Biochemical Systematics and Ecology 69 (2016) 101–107

Contents lists available at ScienceDirect

Biochemical Systematics and Ecology

journal homepage: www.elsevier.com/locate/biochemsyseco

The mitogenomes of three beetles (Coleoptera: Polyphaga: Cucujiformia): New gene rearrangement and phylogeny

Xuejuan Li [1], Jing Ou [1], Zhaoming Wei*, Yaxiao Li, Yingfang Tian

College of Life Sciences, Shaanxi Normal University, Xi'an 710062, China

ARTICLE INFO

Article history:
Received 30 June 2016
Received in revised form 20 August 2016
Accepted 26 August 2016

Keywords:
Polyphaga
Mitogenome
Gene rearrangement
Phylogeny

ABSTRACT

The mitochondrial genome (mitogenome) has been extensively used for studying phylogenetic relationships at different taxonomic levels. Several molecular analyses have been performed, but the phylogenetic relationships among infraorders in Polyphaga have not been well resolved. In this work, three nearly complete mitogenomes of Coleoptera, *Sitophilus oryzae*, *Oryzaephilus surinamensis* and *Callosobruchus chinensis*, were determined. The *O. surinamensis* and *S. oryzae* mitogenomes harbor gene content typical of other Polyphaga mitogenomes, while a gene rearrangement (trnQ) was found in the *C. chinensis* mitogenome. The mitogenomes of these three Coleoptera species each consist of approximately 13 protein-coding genes, 22 tRNA genes, two rRNA genes and one A + T-rich region. Phylogenetic analysis within Polyphaga was carried out based on mitochondrial data. The phylogenetic results within Polyphaga support the basal position of *Cyphon* sp., which belonged to Scirtoidea, Elateriformia. Within Cucujiformia, monophyletic Curculionoidea, Chrysomeloidea and Tenebrionoidea were confirmed.

1. Introduction

Coleoptera is an ancient order and has adapted to a variety of environmental conditions (Crowson, 1981). Coleoptera contains over 390,000 described species and has been divided into four suborders (Adephaga, Archostemata, Myxophaga and Polyphaga) (Crowson, 1960; Nie and Yang, 2013). According to morphological characteristics, Crowson (1960) divided Polyphaga into five infraorders (Bostrichiformia, Cucujiformia, Elateriformia, Scarabaeiformia and Staphyliniformia) and 18 superfamilies. However, Bouchard et al. (2011) subdivided Polyphaga into six infraorders (Bostrichiformia, Cucujiformia, Elateriformia, Scarabaeiformia, Staphyliniformia and Derodontiformia) and 16 superfamilies. These classification systems contain different numbers of infraorders mainly due to different phylogenetic positioning of some superfamilies (Nie and Yang, 2013).

The mitogenomes of most metazoans are circular molecules spanning approximately 16 kb in size, composed of 13 protein-coding genes (PCGs), 22 tRNA genes, two rRNA genes, and usually one large non-coding element (Wolstenholme, 1992). Mitogenome sequences are widely used in population genetics, comparative and evolutionary genomics, reconstruction of phylogenetic relationships, and evolutionary biology (Cameron and Whiting, 2008; Hao et al., 2012).

Mitochondrial gene rearrangements have been reported in insect taxa, primarily Thysanoptera (Shao and Barker, 2003), Hymenoptera (Oliveira et al., 2008) and Orthoptera (Zhao et al., 2010). However, a small number of mitochondrial gene

* Corresponding author.
E-mail address: weizhaoming@snnu.edu.cn (Z. Wei).
[1] The authors contributed equally to this work.

http://dx.doi.org/10.1016/j.bse.2016.08.012
0305-1978/

rearrangements have been observed in Coleoptera. Timmermans and Vogler (2012) and Timmermans et al. (2015) found that gene order rearrangements in Coleoptera mainly affected the trnA-trnR-trnN-trnS-trnE-trnF cluster between the nad3 and nad5 genes. For example, tRNA gene rearrangements of trnA-trnN-trnR-trnS-trnE-trnF in *Mordella atrata* (Wei and Chen, 2011) and *Cyphonistes* sp. (Timmermans et al., 2015), and trnR-trnA-trnN-trnS-trnE-trnF in *Naupactus xanthographus* (Wei and Chen, 2011) and three genera of Chrysomelidae (*Exema*, *Cryptocephalus* and *Pseudocolapsis*) (Timmermans et al., 2015) were found.

