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# The complete mitochondrial genome of the cockroach *Eupolyphaga sinensis* (Blattaria: Polyphagidae) and the phylogenetic relationships within the Dictyoptera

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**Abstract** We present the complete mitochondrial DNA sequence of *Eupolyphaga sinensis*. This closed circular molecule is 15553 bp long and consists of 37 genes that encode for 13 inner membrane proteins, 2 ribosomal RNAs and 22 transfer RNAs. The genome shares the gene order and orientation with previously known Blattaria mitochondrial genomes. All tRNAs could be folded into the typical cloverleaf secondary structure, but the tRNASer (AGN) appears to be missing the DHU arm. The A + T-rich region is 857 bp long and longer than other cockroaches. Based on the concatenated amino acid sequences of all protein coding genes of *E. sinensis* in conjunction with those 23 other arthropod sequences, we reconstruct the phylogenetic tree. Phylogenetic analyses shows that Blataria (including Isoptera) and the Mantodea are sister groups. Furthermore the relationship of the three basal clades of winged insects are different from the three previous hypotheses ((Ephemeroptera + Odonata) +Neoptera, Ephemeroptera + (Odonata + Neoptera), Odonata + (Ephemeroptera +Neoptera)). The Ephemeroptera (*Parafronurus youi*) clusters with the Plecoptera (*Pteronarcys princes*).

**Keywords** Complete mitochondrial genome · Blattaria · Polyphagidae · *Eupolyphaga sinensis* · Dictyoptera · Phylogenetic analysis

## Introduction

Complete mitochondrial genome sequences are popular molecular markers used for phylogenetic, phylogeographic and ecological studies in different animal lineages [1].With the development of molecular biology techniques, more and more mitochondrial genomes have been reported. Since Wolstenholme and Clary [2] reported the first mtDNA sequences, more than 1018 complete metazoa mitochondrial genomes have been determined. However, current knowledge on mtDNAs is very uneven; most complete mt-genomes have been sequenced for vertebrate taxa available in GenBank (http://www.ncbi.nlm.nih.gov/). Insects constitute the most species-rich class among animals with almost a million of taxa described to date [3], but only 114 mitochondrial genomes are from insects (http://www.ncbi.nlm.nih.gov/). Moreover current genomic knowledge of Blattaria is very scanty and the current taxa sampling is extremely poor. In fact, only two species are in Blattaria, *Periplaneta fuliginosa* [4] and *Blattella germanica* (unpublished).

*Eupolyphaga sinensis* Walker belongs to the family Polyphagidae and the order Blattaria. This species is widely distributed in China, but there are few reported in other countries. *E. sinensis* has been used to be a traditional Chinese medicine. Studies showed that it plays an important role in dealing with thrombi [5, 6]. Despite a lot of studies have been reported, they were limited to the researches on morphological, physiological and biological features, few researches on molecular biology have been done yet.

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**Table 1** Sequencing primers used in the analysis of *E. sinensis*

| Primer name | Upstream primers sequences(5′-3′) | Downstream primers sequences(5′-3′) | Anneal temperature |
|---|---|---|---|
| 1 | Ti-J-8:TGCCTGATAAAAAGGRTTAYCKTGATAG | C1-N-2329:CTGTAAATATATGATGAGCTC | 50°C |
| 2 | C1-J-2183:CAACAYTTATTTTGATTYTTTGG | TK-N-3785:GTTTAAAGAACCAWTACTTR | 50°C |
| 3 | C2-J-3640:AAAGCTGATGCAACCCCTGGACGATT | N4-N-8700:GGCTTATATTTTAATAATTGCTCATGG | 52°C |
| 4 | N4-J-8502:GARGGRGGAGCAGCTATATTAS | CB-N-11367:ATTACWCCTCCTAATTTRTTAGGAAT | 50°C |
| 5 | CB-J-11800:CAATGAGTATGAGGAGGATTTGCTGT | SR-N-14750:TGTGCCAGCAGTCGCGGTTATACA | 50°C |
| 6 | SR-J-14588:ATAATAGGTATCTAATCCTAGTTT | N2-N-292:TCTAAGCCTGTTCATACACCT | 55°C |

