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Molecular Phylogenetics of Six Dragonfly Species (Odonata: Anisoptera: Libellulidae) of Udaipur Region (Rajasthan)

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ABSTRACT

In current study, mitochondrial COI gene sequencing of six species of dragonflies (Anisoptera: Libellulidae) collected from Udai Sagar lake of Udaipur region was done using Sanger sequencing protocol. All six reported species shows 100% similarity to the NCBI Genbank database. Molecular phylogenesis was done using BLAST (Basic Local Alignment Search Tool). All six Sequences were submitted to Genbank, which would be useful to identify dragonflies in future studies. This study provides phylogeny trees and idea of evolutionary relationship of six dragonfly species.

INTRODUCTION

Dragonflies are keystone insect group of aquatic food webs and wetland ecosystem. It is important biocontrol agent and predators of integrated pest management. It is also a prominent bioindicator of environment change (Simaika *et al.*, 2012). However dragonflies are easily identifiable because of their large body size and vibrant colours but sometimes sexual dimorphism, polymorphism, cryptic, sibling species and color change due to different environment, make their taxonomy difficult. Sometimes new emerged adults (tenerals) show less vibrant colors than their aged counterparts (Nisar *et al.*, 2021; Philips and Swanson, 2018; Yong *et al.*, 2014). Identification and confirmation of species by sequencing mitochondrial COI gene is significant (Almansoori *et al.*, 2019; Heber *et al.*, 2003). Molecular phylogenetic analysis using DNA barcoding is an effective tool for assessing evolutionary history of an organism (Ahmed and Karim, 2024). Libellulidae is a common family of dragonflies which comes under order Odonata and suborder Anisoptera (Karle *et al.*, 2015; Karthika *et al*, 2012). Anisoptera has 12 families and 3113 species of dragonflies worldwide, while Libellulidae family alone has 1038 species worldwide. 97 species of Libellulidae family are reported in India, out of which 8 species are reported endemic to India (Kalkman *et al.*, 2020; Subramanian and Babu, 2024).

MATERIALS AND METHODS

Adult specimens of all six species of dragonflies were collected by insect net from Udai Sagar lake in non-breeding season (Mar-May, 2025). Collected specimens were preliminary identified in the laboratory with the help of taxonomic keys and field guide (Dheerendra 2022; Subramanian, 2005). After preliminary identification six male specimens were send to Dextrose Technologies Laboratory, Bangalore, Karnataka. Experimental protocol and methods adopted are as follows: DNA isolation and extraction was done using Genei DNA extraction kit. Extracted DNA was eluted in 25 µl of nuclease-free water. PCR amplification was done using universal forward and reverse primers, LCO 1490 (5’-GGTCAACAAATCATAAAGATATTGG-3’) and HCO 2198 (5’TAAACTTCAGGGTGACCA AAAAATCA-3’). Quality check of DNA was done using agarose gel electrophoresis. Amplified DNA was purified enzymatically using Exo-SAP (Applied Biosystem). After the purification, Sanger dideoxy sequencing was done. After getting FASTA sequence, Sequence similarity matching, molecular phylogenetic analysis and phylogeny tree making was done by using BLAST (Basic Local Alignment Search Tool). All sequences are submitted to Genbank. Genbank is the largest nucleotide database available online which is maintained by NCBI (National Centre for Biotechnology Information).

RESULTS AND DISCUSSION

Mitochondrial COI gene sequencing of six species of family Libellulidae Leach, 1815 after preliminary identification was done. FASTA sequence of all six species were run on BLAST for finding sequence similarity and preparation of phylogeny trees. Accession number were due at the time of publication. All six species show 100 percent similarity with available database, which confirm their identification. Summary of results are given in Table 1 and phylogeny trees obtained using BLAST are given in Figure 1. *Brachythemis contaminata* show phylogenetic relationship with genus *Nannophya*. They also show common ancestry with genus *Sieboldius* (family Gomphidae). *Indothemis carnatica* show phylogenetic relationship with genus *Orthetrum*. *Orthetrum sabina* and *Trithemis aurora* both show phylogenetic relationship with *Orthetrum poecilops* and *Trithemis pallidinervis*. Further *Trithemis pallidinervis* also show phylogenetic relationship with *Trithemis festiva*. *Trithemis kirbyi* show phylogenetic relationship with genera *Acisoma* and *Agrionoptera* as well as *genus Synthemis* (family Synthemistidae). However *Trithemis kirbyi* is morphological similar to *Trithemis aurora*.

Table 1. Summary of sequence similarity of six species of dragonflies.