Polyphaga is the largest group in Coleoptera (Nie and Yang, 2013). Although several molecular analyses have been performed, the phylogenetic relationships among infraorders in Polyphaga have not been well resolved (Nie and Yang, 2013). Moreover, the relationships among superfamilies within the Elateriformia and Cucujiformia are not currently understood in great detail (Kukalová-Peck and Lawrence, 1993).

Within Coleoptera, Cucujiformia is a diverse clade that contains more than half of all beetles (Sheffield et al., 2009). However, *Sitophilus*, *Oryzaephilus* and *Callosobruchus*, which have been considered to belong to Cucujiformia, only had a few mitochondrial segments sequenced, such as the cox1 gene, and the corresponding mitogenome data was lacking. In this study, the mitogenome sequences were determined for *S. oryzae*, *O. surinamensis* and *C. chinensis*, which belong to Curculionoidea, Cucujoidea and Chrysomeloidea, respectively (Coleoptera: Polyphaga: Cucujiformia). The mitogenome sequences were described by comparison with each other and with other Polyphaga mitogenomes. Furthermore, the concatenated nucleotide sequences datasets of Polyphaga were utilized in order to gain insight into the phylogenetic relationships.

2. Materials and methods

2.1. Specimen collection and DNA extraction

Specimens of *S. oryzae* were collected in Chengdu Grain Reserves Research Institute, Sichuan Province, China; *O. surinamensis* in QianDong-nan, Guizhou Province, China; and *C. chinensis* in Xi'an, Shaanxi Province, China. Total DNA was extracted from the entire body, excluding the abdomen, using the phenol/chloroform method (Zhou et al., 2007).

2.2. Primer design, PCR amplification and sequencing

Seven primary pairs of PCR primers (Simon et al., 2006) were created to amplify overlapping segments of the entire mitogenomes of three species. All primers used for this study are included in Table S1. Fragments were amplified using r-Taq mix DNA polymerase (Takara, China) with an initial denaturation at 93 °C for 1 min, followed by 35 cycles of denaturation at 92 °C for 10 s, annealing at 48–58 °C for 90 s, and extension at 72 °C for 1–4 min with a final elongation at 72 °C for 6 min after the last cycle. PCR products were purified using a DNA Gel Purification Kit after separation by electrophoresis in a 1.0% agarose gel. PCR products were directly sequenced via primer walking by Beijing Genomics Institute (BGI). In addition, the mitogenome of *C. chinensis* was also sequenced using the Illumina HiSeq 2500 high-throughput sequencing platform at Genesky Biotech, Shanghai, China.

2.3. Sequence assembly, annotation and analysis

The overlapping nucleotide sequences were assembled using Staden package 1.7.0 (Staden et al., 2000). The 13 PCGs were identified using ORF Finder (Rombel et al., 2002). Most of the tRNA genes were identified using tRNAscan-SE 1.21 (Lowe and Eddy, 1997). Two rRNA genes, the A + T rich region and the remaining tRNAs that were not predicted by tRNAscan-SE were identified by sequence comparison with other beetles. Nucleotide composition statistics and codon usage were computed using MEGA 5.0 (Tamura et al., 2011).

2.4. Phylogenetic analyses

For phylogenetic analyses, two types of nucleotide sequence datasets, the 13 protein-coding genes (PCG) and the complete mitogenomes excluding the A + T-rich region (mtDNA) were obtained from 44 Polyphaga species, using two Adephaga species as outgroups (Table S2). The amino acid sequences of protein-coding genes were individually aligned using MEGA 5.0 (Tamura et al., 2011), and then the corresponding nucleotide sequences were retro-aligned. The nucleotides from each rRNA and tRNA were aligned using ClustalX 1.83 (Thompson et al., 1997) with default options.

Phylogenetic trees were estimated via the maximum likelihood (ML) method using RAxML v7.0.3 (Stamatakis, 2006) and Bayesian inference (BI) method using MrBayes v3.2.1 (Ronquist and Huelsenbeck, 2003). The model with the best fit (GTR + I + G) for the two datasets was suggested by MrModeltest 2.2 (Nylander, 2004). For ML analysis, node reliability was estimated using 1000 bootstrap permutations. For BI, four simultaneous Markov chain Monte Carlo analyses were run for 1 million generations, sampling every 10 generations. The first 25% of the generations were discarded as burn-in, and the remaining samples were used to compute the consensus tree and Bayesian posterior probabilities.