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Genetic Variation and Phylogeny Assessment of *Aciagrion Occidentale* (Odonata: Coenagrionidae) Using Mitochondrial Cytochrome Oxidase Subunit I Gene

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Abstract: ***Aciagrion occidentale is a migratory species widely distributed in montane and submontane areas in open grass besides weedy ponds and herbage. Here we used the COI barcode locus (522bp) to clarify the specific taxonomic status of Aciagrion occidentale. Molecular data suggests that this species is having 99% sequence similarity to Aciagrion borneense found in Netherland and confirmed that both are sharing same genus. The N-J tree constructed with BLASTn result depicted that Aciagrion occidentale is phylogenetically very close to damselflies. Anisopterans and Zygopterans are sharing some common characters and the results confirm that Zygoptera is a paraphyletic group derived from the monophyletic groups of Anisoptera and Anisozygoptera.***

Keywords: Odonates, *Aciagrion occidentale*, Mitochondrial DNA, Cytochrome oxidase subunit I gene

1. Introduction

Odonates are often addressed as the ‘guardians of the watershed’, includes dragonflies and damselflies. Damselflies tend to be less robust, appearing weak in flight. Most species hold their wings folded back over the abdomen at rest. Damselflies eyes occupy much of the animal's head, nearly touching each other across the face with typically a gap between the eyes. In nature they appear as the subject of conservation strategy in the case of endangered species and indicators of wetland habitat quality. The habitat selection of adult Odonate species strongly depends on vegetation structure and their larvae develop only in clean water. Thus the different stages of their life cycle are very much sensitive to the ecological disturbances. Therefore, along with birds and amphibians, dragonflies can serve as one of the key bio-indicator groups, whose high species diversity clearly mirrors favourable conservation state of wider wetland ecosystem [1]. About 137 species and subspecies of Odonata spreading over 79 genera, 12 families and 31 subfamilies are reported from Kerala [2]. *Aciagrion occidentale* is one of the important odonate species coming under the family Coenagrionidae, commonly known as ‘Slims’. They are widely distributed across Central and South India, Sri Lanka, Thailand, Cambodia and Vietnam. No major threat to the species has been described and the species is quite common across its range. Suitable habitat is shrub dominated wetlands although it can also be found in bogs, marshes, swamps, fens, peat lands, small streams and permanent freshwater lakes. Males are very small and slender, having 8 abdominal segments with a black elongate dorsal triangular mark. Females are similar to male but much more robust and with a stouter abdomen.

Taxonomy is the identification of species, based on the morphological characters and it requires vast knowledge about varied organisms and their characters. Identification using traditional taxonomy is difficult due to the external changes in the organisms caused by seasonal and geographical variations. Many organisms alter themselves physiologically and morphologically due to the unfavourable conditions in the environment. Thus adoption of manual taxonomy, often leads to wrong identification of the species. This problem has thus influenced the emergence of the molecular taxonomic frame work studies for the conformation and the betterment in the identification of species.

Mitochondrial DNA acts as an excellent genetic marker with the gene flow in its matrilineal inheritance. It represents maternal haploid genome and accumulates nucleotide substitution 5-10 times more rapidly than nuclear DNA, which makes it suitable for examining population and sub population structures among related taxa [3]. Cytochrome oxidase subunit I (COI) gene is one of the most important protein encoding genes of mt DNA and has been utilized in the studies of molecular evolution due to lack of introns, simple alignment, limited exposure to recombination and availability of robust primer sites. Phylogenetic analysis using COI gene sequences were extensively carried out by several workers in different group of organisms like southern house mosquito *Culex quinquefasciatus* [4], *Armigeres subalbatus* mosquito [5] green bottle fly *Lucilia sericata* [6], *Herpetogramma stultalis* [7], white backed plant hopper *Sogatella furcifera* [8], Asian honeybee *Apis cerana* [9], and lepidopteran species [10]. Here we amplified cytochrome oxidase I gene of *Aciagrion occidentale* found in Kerala for its phylogenetic analysis.

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.

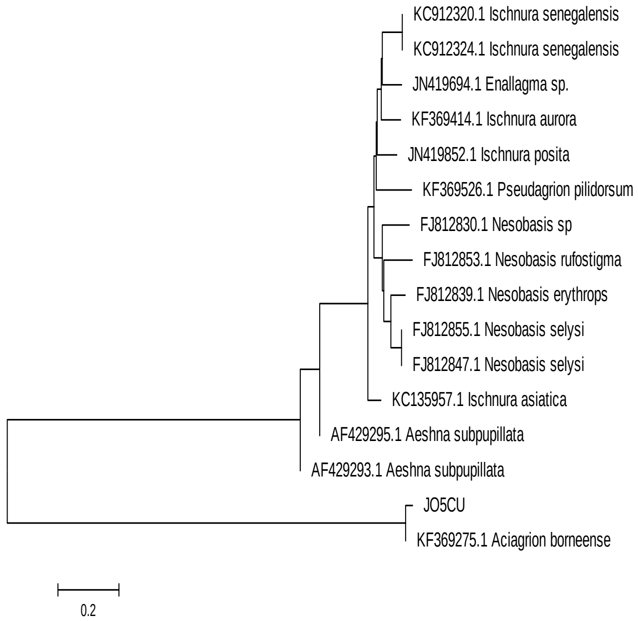

Figure 2: Phylogenetic tree of *Aciagrion occidentale (JO5CU)* using neighbor joining method

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.

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