Research Paper
Impact Factor: 6.089
Peer Reviewed Monthly Journal
www.ijmdrr.com

IJMDRR
E- ISSN –2395-1885
ISSN -2395-1877

"MOLECULAR PHYLO-GENETICS OF SOME ODONATES OF VELLAYANI WETLAND"

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Abstract
Dragonflies and damselflies collectively known as Odonates are prominent and colorful insects of wetlands. Study was carried out on the molecular phylogeny of selected odonates of Alappuzha. Seven samples were collected Kerala, that is Ceriagrion coromandelianum, Pseudagrion microcephalum, Pantala flavescens, Brachythemis contaminata,Trithemis aurora, Diplacodes trivialis, Myrmeleon sp. Whole genomic DNA was isolated using "Phenol chloroform method". Molecular phylogenetic relationship among the species is examined using 540 bp of 16SrRNA gene sequence. Then phylogenetic tree was constructed using ML tree.The analysis of the phylogenic relation between the species subjected to analysis suggest that the Damselflies (Ceriagrion coromandelianumand Pseudagrion microcephalum) and Dragonflies (Pantala flavescens, Brachythemis contaminata, Trithemis aurora and Diplacodes trivialis) apart in to two different clades. The outgroup which was an Antilion (Myrmeleon sp.) forms a separate clade and is a right outgroup, as it is rightly separated out from the Odonate clusters.

Keywords: Dragonflies, Damselflies, Phenol Chloroform Method.

Introduction

Biodiversity is the differences and variety among the living organisms and it explains the variety of life from genes to ecosystem. (Zhang *et al.,* 2011) Kerala is one of the biodiversity rich states of India and is well known for its wetlands. They are about 217 wetland areas in Kerala. They are considered as 'kidneys' of nature.

Dragonflies and damselflies collectively known as Odonates (Order- Odonata) are prominent and colourful insects of wetlands.The major thrust of this study is to develop wider conservation priorities for odonata and to assess the suitability of adult odonata as biological indicators of environmental quality at local scale.(Villalobos- Jimenez *et al.*,2016)

Study area

Vellayani Lake, the second largest freshwater lake of Kerala, is located in the outskirts of Thiruvananthapuram city (8"24'09" - 8"26'30" N; 76°59'08"- 76°59'47" E) and has a water spread area of 450 ha. The lake is situated 29 m above msl, and the lake bed is 0.1 to 1.5 m below the msl

Objective of the Present Study

- To obtain molecular phylogeny, evolutionary status and construct evolutionary trees.
- To explore the efficiency of 16S gene as a barcode to identify Odonates.
- To explore its usefulness as a conservation tool in analyzing the genetic differentiations among Odonates in a wetland ecosystem.

3. Materials and Methods

3.1 Sample Collection

Seven unknown different colour Odonates were sampled from Vellayani wetlands, Trivandrum, Kerala were used for this study. Three were identified as Dragonflies, two as Damselfiles and one as Antilion after primary identification. All species were photographed in field prior to capturing for sampling.

DNA Isolation from Tissue Samples

DNA sampling method has been standardized for this purpose by clipping the legs of Dragonflies. Approximately 25 mg tissue taken from the legs or thorax of the Odnotaes will be used to extract high molecular weight genomic DNA. Tissue collections will be made from the field itself and preserved in DNA friendly fashion (100% ethanol). Specimens were preserved in 70% ethanol for further reference. Tissue samples will be transported to the laboratory in labeled 1.5ml micro centrifuge tube.

Phenol Choloroform Isomamyl alcohol method will be used for the extraction of the DNA. The quality of extracted DNA will be determined through electrophoresis.

PCR Amplification of 16 S RNA Regions

PCR was performed in reaction mixture of 25µl and 38 cycles of PCR were performed in a DNA thermocycler (Eppendorf). Primer details, PCR mixture details and the thermocycling parameters are given in the following tables.

Multiple Sequence Alignment

Sequences were aligned using Clustal W. Length differences were resolved by inserting alignment gaps and positions that could not be aligned unambiguously were excluded.

