



PHYLOGENETICS OF FOUR DAMSELFLY SPECIES (ODONATA: ZYGOPTERA: COENAGRIONIDAE) OF UDAIPUR REGION (RAJASTHAN)

^{1*}Rawal Deepak, ²Dhaked Jaynesh and ³Suthar Yamini

Department of Zoology, Mohanlal Sukhadia University Udaipur, India

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ABSTRACT

In current study, DNA barcoding of four species of damselflies was done to assess molecular phylogeny. Phylogeny trees were obtained for all four species using BLAST. Mitochondrial COI Sequences of all four species match 100% with available nucleotide database. All sequences were submitted to Genbank, which would be part of available database for future studies. This study provides a good idea of evolutionary history of damselflies. This is the first molecular phylogenetic study, which is done on damselflies of Udaipur region.

Keywords: Zygoptera, Phylogeny, COI gene, BLAST, Coenagrionidae.

INTRODUCTION

Odonates are the most ancient winged insects (Carle *et al.*, 2008). Odonates are considered as monophyletic group which includes both dragonflies (Anisoptera) and damselflies (Zygoptera). Damselflies comes under order Odonata and suborder Zygoptera Selys, 1854 (Corbet, 1999; Grimaldi and Engel, 2005). Total 36 families of damselflies with 318 genera and 3337 species reported globally. Coenagrionidae is most ancient family of damselflies. Alone Coenagrionidae family has 1295 species and 108 genera worldwide (Paulson and Schorr, 2024). 59 species under Coenagrionidae family are reported from India, out of which 4 species are endemic to India (Kalkman *et al.*, 2020; Subramanian and Babu, 2024). Members of family Coenagrionidae are commonly known as pond damsels. Observation of wing venation is traditional tool of odonate taxonomy but it has certain limitations like homoplasy, cryptic species, dull colored tenerals, sibling species, polymorphism, sexual dimorphism *etc* (Dijkstra *et al.*, 2013). So DNA barcoding tool provides fast, accurate and authentic identification. COI (cytochrome c oxidase I) gene is a good mitochondrial DNA marker used in this (Zhu and Han, 2000; Zhu and Ou-Yan, 2000). Anisoptera were more studied by molecular phylogenetics as compared to Zygoptera (Hebert *et al.*, 2003).

MATERIALS AND METHODS

Adult specimens of all four species of damselflies 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 four 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 and HCO 2198. Quality check of DNA was done using agarose gel electrophoresis. Amplified DNA was purified enzymatically using Exo-SAP (Applied Biosystem).

After the purification, Sanger sequencing was done. After getting FASTA sequence, Sequence similarity matching, molecular phylogenetic analysis and fast-minimum evolution phylogeny tree making was done using BLAST (Basic Local Alignment Search Tool). All sequences are submitted to Genbank. Accession numbers are due at the time of publication. Genbank is the largest nucleotide database available online which is maintained by NCBI (National Center for Biotechnology Information).

*Corresponding Author: Dr. Rawal Deepak, Faculty, Department of Zoology, Mohanlal Sukhadia University Udaipur, India Email: deepakrawal5@gmail.com.

RESULTS AND DISCUSSION

Mitochondrial COI gene sequencing of four species of family Coenagrionidae Kirby, 1890 after preliminary identification was done. FASTA sequence of all four specimens were run on BLAST for finding sequence similarity and preparation of fast-minimum evolution phylogeny trees. All four species show 100 % similarity with available Genbank nucleotide database, which confirm their identification. *Agriocnemis femina* show phylogenetic

relationship with *Agriocnemis toto*. *Agriocnemis pygmaea* show close relationship with *Agriocnemis femina*. Both *Agriocnemis femina* and *Agriocnemis pygmaea* show common ancestry. *Ceriagrion coromandelianum* show evolutionary relationship with *Ceriagrion sauve* and *Ceriagrion glabrum*. *Ceriagrion olivaceum* also show evolutionary relationship with *Ceriagrion glabrum*. Summary of results are given in Table 1 and phylogeny trees obtained using BLAST are given in Figure 1.

Table 1. Summary of sequence similarity of four species of damselflies.

