



Research Article

MOLECULAR EVIDENCE OF PHYLOGENETIC RELATIONSHIP OF EMBIOPTERA AND RELATED GROUPS

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ABSTRACT

Molecular evidence of phylogenetic relationship of embioptera and related groups has been attempted. Many evidence exists for familial, ordinal and super ordinal relationship with stoneflies (Plecoptera). Also, based on resemblance in habitat, the termites (Isoptera) was included in studying the phylogenetic relationship. Bioinformatics tool was employed to confirm the close relationship. The taxonomic identification of Embiid species involves the study of anatomical differences in the structure of male genitalia which is taken as the main criterion. Bioinformatics tool was employed to confirm the close relationship. In the present study the evolutionary relationships of embiid *Oligotoma saundersii*, stonefly *Acroneuria lycorias* and termite *Odontermes redemani* was studied. Multiple sequence alignment was done for the mitochondrial sequences. The Mega 6 Software was used for this purpose of constructing the phylogenetic tree. The analysis involved 3 nucleotide sequences of the abovementioned species and the evolutionary history was inferred using the Neighbor-Joining method. The optimal tree with the sum of branch length = 7.17153663 is shown. From the present study it was concluded that the termites (Isoptera) showed a closer phylogenetic relationship to the Order Embioptera, now referred to as Embiidina compared to stoneflies belonging to the order Plecoptera.

Keywords: Taxonomic, Phylogenetic, Embioptera, Plecoptera.

INTRODUCTION

The phylogeny of the Order Embiidina remains controversial. As early as the first half of the last century, the genus, Embia was placed nearest to the genus Termes, assuming a close relationship of the embiids to the termite group. Relationships between Embioptera and other orders remain unclear (Klass & Ulbricht, 2009), but proposals about the Embioptera sister group have included Plecoptera (Boudreaux, 1979; Wheeler *et al.*, 2001), Zoraptera (Grimaldi & Engel, 2006; Yoshizawa, 2007, 2011) and Neoptera except Plecoptera (Beutel & Gorb, 2006; Hennig, 1981). Except for the monophyly of cockroaches, mantids and termites, based on various morphological data no consensus could be arrived, (Letsch & Simon, 2013). Less than 20 species of embiids were recognized in a single family (Hagen, 1885). Also studies on embioptera include major comprehensive revisions by (Gunther Enderlein, (1903); Enderlein, (1909a, 1909b). Earlier, numerous new species and higher taxa were made by Krauss (1911).

Most historical taxonomic literature on the group has emphasized descriptions of new species. Relatively few papers have comprehensively addressed the phylogeny or higher classification and fewer of these have incorporated a more modern philosophy emphasizing cladistics or the naming of demonstrably monophyletic groups. Among the small insect orders, Embioptera is a distinct, monophyletic group with representatives distributed throughout warmer regions of the world (Miller, 2009). The polyneopterans may be grouped in three ways:- one lineage includes the Plecoptera, Dermaptera and Grylloblattodea, the second contains the Blattodea, and the third contains the Embiidina, Phasmida, and Orthoptera (Flook & Rowell, 1998). It is not enough to resolve the phylogenetic relationships within Polyneoptera. Analyses of transcriptomic data with reference to Grylloblattodea and Mantophasmatodea, combined with studies on Embioptera and Phasmatodea along with that of Dictyoptera, which includes Mantodea and Blattodea including termites indicates Orthoptera as the sister taxon.

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MATERIALS AND METHODS

Live specimens were exposed from beneath the bark using a chisel and carefully transferred with the help of a fine brush into specimen tubes filled with habitat material viz., crumbs of bark. Straight sided culture tubes (plastic) about 1.5 cm in diameter and 3.5 cm long, stopper with a polythene slip on cap which has a punched out centre-circle with cotton plug inserted to permit air-circulation, while preventing escape of insects were used for embiid culturing. Embioptera are difficult to collect and require rearing to acquire males upon which the classification is based. Once collected, specimens are often difficult to identify or represent undescribed taxa making taxon sampling more challenging than many other taxa. The criteria for identification of the species were the structure of male genitalia.

