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RESEARCH ARTICLE

SPECIES AUTHENTICATION AND TAXONOMIC RELATIONSHIP ASSESSMENT OF *CERIAGRION COROMANDELIANUM* (FABRICIUS) (ZYGOPTERA: COENAGRIONIDAE) USING THE MOLECULAR MARKER CYTOCHROME OXIDASE I GENE

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ABSTRACT

Ceriagrion coromandelianum commonly called 'Coromandel Marsh dart' or 'Yellow Waxtail' is a widely distributed damselfly species in South Asia. It acts as a natural 'biocontrol' agent against paddy pests like leaf hopper, planthopper, midges and flies. The partial sequence of cytochrome oxidase I gene of *C. coromandelianum* was analysed by PCR and the result yielded a gene product of 573 bp length. Phylogenetic tree constructed by Maximum likelihood and Neighbour joining method supported with the bootstrap value, taxonomically confirmed the relationship of this species with other damselflies and depicted that it is closely related to *C. nipponicum* than other *Ceriagrion* members. Evolutionary divergence and tree reveals that all the *Ceriagrion* members are having a monophyletic ancestry originating from a common clade with maximum divergence for *C. whellani* followed by *C. nipponicum*, *C. coromandelianum* and *C. glabrum* and it occurred mainly due to the transitional change of nucleotides.

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INTRODUCTION

Damselflies belongs to a primitive and ancient insect order Odonata, with fossil records dating back to the Permian era of about 230 - 280 million years ago. Unlike dragonflies they have a very narrow rectangular head and widely separated eyes. The hind wings and forewings are similar in shape, size and venation. They are good indicators of environmental changes as they are sensitive to changes in the habitats, atmospheric temperature and weather conditions (Corbet, 1999; Foote, 2005). The abundance of the damselfly family Coenagrionidae is an important biological indication of certain environmental aspects like pollution free area of the wetland. *Ceriagrion coromandelianum* is a widely distributed Coenagrionidae species known from India (Prasad *et al*., 1995), Srilanka, Nepal and Pakistan and there are also certain suspected records from China (Needham, 1931). They are often seen along the banks of ponds, rivers and canals and their breeding generally takes place in shallow water bodies with abundant growth of grass and other aquatic plants (Subramanian, 2009).

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This species are known to be ecologically important as they are predators of leafhoppers, plant hoppers, midges and flies (Krishnasamy *et al.,* 1984) and hence has got a pest management strategy. It is a medium sized bright yellow coloured damselfly with marked sexual dimorphism. Females have 29-32 mm long abdomen, uniformly olivaceous with an ochreous or golden brown tint colour on dorsal side of the body, golden brown coloured thorax, with eyes olivaceous above and pale greenish below. Males have 28-30 mm long abdomen, 18-20 mm long hind wing, and yellow with black colour spines on legs and eyes are olivaceous above and pale greenish yellow below.

Srivastava *et al.* (1953) showed that *C. coromandelianum* exhibits heteropycnosis in the autosomal segment of its chromosome. Andrew *et al.* (2011) reported that *C. coromandelianum* uses certain tactile and visual clues for the leaf lamina preferentially at the lateral region to oviposit. They also concluded that eggs of this species are cylindrical and composed of exochorion and endochorion surrounded by 5 micropylar orifices. Phylogentic relationships of different damselflies under the genus *Ishnura, Calopteryx* and *Aciagrion* are well studied by using the molecular markers like 12S, COII, 16S and COI mitochondrial genes (Paul *et al.*, 1999*;* Benahard *et al*., 2000; Jisha and Sebastian, 2015).

Most of the phylogenetic studies of Odonates strongly supported the monophyletic origin in Anisopterans and paraphyletic origin in Zygopterans. But molecular and phylogenetic studies of *Ceriagrion coromandelianum* are not yet reported in detail and hence this study is relevant in this scenario.

MATEIALS AND METHODS

Ceriagrion coromandelianum was collected from the rice fields of Kerala, India and it was stored at -20°C until the DNA was extracted. The genomic DNA was isolated using Origin DNA Prep Kit. The 5' end of the mitochondrial cytochrome oxidase subunit I (COI) gene was amplified using the forward primer, 5'-TCGGTCATGAGCAGTATAGTAGTAC-3' and reverse primer, 5'-AATAGGATCTCCTCCACCTGCTG-3'. The PCR product was column purified using MoBio UltraClean PCR Clean-up Kit (MoBio Laboratories, Inc. California). The purified PCR product was sequenced from both ends using the forward and reverse primers using the Sanger's sequencing method. The forward and reverse sequences were assembled by using Clustal W after removing the forward and reverse primers and the consensus was taken for the analysis. The phylogenetic analysis was done using MEGA6 software (Tamura *et al.,* 2013).

RESULTS AND DISCUSSION

Ceriagrion coromandelianum under the family Coenagrionidae is a natural biocontrol agent in the paddy fields since it feeds on a large variety of leaf hoppers, plant hoppers, midges and flies (Das *et al.,* 2015). The top of the head is bright orange in colour. Eyes are bluish above and greenish below. Legs are greenish near thorax, which merge to become yellow at the end. Thorax is light green in color. Abdomen is yellow, with slight orange colour at the tip. Wings are transparent, wing spots are pale brown-yellow. Genetic analysis of this species is not yet reported from India. The PCR amplification of partial mitochondrial COI gene of *C. coromandelianum* yielded a single product with about 573 bp in size. The sequence obtained after removing the primers used for PCR amplification was submitted to NCBI GenBank (GenBank Accession: KT 222949) for future references.