Species	Common name	Base pair	Similarity percentage
<i>Agriocnemis femina</i> (Brauer, 1868)	Pinhead wisp	531	100
<i>Agriocnemis pygmaea</i> (Rambur, 1842)	Pygmy wisp	607	100
<i>Ceriagrion coromandelianum</i> (Fabricius, 1758)	Coromandel marsh dart	664	100
<i>Ceriagrion olivaceum</i> (Laidlaw, 1914)	Rusty marsh dart	641	100

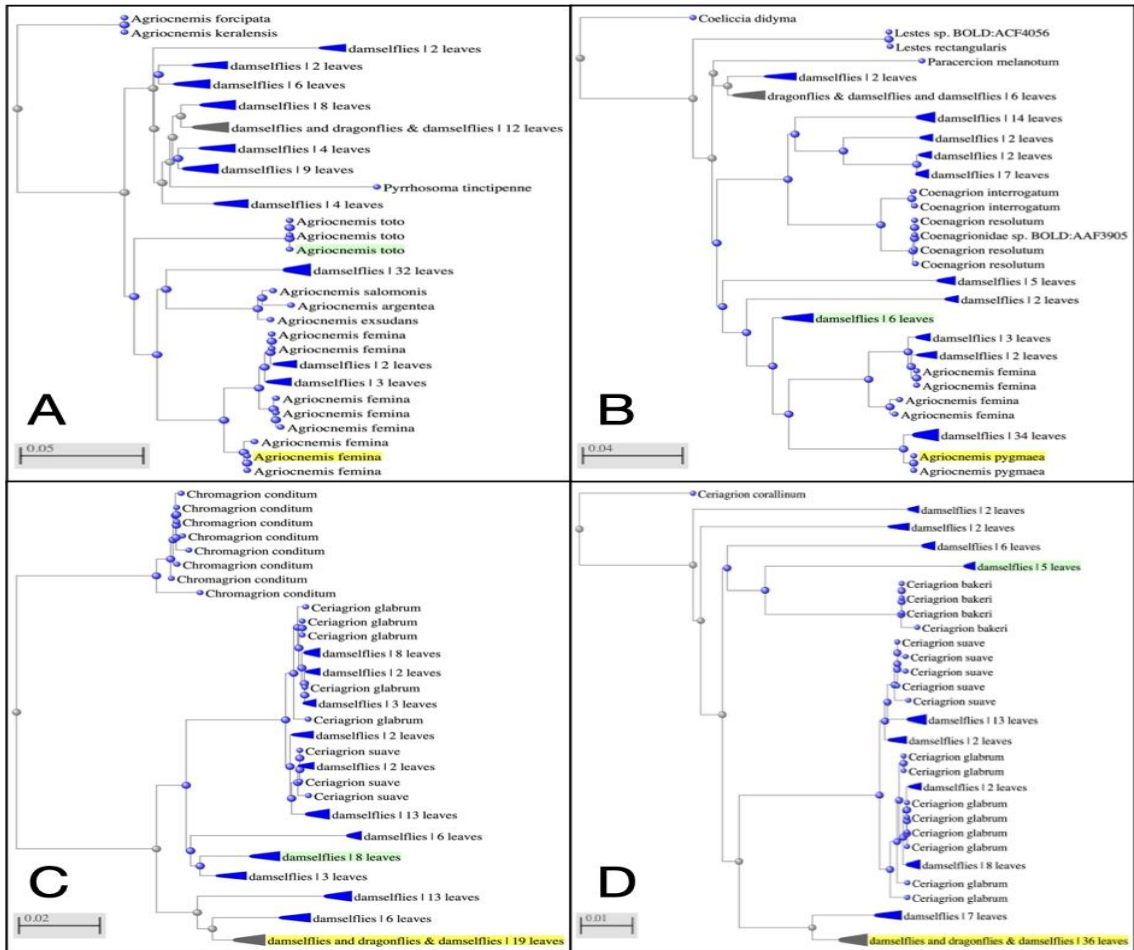


Figure 1. Fast-minimum evolution phylogeny trees of *Agriocnemis femina* (A), *Agriocnemis pygmaea* (B), *Ceriagrion coromandelianum* (C) and *Ceriagrion olivaceum* (D).

CONCLUSION

This study confirms the species identification and provides evolutionary phylogeny trees of four reported damselflies species. Phylogenetic relationship of four damselflies shows their association with other odonates. By chance, mitochondrial COI sequences of all four species match 100 percent with available Genbank nucleotide database. DNA barcoding is a promising tool for identification and authentication of known and unknown species. Only limitation is unavailability of complete database. Genbank has done good work by providing open access to public. BLAST further ease the work and provide best result. This is the first attempt to assess molecular phylogeny of damselflies of Udaipur region, which would help researcher in future studies.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest

ETHICS APPROVAL

Not applicable

AI TOOL DECLARATION

The authors declares that no AI and related tools are used to write the scientific content of this manuscript.

DATA AVAILABILITY

Data will be available on request

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