Species	Common name	Base pair	Query cover (Similarity)
Brachythemis contaminata (Fabricius, 1793)	Ditch jewel	541	100%
Indothemis carnatica (Fabricius, 1798)	Black marsh skimmer	539	100%
Orthetrum sabina (Drury, 1770)	Green marsh hawk	634	100%
Trithemis aurora (Burmeister, 1839)	Crimson marsh glider	615	100%
Trithemis kirbyi (Selys, 1891)	Scarlet rock glider	658	100%
Trithemis pallidinervis (Kirbyi, 1889)	Long-legged marsh glider	670	100%

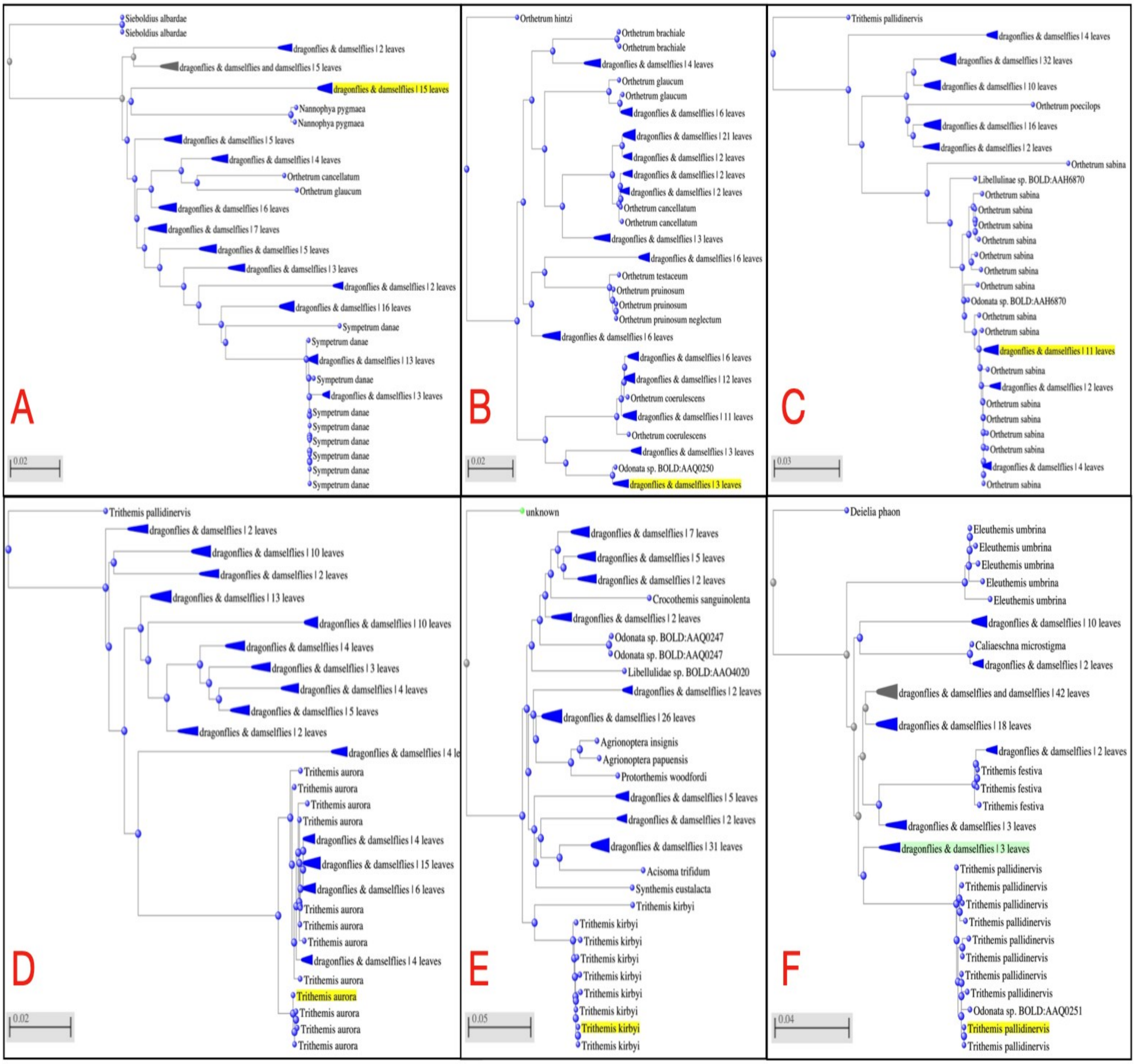

Fig 1. Neighbor-joining phylogeny trees of *Brachythemis contaminata* (A), *Indothemis carnatica* (B), *Orthetrum sabina* (C), *Trithemis aurora* (D), *Trithemis kirbyi* (E) and *Trithemis pallidinervis* (F).

CONCLUSION

This study confirm the identification and provides phylogeny trees of six reported dragonflies species. Phylogenetic relationship of six dragonflies show their association with other odonates. Fortunately COI sequences of all six species match 100 percent with available nucleotide database. DNA barcoding is a promising tool for identification and confirmation of known and unknown species. Only problem is unavailability of complete database. However in assessing molecular phylogeny, Genbank has done good work by providing open access to public. BLAST like software further ease the work and provide great result with accuracy. This is the first attempt to assess molecular phylogeny of dragonflies in Udaipur region, which would help researcher in future studies.

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