There are many arguments about the phylogenetic relationships among winged insects. The phylogenetic relationship of the Dictyoptera has always been a hot spot. Henning [7] and Kristensen [8] suggest that Blattaria is the sister group to Isoptera, forming the group Dictyoptera (Mantodea + (Blattaria + Isoptera)). But some studies [9–11] are in conflict with their view. They reckon that the phylogenetic relationship of the Dictyoptera should be Isoptera + (Blataria + Mantodea). Besides, the relationships among Ephemeroptera, Odonata and Neoptera are ambiguous. As Zhang et al. [12] reported, there are three main hypotheses as follows: the basal Paleoptera hypothesis ((Ephemeroptera + Odonata) + Neoptera), the basal Ephemeroptera hypothesis (Ephemeroptera + (Odonata + Neoptera)) and the basal Odonata hypothesis (Odonata + (Ephemeroptera +Neoptera)). With the application of molecular markers, all the three hypotheses were supported by some researchers respectively [12–18].

In this study, we were able to sequence and describe the complete mitochondrial genome of *E. sinensis*. The newly determined mtDNA is the first complete sequence for the family Polyphagidae and the third for the order Blattaria. On the one hand, the sequences given in the recent study may not only provide useful information to phylogenetic researches of Blattaria and other insects, but also for developing mitogenome genetic markers for species identification in the *Eupolyphaga* species complex. On the other hand, it may accelerate the researches on molecular biology of *E. sinensis*.

## Materials and methods

### Samples and DNA extraction

Specimens of *E. sinensis* were sampled from the cockroach isolated from our laboratory. Total genomic DNA was extracted from the muscle of the fresh specimen's femora using the standard Proteinase K and phenol/chloroform extraction method. An aliquot of the re-suspended DNA was used as the template for the subsequent PCR reactions.

### Primer design and PCR amplification

The primers were designed based on universal primers [19] and compared with the sequence of *B. germanica* (unpublished). Six pairs of specific primers were designed to amplify the complete mitochondrial genome in six fragments of approximately 2 kb (1), 1.6 kb (2), 5 kb (3), 2.8 kb (4), 2.8 kb (5), 1.5 kb (6), via long PCR (Table 1).

Long PCR conditions were the following: an initial denaturation for 2 min at 94°C, followed by 15 cycles of denaturation 30 s at 94°C, annealing 30 s at 50–55°C (depending on primer combinations), elongation 60–300 s (depending on putative length of the fragments) at 68°C; then followed the other PCR program by 15 cycle of denaturation 30 s at 94°C, annealing 30 s at 50–55°C, elongation 60–300 s + 8 s/cycle at 68°C and a final extension period of 68°C for 10 min.

Amplifications were performed on a DNA Engine ® Peltiter Thermol Cycler (BIO-RAD) in 25 μl reaction volume composed of: 13.5 μl of sterilized distilled water, 2.5 μl of LA PCR Buffer II (Takara), 2.5 μl of 25 mM $MgCl_2$, 4 μl of dNTPs mix, 1 μl of each primer (10 μM), 0.5 μl of DNA template and 0.25 μl (1.25 U) of TaKaRa LATaq polymerase (Takara).

### Purifying, sequencing and sequence assembling

PCR products were tested by electophoresis on an agarose gel. When a single band was observed in the PCR products, long-PCR fragments were purified using the V-gene PCR Clean-up purification Kit. If more than one band present, the appropriately sized PCR product was cut from the gel and extracted using a Biospin Gel Extraction Kit. Four fragments were sequenced in both directions directly, and large PCR products were sequenced by primer walking strategy. Two exceptional fragments amplified by N4-J-8502/CB-N-11367 and C2-J-3640/N4-N-8700 were ligated to the pGEM-T Easy Vector (Promega). Each resulting clone was sequenced as well as other four fragments. Sequences were manually checked and assembled using

the software BioEdit and Chromas 2.22 and Seqman (DNASTAR, Steve, 2001).

