EVOLUTIONARY DIVERGENCE AND PHYLOGENETIC RELATIONSHIPS OF SELECTED DRAGONFLIES USING CYTOCHROME OXIDASE I GENE

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Abstract

Dragonflies are candidates among ancient flying insects existed in the carboniferous period. Libellulidae is the largest and cosmopolitan dragonfly family of the order Odonata. Here we assessed the phylogenetic relationships of 5 dragonfly members (Rhyothemis variegata, Acisoma panorpoides, Orthetrum sabina, Anaeciashna jaspidea and Trithemis pallidinervis) using cytochrome oxidase I (COI) marker. The partial PCR product of this gene yielded 450bp, 479bp, 500bp, 591bp and 580bp DNA respectively. The nucleotide BLAST analysis confirmed the taxonomic identity of these entire species. Phylogenetic tree constructed by Neighbour joining method showed that Libellulidae members are having a monophyletic ancestry due to the divergence from a common ancestor. Among these members, Orthetrum sabina is sharing a sister clade relationship with Trithemis pallidinervis which remained in the same clade and Rhyothemis variegata with Anaeciashna jaspidea in another clade. The average A+T content of all these species is 66.26% while G+C content is 33.74% showing a strong A+T bias. The nucleotide substitution analysis states that Acisoma panorpoides, has the highest substitution rate followed by Rhyothemis variegate, Anaeciashna jaspidea, Orthetrum sabina and Trithemis pallidinervis. The high A+T content along with second codon change reflects evolutionary divergence of all these species. Thus the present study concluded that cytochrome oxidase I is an effective tool for predicting phylogenetic relationships and evolutionary divergence of closely related species.

***Keywords**: Libellulidae, DNA barcoding, Cytochrome oxidase I gene, Molecular phylogeny*

Introduction

Odonata is a well known insect order of dragonflies and damselflies distributed widely over the world [1]. Approximately 6500 extant species in over 600 genera and 28 families are known to exist [2]. Libellulidae commonly called 'Skimmers' are the largest and cosmopolitan family of dragonflies consisting about 1000 species distributed in 11-13 subfamilies under 140 genera[3]. They are often seen during April to December and prefer to live in ponds, meadows, water filled ditches etc. The most diagnostic feature of this family is the presence of wing triangles and anal loop in forewings and hindwings. The most notable pre-cladistic studies of Odonata were mostly based on wing venation, secondary male genitalia and the prehensile labial mask of the larvae [4].

DNA barcoding is considered to be a very easy and cost effective molecular identification tool across metazoan taxa [5]. Insect mitochondrial cytochrome oxidase I (COI) genes are used as a model to examine the gene heterogeneity of evolutionary rate and its implications for evolutionary analyses [6]. These genes typically lack recombination and promote to the fixation of mtDNA halotypes for species identification [7].

Molecular phylogenetic studies of this order are well known.[8] inferred the monophyletic ancestry of this order using mitochondrial genes (COI,16S rRNA) and nuclear genes (28S rRNA, EF-1α).[9] developed a well sustained phylogeny of the Libellulidae from 2 gene fragments of 16S and 28S. Bayesian likelihood and parsimony analysis concludes that Macromiinae, Cordullidae and Libellulidae families of Odonates are monophyletic. In the present study, the cytochrome oxidase I gene of certain selected dragonflies was amplified from Kerala to predict their evolutionary divergence and phylogenetic relationships.

Materials and Methods

Sample Collection and Preservation

Dragonflies were collected by hand sweep netting and random field sampling method was used to cover entire study area. Morphological identification was done from taxonomic experts. Each specimen was then placed in a separate collecting bottle, assigned a code number and stored in 70% ethanol until further use. Morphological identification was done from taxonomic experts. To extract DNA, the middle and hind leg of each specimen was removed leaving the rest of the specimen as vouchers.

DNA Amplification and Sequencing

DNA was extracted using Origin DNA preparation kit. The obtained DNA was amplified using Takara PCR thermo cycler. The thermo cycler conditions were slightly modified as follows; 1 initial cycle of 5 minute at 95°C followed by 30 cycles of 95°C for 10 seconds, 50°C for 1 minute, 72°C for 45 seconds. This is followed by a final step of 72°C for 3 min. The obtained PCR product was checked using 2 % agarose gel electrophoresis and was sequenced with both, the forward and reverse, primers using an automated sequencer ABI 3730XL by Sanger's sequencing method. Phylogenetic analysis was done by MEGA software [10].