The tenth tergum is triangular and un-modified; the eleventh (epiproct) is represented by a small, rudimentary sclerite (EP) just beneath the acute apex of the tenth; the ninth sternite is transversely quadrate; the anus is flanked by large, triangular, convex paraprocts (LPPT and RPPT); the cerci and their fleshy basipodites (LCB and RCB) are symmetrical. Later in the penultimate instar future changes are presented by distortions due to developments within; the tenth tergum may enlarge toward its left side, the ninth sternite (H) may develop a small medial lobe (HP), laterotergites 7th tergum 8th tergum 9th tergum spiracle. 10th tergum 7th pleurites storm pleurite-laterotergites and its left cercus-basipodite often exhibits signs of profound changes. The appearance of highly modified adult structures following ecdysis and reduction of some nymphal structures, e.g., the right paraproct, is most interesting. With these characteristics the embiid species was identified as *Oligotoma saundersii* (Westwood).

RESULTS AND DISCUSSION

Embiid ancestors may have had a prognathous head with chewing mouthparts and long filiform antennae, as in extant embiids. Legs moderately long as in Plecoptera; tarsi five-segmented (Figure 1) Wings present in both sexes, subequal in size and shape except for a relatively large

anajugal area in hindwings; veins uniformly sclerotized, none developed as blood sinuses. Male abdominal terminalia-Symmetrical, cerci more than two segmented. Food weathered Habitat and outer bark and dead leaf litter. Defensive biting weak repugnant secretion is absent. The male abdominal terminalia distinctions are an important criterion in systematic studies.

Early in the penultimate instar a male's abdominal apex is identical to that of other nymphal stages. The tenth tergum in adult males is divided incompletely or completely into two, namely the right hemitergite (10R) and the left hemitergite (10L); 10 RP₁ and 10 RP₂ are the outer or major process of 10R respectively. 10 RP₁ is larger than 10 RP₂. Also, 10 LP which is the process of the left hemitergite, may be short and straight or long and bent, with its apex either simple or complex being thickened with dorsal or ventral appendices, H, which is the hypandrium or 9th sternite, is a triangular or quadrangular plate, produced caudad into a rounded or elliptical hypandrial process (hP); LPPT or the left paraproct is homologous to the 11th abdominal plate and it often takes the form of a sclerotic spinebearing an outer and an inner process or arm, shaped like the letter 'S' or 'U' in Oligotomids.

Molecular phylogeny -Sequence alignment is a basic tool of bioinformatics, which provides information about two sequences whether the two sequences are similar, what is the level of similarity, where the two sequences are similar and where they differ. Sequence alignment is very useful for discovering functional, structural and evolutionary information in biological sequences. Given two or more sequences, sequence alignment studies would throw light on the following aspects of these sequences. Measure their similarity: Statistical measure of similarity could be provided by sequence alignment. Multiple sequence alignment not only depicts residue-residue correspondence, but also helps in search for weak but significant similarities in sequence databases. Demonstration of homology between sequences, Molecular Phylogeny-Inferring an evolutionary relationship based on Multiple Sequence Alignment is studied in Molecular phylogenetic.

Sequences

EF623251.1 *Acroneuria lycorias* 16S ribosomal RNA gene, partial sequence; mitochondrial

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TCGCCTGTTTATCAAAAACATGTCTTCTTGGGTAAAAATATGAAGTCTGACCTGCCACTGAATATGAATTT
TGAAGGGCCGCGGTATTTTGACCGTGCAAAGGTAGCATAATCATTAGTCTTTTAATTGGAGGCTGGATGAAT
GGTTGGACGAGGCACTGACTGTCTCATTTTGATTGAGGTTAAATTTAACATTTGAGTGAAAAGCTTAAATG
TTTTAAAGGACGAGAAGACCCTATAGAGCTTGATATTAACCTGTATATAAATTATTTAGAGTTTATGTTTTT
TATGGGGTTTAGTATTTTATTGGGGTGATAGGAAGATAAGAAAACTCTTTTATGTGAAGTTTGTACATTGAT
TTATGATTTATTGATCCGTTTCATAGCGATTATAAGACTAAGTTACCTTAGGGATACAGCGTAATCTTTTTTGA
GAGTTCTTATCGACAAGAAGGGTTGCGACCTCGATGTTGGATTAAGGTAATTTGGGTGTAGATGTTCAAAT
TTTTAGGTCTGTTTCGACCTTTAAATCCTTACATGATCTGAGTTCAA CCGGAGA
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>KU574647.1 *Odontotermes redemani* 16S ribosomal RNA gene, partial sequence; mitochondrial