### Data analysis: gene annotation and analysis

Genes encoding proteins, ribosomal RNAs (rRNA) and transfer RNAs (tRNA) were identified according to their amino acid translation or secondary structure features, respectively. Individual gene sequences were compared with the available homologous sequence of *Periplaneta fuliginosa* in GenBank. The secondary structure of tRNA genes was manually reconstructed using the software DNASIS (Ver.2.5). Protein-coding genes were identified using SEQUIN (Ver.5.35) and compared with other related insects.

The nucleotide and amino acid sequences were aligned by ClustalX1.8 [20]. Phylogenetic analysis were performed on the complete amino acid sequences of all the protein coding genes (PCGs) using a Bayesian approach as implemented in MrBayes 3_0b4 [21]. Following the conclusion of Boore et al. [22] and the results of Carapelli et al. [23], we used the corresponding sequences of *Pagurus longicarpus* and *Penaeus mondon* of Decapoda as outgroups for rooting.

## Results and discussion

### Gene content and genome organization

The mtDNA genome of *E. sinensis* is a circular molecule of 15553 bp. It contains the entire set of 37 genes usually found in metazoans: 13 PCGs, 22 tRNA genes, and 2 ribosomal genes (Fig. 1; GenBank accession number FJ830540). The length in *E. sinensis* is 557 and 531 bp longer than that in *P. fuliginosam* [4] and *B. germanica* (unpublished) respectively. The gene order is identical to *Drosophila melanogaster*, in spite of one *trnF* in *D. melanogaster* has changed to *trnM* in *E. sinensis*. The structure of mtDNA is conserved in divergent insect orders and even some crustaceans [24, 25]. Such an extensive conservation in orientation and gene order in divergent insects has been inferred to be ancestral among insects [22]. The majority of genes are found on the plus or J-strand, the remainder having opposite polarity and being oriented on the minus or N-strand. The mtDNA genome of *E. sinensis* also contains 10 non-coding regions, spanning 1–857 bp. The largest non-coding region is located at the gene junctions *rrnS/trnI,* which is presumed to be homologous to the A + T-rich region of other hexapods by positional homology and shared features. This region is considered to regulate and initiate the replication and transcription of the mitochondrial genome [26].

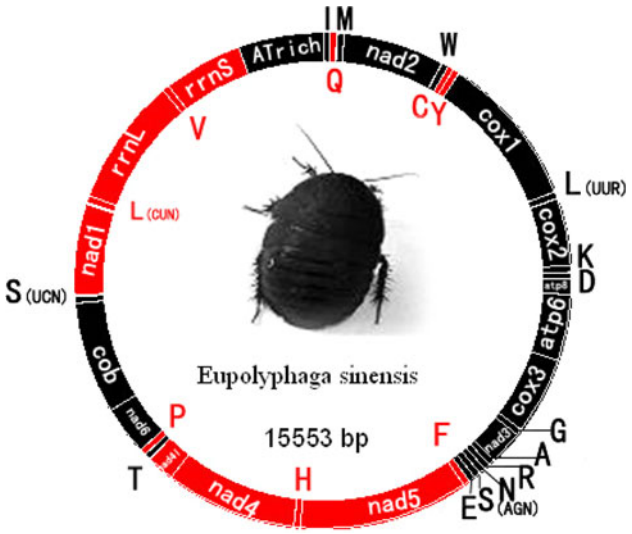


**Fig. 1** The mitochondrial genome organization of *E. sinensis*. Genes for proteins and rRNAs are indicated with standard abbreviations, whereas those for tRNAs are designated by a single letter for the corresponding amino acid. Black color is used for the genes (I, M, *nad2*, W, *cox1*, L(UUR), *cox2*, K, D, *atp8*, *atp6*, *cox3*, G, *nad3*, A, R, N, S, E, T, *nad6*, *cob*, S (UCN)) oriented on the J-strand, red for those (Q, C, Y, F, *nad5*, H, *nad4*, *nad4L*, P, *nad1*, L(CUN), *rrnL*, V, *rrnS*) with opposite polarity