Data analysis

Mitochondrial COI sequence data for the selected dragonflies was sequenced and submitted in GenBank. These sequences were edited using basic BLAST tool with CDS analysis. The aligned sequences were used for species identification using BLAST. The sequences from GenBank were retrieved and sequences of each species generated from this study were compared and aligned using the CLUSTALW program.

Results

Morphological identification from the taxonomic experts confirmed the selected dragonflies as *Rhyothemis variegate, Acisoma panorpoides, Orthetrum sabina, Anaciaeschna jaspidea and Trithemis pallidinervis*. The partially amplified product of this gene yielded 450bp, 479bp, 500bp, 591bp and 580bp DNA respectively. The databases revealed definite identity matches in the range of 99%-100%. Phylogenetic tree constructed by Neighbour joining method and the table of nucleotide substitution is given below.

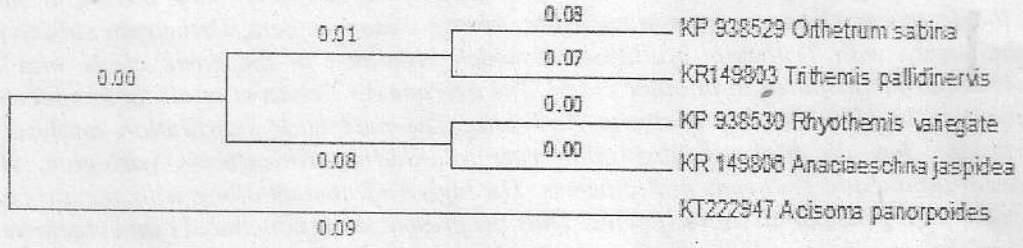

FIG 1: The evolutionary history was inferred using the Neighbour-Joining method

Table 1: Maximum Likelihood Estimate of Substitution Matrix

	A	T/U	C	G
A	-	9.43	4.34	6.33
T/U	8.08	-	9.35	4.58
C	8.08	20.3	-	4.58
G	11.17	9.43	4.34	-

Discussion

Mitochondrial DNA sequence proved to be a valuable tool for determining the phylogentic elationships [11]. This gene provides deeper phylogenetic insights than other mitochondrial genes because hanges in its amino acid sequence occur more slowly than other mitochondrial gene [12]. The cytochrome xidase I sequence of selected dragonflies were submitted to GenBank Accession numbers as KP938529, CP938530, KR149803, KR149806 and KT222947 for future references. The nucleotide BLAST analysis howed 99-100% sequence similarity to the similar sequences already in the data base. The multiple sequence lignment of the similar sequence was done by Clustal W and the evolutionary history was inferred using the leighbour joining method [13]. Phylogenetic tree interprets that Libellulidae members are having a ıonophyletic ancestry due to the divergence from a common ancestor. Among these members, *Orthetrum abina* is sharing a sister clade relationship with *Trithemis pallidinervis* which remained in the same clade and *Rhyothemis variegata* with *Anaeciashna jaspidea* in another clade.(Figure 1). Most of the phylogenetic studies f Odonates using COI gene depicted monophyletic ancestry [14].

The amplified product of all these sequences is rich in AT content with thymine is the most frequently een followed by Adenine, Cytosine and Guanine. The AT content stood 66.26 % in comparison with the GC ontent of 33.74 %. [15] also confirmed the high A+T content among Odonates. The high A+T content along ith second codon change reflect the higher evolutionary divergence of these species. The nucleotide ıbstitution analysis showed that *Acisoma panorpoides* is having high divergence than other members. litochondrial gene thus can be useful for unravelling phylogenetic relationships among closely related species.

eferences

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2. Methodology

Genomic DNA was extracted from one of the thoracic legs of the experimental insect, *Aciagrion occidentale* (Figure 1). The tissue was homogenized and genomic DNA in the homogenate was isolated using Ultrapure Mammalian Genomic DNA Pre Kit. About 2 ng of genomic DNA was amplified for mitochondrial cytochrome oxidase subunit I (COI) gene using forward Primer, 5'-CATTGGAGATGACCAAATTTA-3'and reverse primer, 5'ATTGGATCTCCACCACCTGC-3'. The The PCR products were resolved on a 1% TAE- agarose gel, for confirmation of the target gene amplification. The PCR product was column purified using UltraClean PCR Clean-up Kit (Mo Bio Laboratories, Inc. California). The purified PCR product was sequenced from both ends using Sanger's sequencing method[11]. Sequences obtained were assembled by using ClustalW and the consensus was taken for the analysis. The final sequence was searched for its similarity using BLAST of NCBI (www.ncbi.nlm.nih.gov/) and submitted in the GenBank for worldwide accession. The phylogenetic tree was plotted in Neighbor Joining method using by MEGA6 software [12].

