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Mitochondrial genomes of two luminous beetles, *Rhagophthalmus lufengensis* and *R. ohbai* (Arthropoda, Insecta, Coleoptera)

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Abstract

We determined the mitochondrial DNA (mtDNA) sequences of two luminous beetles (Arthropoda, Insecta, Coleoptera), *Rhagophthalmus lufengensis* from Yunnan, China and *Rhagophthalmus ohbai* from Yaeyama Island, Japan. We identified all the 37 mtDNA genes of *R. lufengensis* (15,982 bp) and the 34 genes of *R. ohbai* (15,704 bp). *R. lufengensis* and *R. ohbai* genomes have higher A + T contents than other coleopteran genomes although the gene arrangements are similar. Interestingly, in a study of the evolutionary relationship among *R. lufengensis*, *R. ohbai* and the firefly *Pyrocoelia rufa*, the phylogenetic tree inferred from lrRNA genes from mitochondrial genomes indicates a biogeographic relationship among the bioluminescent insects in East Asia and the phylogenetic tree inferred from luciferase-related genes from nuclear genomes shows an appropriate relationship among coleopterans, reflecting the evolutionary origin of bioluminescence. Thus, the mtDNAs of luminescent beetles can provide an insight into their evolutionary origin and biogeography.

Keywords: Mitochondrial genome; Luminous beetle; Coleoptera; *Rhagophthalmus lufengensis*; *Rhagophthalmus ohbai*; Molecular phylogeny

1. Introduction

With a few exceptions, mitochondrial DNA (mtDNA) in metazoans is a single circular DNA molecule ranging in size from 14 to 42 kbp. It encodes 37 genes: 13 protein-coding genes (ATP6 and ATP8, COI–III, CYTB, ND1–6 and 4L), two ribosomal RNA genes (lrRNA and srRNA), and 22 tRNA genes (one for each amino acid except leucine and serine, which have two genes each) (Wolstenholme, 1992; Boore, 1999). Additionally, a large noncoding region is typically present, which contains sequences essential for the initiation of transcription and gene replication (Shadel and Clayton, 1997). This is called the control region and is also the main source of variation in genome length (Inohira et al., 1997). The mitochondrial genome is very useful for the studies of evolutionary origin and biogeography. Because of features such as the presence of orthologous genes, uniparental inheritance (usually maternal), very low recombination rate, small size, constant gene content and relatively high mutation rate, mtDNA sequences have been extensively used in studies of evolutionary genomics and for the investigation of population structures and phylogenetic relationships at various taxonomic levels (Avise, 1994; Saccone et al., 2002). In addition, because of the low rearrangement rates, mitochondrial genomes promise to be a useful data set for the study of deep metazoan divergences (Boore and Brown, 1998). For example, the ancient phylogenetic relationship between insects and crustaceans is strongly supported by mtDNA rearrangement data (Boore et al., 1998).

Abbreviations: A, adenine; ATP 6 and 8, ATPase subunits 6 and 8; C, cytosine; COI–III, cytochrome oxidase subunits I–III; CYTB, cytochrome *b*; G, guanine; lrRNA, large subunit rRNA; mtDNA, mitochondrial DNA; ND1–6 and 4L, NADH dehydrogenase subunits 1–6 and 4L; nt, nucleotide(s); PCR, polymerase chain reaction; rRNA, ribosomal RNA; srRNA, small subunit rRNA; T, thymine; tRNA, transfer RNA.

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Luminous beetles are distributed in four main families: Lampyridae (fireflies), Rhagophthalmidae (glowworms), Phengodidae (railroad worms) and Elateridae (click beetles). However, Rhagophthalmidae was once thought to be subfamily (Rhagophthalminae) classified under Lampyridae (McDermot, 1964, 1966) or Phengodidae (Crowson, 1972). Although Wittmer and Ohba (1994) revised the status of Rhagophthalmidae as a separate family, the phylogenetic relationship between Rhagophthalmidae and Lampyridae remains controversial (Branham and Wenzel, 2001, 2003; Ohmiya et al., 2000; Suzuki, 1997; Kobayashi et al., 2001, 2002, 2003). In fact, Lampyridae is widely distributed in all continents except the Antarctic and its all larvae are luminous although adults of some species are non-luminous. Rhagophthalmidae is only distributed in East Asia, and all larvae and larviform female adults are luminous, but male adults are non-luminous. Species belonging to the Phengodidae are found in the neotropical regions of South and Central America and in the South Pacific islands, its luminous form is found only in larvae and female adults. Although elaterids are common beetles, only some species of Elateridae are luminous and they are mainly found in South and Central America. The relationships among these luminous beetles dispersed in these four families and the origin and evolution of their bioluminescence are not clear.