The composition of the j-strand of *E. sinensis* mtDNA is 31.6% T, 40.4% A, 17.5% C, and 10.5% G, with a total A + T content of 72.0%.This value is well in the range of other arthropods, which show a remarkable variability, from 69.5% to 84.9% A + T content [27, 28] But it is lower than *B. germanica* and *P. fuliginosa* (74.56% and 75.14%).

### Protein-coding genes and codon usage

The mtDNA of *E. sinensis* contains the full set of PCGs usually present in animal mtDNA. Canonical initiation codons (ATA or ATG), encoding the amino acid methionine, are used in 8 PCGs (*nad2-4, cox2-3, atp6, nad4L, cob*), whereas five other genes start with non-standard codons (c*ox1, atp8, nad6, nad1, nad5*) (Table 2) as it often happens in animal mtDNAs. It is noteworthy that the cytochrome oxidase subunit I (*cox1*) gene often starts with non-standard codon in other species. It has been extensively discussed in several arthropod species [29]. Tetranucleotides (ATAA, TTAA, and ATTA) and a hexanucleotide (ATTTAA) were postulated as the initiation codon of the *cox1* gene, but the initiation codon of the *cox1* gene in *E. sinensis* is the trinucleotide ATT.

Complete termination codons TAG and TAA were found in three (*nad1-3*) and six (*atp8*, *atp6*, *nad5*, *cox3*, *nad6*, *cob*) PCGs, respectively. The remaining four genes are supposed to end with TA (*cox1*, *cox2*) or a single T (*nad4*, *nad4L*). Not surprisingly, their function would be recovered into a complete TAA stop codon by a post-