3. Results and Discussion

The partial mitochondrial cytochrome oxidase I (COI) region of *Aciagrion occidentale* yielded a 522bp long fragment (GenBank Accession number: KM 096996) showing 99% sequence similarity to *Aciagrion borneense* (GenBank Accession number: KF 369275) found in Netherland.

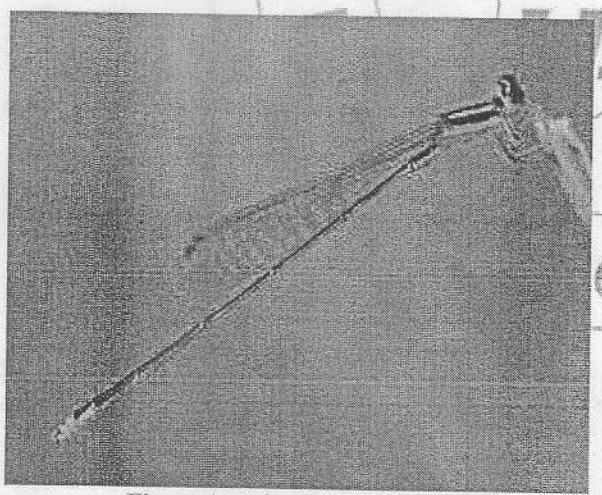

Figure 1: *Aciagrion occidentale*

Odonates are the indicators of environmental quality, evolutionary developmental biology and conservation biology [1]. They generally belongs to the base of winged insect (Pterygota), making them unique and exciting. This phylogenetic position shows that they can provide most useful information on the evolution of the morphological diversification and mechanics of insect wings and the general body plan of the winged insects. The effect of changing environmental condition related to climate change, environmental pollution, and water quality, habitat fragmentation on life history strategies, population dynamics and adaptability of animals in the field rather than in the laboratory makes them an excellent model system for study purpose [2]. *Aciagrion occidentale* is a member of Coenagrionidae family of Odonates. This damselfly occurs in a wide variety of habitats chiefly along streams, ponds and swamps.

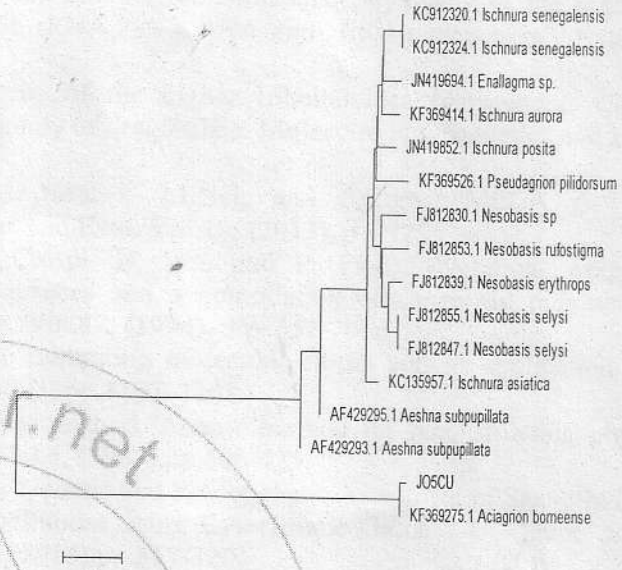

Figure 2: Phylogenetic tree of *Aciagrion occidentale (JO5CU)* using neighbor joining method

GenBank data analysis showed that *Aciagrion occidentale* (KM 096996) is having 99% sequence similarity to *Aciagrion borneense* COI gene (KF 369275) reported from Netherland [1]. This indicates that both of them are sharing a common ancestor. The N-J tree constructed with BLASTn result depicted that *Aciagrion occidentale* is phylogenetically very close to the other species of damselflies reported from different geographical locations.

Acknowledgement

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