Luminous beetles are characterized by the bioluminescent luciferin–luciferase reaction in which luciferase is a light-emitting enzyme belonging to the adenylate family of enzymes (Viviani, 2002). The luciferase genes of more than 18 species have been cloned and are registered in the NCBI database (http://www.ncbi.nlm.nih.gov/). The luciferase-related genes including those for long-chain acyl-CoA synthetase, 4-coumarate-CoA ligases, and EntF nonribosomal peptide synthetases, have been cloned from species ranging from bacteria to mammals. A phylogenetic tree inferred from luciferase-related proteins supports the idea that the genera of luminous beetles represent the four families (Viviani et al., 2004), although mtDNAs or other proteins have not been used to confirm these relationships.

In East Asia, including China and Japan, there are two major families of luminous beetles, Lampyridae and Rhagophthalmidae. More than 40 species of Lampyridae are found in Japan and more than 116, in China; one Rhagophthalmidae species is found in Japan, and several species are found in China. However, the phylogenetic and biogeographic relationships of these luminous beetles in East Asia are not clear. Recently, Bae et al. (2004) determined the mtDNA genome of a lampyrid species, *Pyrocoelia rufa*, from Korea. The partial sequences of mtDNA, including COI (Kim et al., 2000; Lee et al., 2003), COII (Suzuki et al., 2002, 2004), and lrRNA (Suzuki, 1997; Kim et al., 2000; Li et al., 2006), have also been determined and used to investigate the evolutionary and biogeographic relationships of this family in East Asia. For example, phylogenetic analysis has shown that the firefly *Luciola cruciata* dates back ~0.5 million years in the Japanese islands and that there are genetic differences between the eastern and western Japanese forms (Suzuki et al., 2002). On the other hand, Wittmer and Ohba (1994) discovered *Rhagophthalmus ohbai* on Iriomote Island and the nearby islands of Japan and Li (the first author of this paper) et al. recently discovered *Rhagophthalmus lufengensis* (Li et al., submitted for publication) in Yunnan. The Rhagophthalmidae is not well known, not only because their geographic distribution is limited in East Asia but also because only female adults and larvae are luminous and collection of non-luminous male adult is not easy. Their taxonomic status, in relation to the Lampyridae, is unclear. Thus, the mtDNA genomes of both families could provide an insight into the evolutionary origin and biogeography of bioluminescent insects in East Asia.

In this study, we determined the mitochondrial genomes of two species of Rhagophthalmidae, *R. lufengensis* and *R. ohbai*, and examined their nucleotide composition and genomic arrangement. In addition, we discuss the evolutionary origin of bioluminescent insects in East Asia based on phylogenetic trees constructed using mitochondrial proteins and luciferases.

2. Materials and methods

2.1. Samples and DNA extraction

R. lufengensis was collected from the roadside adjacent to forests and rice fields in Jiuzhuang Township, Lufeng County, Yunnan Province, southwest China (N 25.09°, E 101.80°; altitude ca. 1827 m), and *R. ohbai* was collected from Sonai on Iriomote Island, Okinawa Prefecture, Japan (N 24.06°, E 123.12°; altitude ca. 6 m). The collected samples were immediately placed in a 100% ethanol solution. For DNA extraction (using the DNAeasy Tissue Kit of Qiagen) 3–5 mm of the abdomen of a single larviform female adult (Fig. 1A and B) was cut out with a scalpel under a microscope. At least one additional specimen from each locality was pinned or preserved in 75% ethanol as a voucher specimen. The voucher specimens of *R. lufengensis* were deposited in Kunming Institute of Zoology (KIZ), the Chinese Academy of Sciences (CAS) in China and Yokosuka City Museum (YCM) in Japan. The voucher specimens of *R. ohbai* were deposited in Yokosuka City Museum in Japan.

2.2. Determination of partial sequences

Partial fragments of the COII and lrRNA mtDNA genes were amplified using two sets of primers, designed by authors according to homological fragments of other fireflies deposited in NCBI. The COII primers were HmCO2-1F [5′-ataga rcaat taacm ttytt ycayg ayca-3′] and HmCO2-2R [5′-grttt rtwcc rcara tctcw garca ttg-3′], while the lrRNA gene primers were Hm16s-1F [5′-ataat ttaar rtytr ayctg ctcaa tga-3′] and Hm16s-2R [5′-araaa twacg ctgtt atccc yaagg taa-3′]. Polymerase chain reaction (PCR) was carried out in a GeneAmp PCR System 2700 (Applied Biosystems). The cycling protocol included an initial denaturation step at 94 °C for 2 min, followed by 25 cycles of 30 s denaturation at 94 °C, 30 s annealing at 50 °C and 1 min extension at 72 °C, then an additional extension of 7 min at 72 °C. PCR was performed in a 25 μL reaction mixture consisting of 11.4 μL of sterilized distilled water, 2 μL of