**Table 2** Annotation, nucleotide composition and other features of the mitochondrial genome of *E. sinensis*

| Gene | Strand | Sites | Seq position | %T | %C | %A | %G | %A + T | Start codon | Stop codon |
|---|---|---|---|---|---|---|---|---|---|---|
| *trnI* | J | 69 | 1–69 | 34.8 | 11.6 | 33.3 | 20.3 | 68.1 | | |
| *trnQ* | N | 69 | 67–135 | 44.9 | 5.8 | 31.9 | 17.4 | 76.8 | | |
| *trnM* | J | 68 | 144–211 | 25 | 22.1 | 38.2 | 14.7 | 63.2 | | |
| *nd2* | J | 1020 | 212–1231 | 34.4 | 18.7 | 36.2 | 10.7 | 70.6 | ATG | TAG |
| *trnW* | J | 67 | 1236–1302 | 31.3 | 14.9 | 44.8 | 9 | 76.1 | | |
| *trnC* | N | 68 | 1295–1362 | 33.8 | 10.3 | 38.2 | 17.6 | 72 | | |
| *trnY* | N | 66 | 1363–1428 | 31.8 | 9.1 | 33.3 | 25.8 | 65.1 | | |
| *cox1* | J | 1544 | 1430–2973 | 35.9 | 17.2 | 31 | 15.9 | 66.9 | ATT | TAN |
| *trnL*(UUR) | J | 70 | 2974–3043 | 34.3 | 12.9 | 35.7 | 17.1 | 70 | | |
| *cox2* | J | 680 | 3049–3728 | 34.1 | 17.5 | 35.6 | 12.8 | 69.7 | ATG | TAN |
| *trnK* | J | 70 | 3729–3798 | 30 | 17.1 | 38.6 | 14.3 | 68.6 | | |
| *trnD* | J | 68 | 3800–3867 | 36.8 | 10.3 | 41.2 | 11.8 | 78 | | |
| *atp8* | J | 156 | 3868–4023 | 34.6 | 16.7 | 44.2 | 4.5 | 78.8 | ATT | TAA |
| *atp6* | J | 681 | 4017–4697 | 37.7 | 16.2 | 36 | 10.1 | 73.7 | ATG | TAA |
| *cox3* | J | 789 | 4697–5485 | 34.3 | 18.4 | 32.8 | 14.4 | 67.1 | ATG | TAA |
| *trnG* | J | 63 | 5485–5547 | 33.3 | 9.5 | 49.2 | 7.9 | 82.5 | | |
| *nd3* | J | 354 | 5545–5898 | 36.4 | 18.4 | 33.9 | 11.3 | 70.3 | ATA | TAG |
| *trnA* | J | 63 | 5897–5959 | 34.9 | 9.5 | 41.3 | 14.3 | 76.2 | | |
| *trnR* | J | 63 | 5959–6021 | 39.7 | 12.7 | 38.1 | 9.5 | 77.8 | | |
| *trnN* | J | 66 | 6020–6085 | 33.3 | 10.6 | 45.5 | 10.6 | 78.8 | | |
| *trnS*(AGN) | J | 68 | 6085–6152 | 35.3 | 13.2 | 30.9 | 20.6 | 66.2 | | |
| *trnE* | J | 65 | 6152–6216 | 40 | 10.8 | 44.6 | 4.6 | 84.6 | | |
| *trnF* | N | 70 | 6215–6284 | 35.7 | 4.3 | 41.4 | 18.6 | 77.1 | | |
| *nd5* | N | 1731 | 6279–8009 | 46.7 | 9.3 | 26.3 | 17.7 | 73 | GTG | TAA |
| *trnH* | N | 65 | 8010–8074 | 47.7 | 4.6 | 29.2 | 18.5 | 76.9 | | |
| *nd4* | N | 1345 | 8063–9407 | 48.5 | 9 | 24.8 | 17.8 | 73.3 | ATG | TNN |
| *nd4L* | N | 280 | 9406–9685 | 51.1 | 6.8 | 27.5 | 14.6 | 78.6 | ATG | TNN |
| *trnT* | J | 63 | 9688–9750 | 38.1 | 7.9 | 42.9 | 11.1 | 81 | | |
| *trnP* | N | 64 | 9751–9814 | 45.3 | 4.7 | 32.8 | 17.2 | 78.1 | | |
| *nd6* | J | 495 | 9817–10311 | 31.9 | 16.2 | 42.2 | 9.7 | 74.1 | ATT | TAA |
| *cob* | J | 1134 | 10311–11444 | 35.7 | 18.7 | 33.2 | 12.4 | 68.9 | ATG | TAA |
| *trnS*(UCN) | J | 67 | 11442–11508 | 35.8 | 7.5 | 43.3 | 13.4 | 79.1 | | |
| *nd1* | N | 921 | 11524–12444 | 49.5 | 9.8 | 20.7 | 20 | 70.2 | ATT | TAG |
| *trnL*(CUN) | N | 69 | 12465–12533 | 34.8 | 10.1 | 36.2 | 18.8 | 71 | | |
| *rrnL* | N | 1293 | 12534–13826 | 44.9 | 6.9 | 29.8 | 18.4 | 74.7 | | |
| *trnV* | N | 69 | 13827–13895 | 39.1 | 11.6 | 31.9 | 17.4 | 71 | | |
| *rrnS* | N | 801 | 13896–14696 | 43.4 | 8.9 | 29.3 | 18.4 | 72.7 | | |
| *A + T-rich* | | 857 | 14697–15553 | 37.6 | 14.9 | 40.3 | 7.2 | 77.9 | | |

transcriptional polyadenylation [30]. Three (*cox1*, *cox2, nad4*) are exactly adjacent to tRNAs, and one (*nad4L*) is exactly adjacent to the beginning of another gene (*nad4*). Thus, it is highly probable that during mRNA processing the U is exposed at the end of an mRNA molecule and polyadenylated, forming the complete UAA [31].

