18]. 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, Turkey, Poland, Czech Republic, Germany, Austria, former Yugoslavia and Gansu, Jilin, Liaoning, Heilongjiang, Neimenggu, and Ningxia provinces in China [19]. 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 [19, 20]. The morphology, biology and biological and chemical control of P. poligraphus have been studied [21–23]. 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 1000 partially sequenced mitogenomes placed in the GenBank database for Coleoptera (last visited on March 25, 2021) [24]. 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 of these species 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, construct of tRNA secondary structures and phylogenetic relationships of the P. poligraphus mitogenome.

Materials

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 -80°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 NEBNext® Ultra™ DNA Library Prep Kit according to the manufacturer’s instructions.

**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 [25]. Subsequently, the mitochondrial genomes of *P. poligraphus* were annotated with Geneious 10.1.3 ((http://www.geneious.com/) 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) [26]. Strand asymmetry was calculated according to the formulas: AT-skew = [A-T] / [A + T] and GC-skew = [G-C] / [G + C] [27]. The A + T content, AT-skew, GC-skew, were graphically plotted by OriginPro 9.1 [28]. The base composition and the relative synonymous codon usage (RSCU) were calculated using MEGA version 7.0 [29].

**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*, *Cyrtyotrachelus buqueti* and *Rhynchophorus ferrugineus*) were downloaded from GenBank (Table 1). Ahead of Bayesian inference analysis, the Bioedit version 7.2.5 [30] was used to reconfirm the gene boundaries and remove the ambiguous sites by aligning the *P. poligraphus* and previously reported Scolytinae 13 PCGs. The nucleotide substitution model was individually selected for each of two rRNAs and three codon positions of concatenated PCGs using jModelTest version 2.1.4 [31] using the Akaike Information Criterion (AIC) [32]. The best-fit nucleotide substitution models were selected as ‘GTR + G + I’. The phylogenetic tree was reconstructed using Bayesian 3.2.0 [33] based on 13 mitochondrial protein-coding genes.
Table 1
List of species used to construct the phylogenetic tree

| Family       | Subfamily | Species             | Accession number |
|--------------|-----------|---------------------|------------------|
| Curculionidae| Scolytinae| *Anisandrus dispers*| 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   |

Results And Discussion

Genome organization and base composition

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 protein-coding genes (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 2).
| 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         |                        |
| Feature | Strand | Location       | Size(bp) | Start code | Stop codon | Anticodon | Intergenic nucleotides |
|---------|--------|----------------|----------|------------|------------|-----------|------------------------|
| 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      |            |            | ——        |                        |

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 3). Generally, the whole mitogenomes of Scolytinae exhibited a strong base composition bias toward 66.15% (*Gnathotrichus materiarius*) − 77.46% (*Hylastes brunneus*) for A + T content. The entire mitogenomes with a high A + T content benefit from the composition of PCGs, tRNAs and rRNAs. Scolytinae. 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 [27, 34]. 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 less 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 [34].
Table 3
Composition and skewness in the *Polygraphus 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  |

**Protein-coding genes and codon usage**

The PCGs of the mitogenome were 11,061 bp long for *P. poligraphus* (Table 3). 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 2). 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 [35, 36].

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 Fig. 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 2). As shown in Fig. 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 [1, 24, 37–41]. 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 [24, 42].

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 2). The rRNA genes presented a heavy AT nucleotide bias, with A + T content 74.88% in *P. poligraphus* (Table 3). 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 the 20 Scolytinae species and three outgroups (*S. zeamais, C. buqueti* and *R. ferrugineus*) 13 mitogenomes PCGs using Bayesian 3.2.0 (Fig. 5). Phylogenetic analysis showed that the *P. poligraphus* mitogenome clustered clearly with the *G.*
materiarius and P. pubescens mitogenomes in a monophyletic manner. The phylogeny of these three genera of Scolytinae is presented as Polygraphus + (Gnathotrichus + Pityophthorus). The result is consistent with previous results based on traditional classification analyses. The principal aim of this study was to determine the phylogeny of Scolytinae and the location 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 [43, 44], 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.

Declarations

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Ethics approval and consent to participate

All experimental procedures were approved by the School of Agriculture of Ningxia University.

Consent for publication

Not applicable.

Competing interests
The authors declare that they have no competing interests.

**Availability of supporting data**

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.

**Authors’ contributions**

XM S 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. YC Z and P Z assisted in collecting *Polygraphus poligraphus*. YX M participated in the experiments. M B and XP W supervised this study, contributed to the design of the study and drafting the manuscript. All authors read and approved the final manuscript.

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**Figures**
Figure 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.
Figure 2

Comparison of the A + T contents, nucleotide skewness of twenty species of Scolytinae. (A) A + T content; (B) AT-skew; (C) GC-skew.
Figure 3

Relative synonymous codon usage (RSCU) of the mitogenomes of twenty species of Scolytinae.
Figure 4

Secondary structure for the tRNAs of Polygraphus poligraphus
Figure 5

Phylogeny of 20 species within the Scolytinae based on the Bayesian analysis of 13 mitochondrial protein-coding genes. ‘GTR+G+I’ was used as the best-fit nucleotide substitution model. The support values are shown next to the nodes. Three species within the subfamily Dryophthorinae were included as the outgroup taxa.