Sl. No:	Genes	Primer Sequence	Product Length	Reference
1.	16S rRNA	F- 5'-CGCCTGTTTATCAAAAACAT-3' R- 5'-CCGGTCTGAACTCAGATCACGT-3'	540 bp	Palumbi et al. 1991

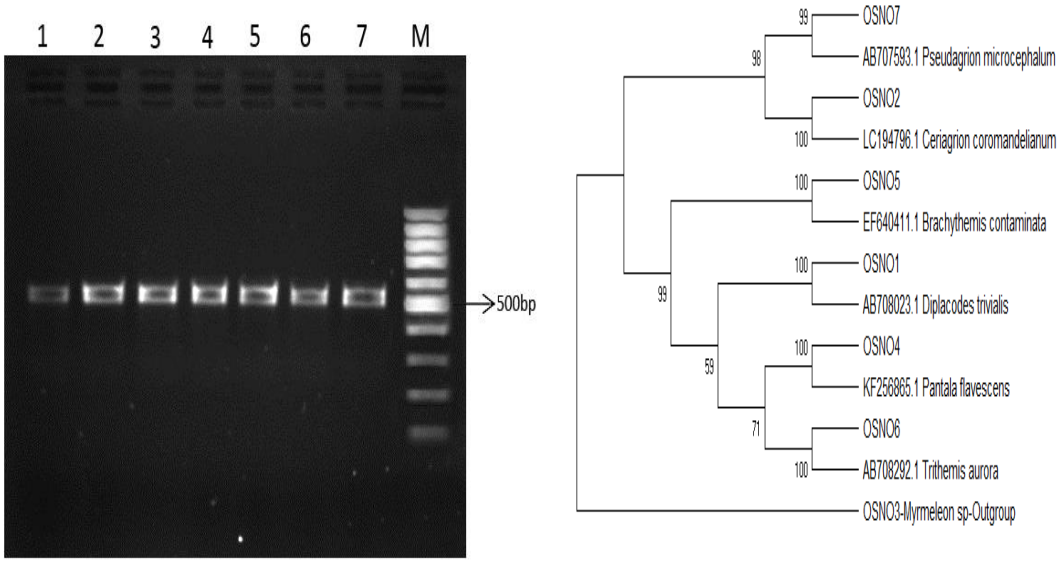
Genetic Diversity and Phylogenetic Analysis

- Gene sequences (AB708292, KF256865.1, AB708023.1, EF640411.1, LC194796.1 and, AB707593.1) were retrieved from Gene bank.

Result

In the course of this study a total of 7 individuals were used for partial sequence analysis of 16S rRNA mitochondrial gene. Finally, it is concluded after sequencing that the six unknown Odonate species (OSNO1, OSNO2, OSNO4, OSNO5, OSNO6 and OSNO7) used in the study belongs to *Diplacodes trivialis, Ceriagrion coromandelianum, Pantala flavescens, Brachythemis contaminata, Trithemis aurora* and *Pseudagrion microcephalum* respectively, partial sequence information of 16S rRNA gene can be used as a diagnostic molecular marker in identification and resolution of taxonomic ambiguity of Odonates. The study has also supported the claim of robustness of universal primers for 16S rRNA mt genes.

Fig. 1 Gel picture of 16S rRNA gene amplified products (1-7 = samples; M = 100 bp DNA Ladder)



Conclusion

Under this study, we have studied some odonates of Vellayani wetland.A total of seven specimen were collected, taxonomically identified and preserved with tissue sampling for DNA isolation .Isolated DNAs were amplified using universal primer pairs and sequenced for 16S rRNA . A total of 7 sequences were generated having sequence length in the range of 540 bp.All sequence were analysed for closest match in NCBI using the program BLAST.Sequence divergence calculated by K-2-P method and phylogenetic tree were reconstructed by ML method .The mean genetic distance computed for all the species used in this study was found to be 0.410. The ML tree showed the clustering pattern of 13 individuals which belongs to 7 different species and phylogenetic relationships of the seven samples were also examined using the 16S rRNA mitochondrial gene to elucidate species relationship. The analysis of the phylogenic relation between the species subjected to Analysis suggest that the Damselflies (*Ceriagrion coromandelianum* and *Pseudagrion microcephalum*) and Dragonflies (*Pantala flavescens, Brachythemis contaminata, Trithemis aurora* and *Diplacodes trivialis*) apart in to two different clades.The out-group which was an Antilion (*Myrmeleon sp*.) forms a separate clade and is a right outgroup, as it is rightly separated out from the Odonate clusters.

Reference

1. Villalobos-Jimenez, G., Dunn, A. and Hassall, C., 2016. Dragonflies and damselflies (Odonata) in urban ecosystems: a review. European journal of Entomology, 113, pp.217-232.
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