Relative synonymous codon usage [32] values were calculated for the PCGs (Table S1, Supplementary Material). Obviously, codon usage is out of harmony with the implicit assumption that synonymous codons are equally used and most values differ from 1 (frequency at equilibrium).

## Transfer RNAs

All the 22 typical tRNA genes were identified in the mtDNA of *E. sinensis* according to their secondary structures and primary sequences of the corresponding

anticodon (Fig. S1, Supplementary Material). The 22 tRNA genes were interspersed in the genome and range from 63 to 70 bp in length. Apart from *trnS (*AGN), all of them showed typical clover-leaf secondary structures and their anticodons are similar to those found in other metazoan animals. The only peculiar feature is the lack of the DHU arm in trnS (AGN). This feature is shared with some other animals, but is not a general feature of Blattaria mtDNA as proved by *B. germanica* which has all tRNAs with a complete clover-leaf structure (unpublished). Though it can't show the typical clover-leaf secondary structure, it can form the correct tertiary structure. Moreover, overlaps between *trnW* and *trnC* genes were 8 bp, as reported by Lessinger et al. [33] which makes the genes producing separate transcripts with their opposite directions.

## Ribosomal RNAs

As reported for other animals, two genes for ribosomal RNAs were fond in *E. sinensis*, one for the small (srRNA) and one for the large ribosomal subunit (lrRNA). The srRNA was located between *trnV* and A + T-rich region, and the lrRNA was located between *trnV* and *trnL*.

## A + T-rich region (control region)

The A + T-rich region in *E. sinensis* mtDNA, comprising the region between srRNA and *trnI* was 857 bp long. This length was well in the range of other arthropods, which shows a remarkable variability; from 263nt for *Rhipicephalus* to 4,601nt for *D. melanogaster* [34]. It is 604 bp and 649 bp longer than *P. fuliginosa* and *B. germanica*. Previous analysis of the *Drosophila* A + T-rich region identified the signals responsible for the origin of replication of the major and minor strands (OJ and ON, respectively) and confirmed that, at least in this genus, the mtDNA replicates with a strand-asynchronous, asymmetric mechanism.

## Phylogenetic analysis

Phylogenetic analysis were performed on the concatenated amino acid sequences of all PCGs of *E. sinensis* in conjunction with those 23 other arthropod sequences whose complete mtDNA sequences were available in GenBank, using a Bayesian approach implemented in MrBayes 3_0b4 [21]. The sequences were as follows: *Reticulitermes virginicus* (NC_009500) [23], *R.flavipes* (NC_009498) [23], *Pteronarcys princes* (NC_006133) [35], O*rthetrum triangulare melania* (AB126005) [4], *Trigoniophthalmus alternatus* (NC_010532) [36], *Nesomachilis australica* (NC_006895) [37], *Petrobius brevistylis* (NC_007688) [38], *Friesea grisea* (NC_010535)[36], *Cryptopygus antarcticus* (NC_010533) [1], *Orchesella villosa* (NC_010534) [36], *Tamolanica tamolana* (NC_007702) [39], *Sclerophasma paresisense*(NC_007701) [39], *Periplaneta fuliginosa* (NC_006076) [4], *Blattella germanica* (EU854321; unpublished), *Thermobia domestica (*NC_006080) [40], *Atelura formicaria* (NC_011197) [41], *Locusta migratoria* (NC_011119; unpublished), *Calliptamus italicus* (NC_011305) [42], *Parafronurus youi* (NC_011359) [12], *Campodea lubbocki* (NC_008234) [43], *Japyx solifugus* (NC_007214) [44], *Penaeus monodon* (NC_002184) [45] and *Pagurus longicarpus* (NC_003058) [46].