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CGCCTGTTTATCAAAAACATCTTTTCTCGTTAGTTTTTGAAGTATGGCCTGCCACTGACTGTTGTTGAAGGC
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CGCGGTATTTTGACCGTGCAAAGGTAGCATAATCATTGGTTCTTTAATTGTGATCTGGTATGAATGGCTTGAC
 GAGACATAAGCTGTCTTGGAAAGTATTATTTATTGAATTTGGTTTTTGGAGTTAAAATTCTTAAATGTTTTTATGG
 GACGAGAAGACCCTATAGAGTTTTACAATTTCCCATTTATTTGTTGTTTGTGTTTTGTTGAGTGGGAAGG
 ATTGTTTTGTTGGGGTGAGGGGGAATAATTTAACTCCTCTTTGATTTTGTATATTTATTTATTTGTATTG
 ATCCATTTATTTGATTATAAGATTAAATTACCTTAGGGATAACAGCGTAA

>EU157039.1 *Oligotoma saundersii* 16S ribosomal RNA gene, partial sequence; mitochondrial

GGCTTTAAGATTTTTTATTTAAGGTTAGTTCTGCTCAATGATTGTTTTAAATGGCTGCGGTAAATTGACTGTGT
 GAAAGTAGCATAATAATTAGTTTATTAATTGTGAACTGGTATGAATGGATTGATGAAATATATACTGTCTTTA
 GTTTAAATATGTGCAATTTTATATTCGGGTTAAAAGGCTTGAATTTTTTGTGAGGACGAGAAGACCCCTTAGA
 ACTTTATTTTTTGTAGTTATTTTTGGGCTTTAAAAGGATTTTGTGAGGTTGACAGGTAGATTTTGTAAATTT
 ATTTGATTTTTGCATTTATTGGTGTGTGTTTTATGAATTTTTTTATTTATTAGATTAAGTTACCTAAGGGATA
 ACAGCGTTATTTATATGGAGAGTTCTTATTGATATATAAGTTTTCGACCTCGATGTTGAATTAAGATTATTT
 TGGAGAAGGGTTAGAATTATTAGGTCTGTTTCGACCTTTAAAATCTTACA

Figure 1. Evolutionary relationships of taxa, the Neighbor-Joining method were used to infer the evolutionary history.

The optimal tree with the sum of branch length = 7.17153663 is shown. The phylogenetic tree has branch lengths indicated by same units as the evolutionary distances and is drawn to scale. The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site. The analysis involved 3 nucleotide sequences. Also to avoid ambiguity gaps and missing data were eliminated in the analysis. There were a total of 420 positions in the final dataset. Evolutionary analyses were conducted in MEGA6. Three sequences belonging to Plecoptera-*Acroneuria lycorias*, Isoptera-*Odontotermes redemani* and Embioptera-*Oligotoma saundersii* were studied (Figure 1). Sequence alignment is a basic tool of bioinformatics, which provides formation about two sequences whether the two sequences are similar, what is the level of similarity, where the two sequences are similar and where they differ. Sequence alignment is very useful for discovering functional, structural and evolutionary information in biological sequences. Given two or more sequences, sequence alignment studies would throw light on the following aspects of these sequences Measure their similarity: Statistical measure of similarity could be provided by sequence alignment. Multiple sequence alignment not only depicts residue- residue correspondence, but also helps in search for weak but significant similarities in sequence databases. Demonstration of homology between sequences and inferring an evolutionary relationship based on Multiple Sequence Alignment is studied in Molecular phylogenetics.

Multiple Sequence Alignment: In MSA we consider alignment of more than two homologous sequences at one time. In this, the sequences are aligned optimally by bringing the greatest number of similar characters into register in the same column of alignment. Biological goals of MSA include-generating concise information and summary of sequence data, to illustrate similarity/dissimilarity among a group of sequences and to develop a model useful for testing evolutionary hypothesis Evolutionary relationships of taxa. The Neighbor-Joining method was used to infer the evolutionary history. The

tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura *et al.*, 2004) and are in the units of the number of base substitutions per site. The analysis involved 3 nucleotide sequences. Also to avoid ambiguity gaps and missing data were eliminated in the analysis. There were a total of 420 positions in the final dataset. MEGA6 software was used for performing the Evolutionary analyses, (Tamura *et al.*, 2013). Neighbor joining (NJ) is a clustering method related to UPGMA (unweighted pair groupmethod using arithmetic mean). It does not make the assumption of additively. It is also quiet fast computationally and so is a better choice of method, and is used to estimate trees from multiple sequence alignments. The two taxa with the greatest similarity are chosen and and these taxa are known as neighbors.

CONCLUSION

Maximum likelihood method was used to analyse the multiple sequence alignment showed more similarity of the embiid sequence (*Oligotoma saundersii*) to termite (*Odontotermes redemani*) than to stone fly (*Acroneuria lycorias*).

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