Every set PCG sequences was concatenated gene-by-gene and produced 24 sets sequences of PCGs, then aligned them by ClustalX1.8 [20]. After removing the unreliable alignment sites and gaps manually inspected, the final alignment resulted in 2974 amino acid sites, 726 were constant, 2248 were variable, and 1581 were parsimony informative.

What's the phylogenetic relationships among Dictyoptera: Mantodea + (Blattaria + Isoptera) or Isoptera + (Blataria + Mantodea)? The argument has been lasting for many years. Some studies confirmed the first postulate [7, 8], but were in conflict with others [9–11]. However, DeSalle et al. [47] reckoned that Mantodea and Isoptera were the sister groups. But in this study, a peculiar finding of this analysis is that *E. sinensis* clusters with Isoptera insects (*Reticulitermes flavipes* and *R. virginicus*), then they cluster with another Blattaria insect (*P. fuliginosa*). In another word, all the Blattaria insects do not cluster together. As reported by Inward et al.[48], molecular phylogenetic analysis showed that termites are social cockroaches, no longer meriting being classified as separate order (Isoptera) from the cockroaches (Blattodea). Instead, they proposed that they should be treated as a family i.e.Termitidae within Blattaria. Obviously our study supplied the molecular evidences for the postulate (Fig. 2). Since the Isoptera group died [48], we proposed that the Blataria (including Isoptera) and the Mantodea are sister groups.

Our phylogenetic tree reflected the most widely accepted interpretation, based on morphological and molecular data, the basal splitting of the Insecta separates the Archaeognatha from the Dicondylia (Zygentoma + Pterygota) [8, 13, 16, 17, 36]. Within Archaeognatha and Zygentoma, relationships are stable across different analysis and congruent with the accepted taxonomy. But the relationships among Ephemeroptera, Odonata and Neoptera are ambiguous. For them, three hypotheses were found as before. With the application of molecular markers, all the three hypotheses were supported by some researches respectively [14–16, 18]. As Ogden and Whiting [15] reported, their result based on 18S rDNA, 28S rDNA and the Histone 3 protein-coding gene (H3) supported the basal Ephemeroptera hypothesis. But

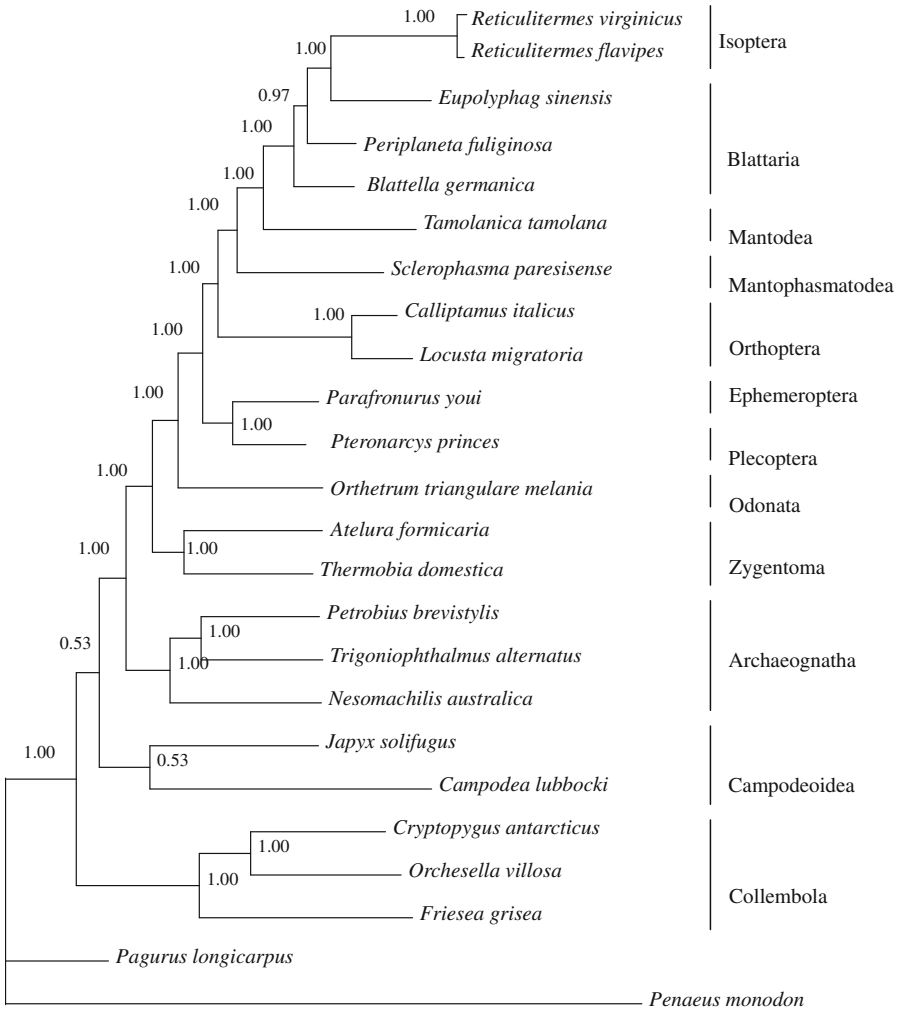


**Fig. 2** Phylogenetic relationships of insects inferred from amino acid sequences of 13 mitochondrial protein-coding genes of 22 Hexapoda by Bayesian analysis. Tree was rooted by branchipod crustacean *Pagurus longicarpus* and *Penaeus mondon*. Numbers at nodes indicate posterior probabilities (×100). Vertical lines indicate monophyletic orders

other 18S/28S analysis supported the basal Palaeoptera hypothesis [14]. Also the basal Odonata hypothesis had been supported by different molecular markers: 18S rRNA [16, 18, 49] and combined complete 18S rRNA and 28S rRNA [17]. Furthermore, based on amino acid data, Carapelli et al.[36] proposed that the odonatan is the basal lineage of the Pterygota. Unfortunately, the absence of the Ephemeroptera from their analysis prevents them to draw conclusions of the relationships. Zhang et al. [12] first sequenced the complement complete mitochondrial genome of *Parafronurus youi*. Their phylogenetic tree based on 12 mitochondrial PCGs supported the basal Ephemeroptera hypotheses (Ephemeroptera + (Odonata + Neoptera)). Surprisingly, our study is different from the three hypotheses. The Ephemeroptera (*Parafronurus youi*) clusters with the Plecoptera (*Pteronarcys princes*) (Fig. 2). Hence, we think the relationships among Ephemeroptera, Odonata and Neoptera should be studied further.

A monophyletic Collembola was recovered. Low or no support was found for the deeper nodes, including the monophyly of the Collembola. Two nodes, *Japyx solifugus* + *Campodea lubbocki* and Collembola + (Campodeoidea + Insecta) were poorly supported (53%, both) in analysis, suggesting that further researches on the relationships of Campodeoidea and Collembola should be carried on (Fig. 2).

## Conclusion

The mitochondrial genome of *E. sinensis* is the first sequenced mtDNA for a representative of the Polyphagidae and the third for the Order Blattaria. The newly determined genome shares the gene order and orientation with previously known Blattaria genomes. It is rich in A + T throughout the entire mitochondrial genomes. The phylogenetic analysis shows that Blataria (including Isoptera) and Mantodea are sister groups. Furthermore the relationships of the three basal clades of winged insects are different from the three previous hypotheses ((Ephemeroptera + Odonata) + Neoptera, Ephemeroptera + (Odonata + Neoptera), Odonata + (Ephemeroptera + Neoptera)).

The Ephemeroptera (*Parafronurus youi*) clusters with the Plecoptera (*Pteronarcys princes*). Therefore, we tentatively conclude that mitochondrial genomes can answer some questions of the basic relationships among the Hexapoda. In addition, to obtain the unambiguous phylogenetic relationships, a larger number of complete mitochondrial genomes should be determined.

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