Table 1. Evolutionary divergence between sequences of closely related species

Organism with Accession No	% of Divergence
Ceriagrion coromandelianum KT 222949	0
Ceriagrion glabrum KF 369334	0.12
Chromagrion conditum JN 419474	0.13
Ceriagrion nipponicum KF 257114	0.12
Ceriagrion whellani KF 369335	0.17
Ischnura senegalensis AB 758091	0.18
Teinobasis cryptica KF 369559	0.17
Ischnura senegalensis AB758002	0.18

Phylogenetic tree constructed by both Neighbour-joining and Maximum likelihood method confirmed that this species is taxonomically more close to *Ceriagrion nipponicum* than other damselfly members. All the *Ceriagrion* members found in the tree inferred a monophyletic ancestry because all the members were originated from one clade having 29 as the Bootstrap value.

Table 2. Maximum Composite Likelihood Estimate of the Pattern of Nucleotide Substitution

	A	*T*	*C*	G
A	-	*1.75*	*0.86*	*21.33*
T	*1.57*	-	*9.41*	*0.84*
C	*1.57*	*19.27*	-	*0.84*
G	*39.96*	*1.75*	*0.86*	-

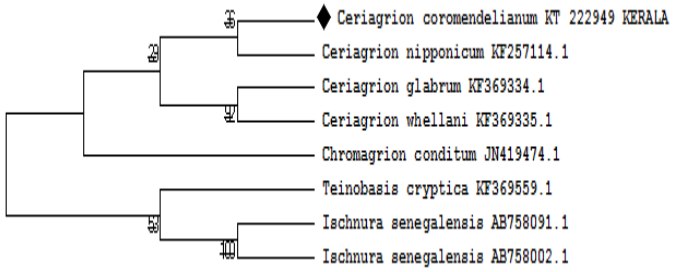

Figure 1. Evolutionary tree constructed by Neighbour joining method

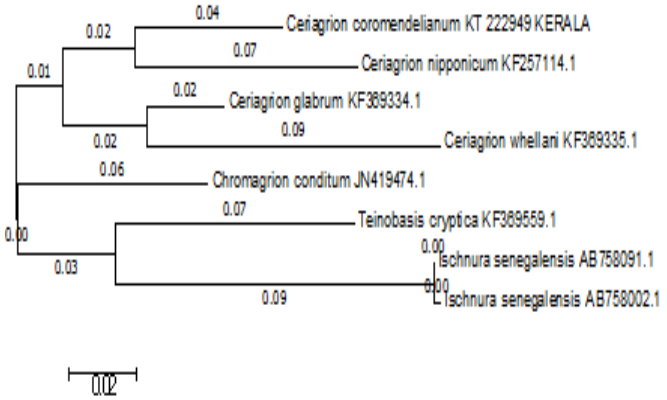

Figure 2. Evolutionary tree constructed by Maximum likelihood method

After a period of time they may have got splitted into two sister clades with one clade containing *C. coromandelianum* and *C. nipponicum* and the other with *C. glabrum* and *C. whellani.* Bootstraping is one of the important statistical methods for constructing phylogenetic tree. Bootstrap value f 90 clearly indicates that *C. glabrum* and *C. whellani* are taxonomically very close. This is supported by the view of Joseph Felsenstein who states that if the bootsrap value is above 90, the taxa will be very close together (Figure 1). Maximum likelihood analysis with branch length showed that *C. whellani* is the most diverged species in relation with DNA sequence which is followed by *C. nipponicum* and *C. glabrum* (Figure 2). BLAST program also says that the genus Ceriagrion is more related to *Chromagrion* followed by *Ishnura* members (Table 1). Maximum composite likelihood showed the nucleotide substitutions in the COI sequences with the nucleotide frequencies in the ratio 31.26% (A), 34.98% (T/U), 17.07% (C), and 16.68% (G) which shows maximum A+T content.

This result is supported by the reports of Chippindale *et al.*, *1999;* Artiss *et al*., 2001 and Freeland & Conrad, 2002 who

stated that the overall A + T content was high (67.6%), within the order Odonata. The table clearly indicates that nucleotide divergence happened mainly due to high transition rate of Adenine and Guanine than compared to transversion (Table 2). The evolutionary history was inferred using the Neighbour-Joining method (Saitou and Nei, 1987). The bootstrap consensus tree inferred from 500 replicates is taken to represent the evolutionary history of the taxa analyzed. The evolutionary distances were computed using the Jukes-Cantor method (Jukes and Cantor, 1969). The evolutionary history was inferred by using the maximum Likelihood method. The tree with the highest log likelihood -1969.2371 is shown. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site (Figure 2). The number of base substitution per site from between sequences is shown in Table 2. Analysis was conducted using maximum likelihood model. Rates of different transitional substitutions are shown in bold and those of transversional substitutions are shown in italics.

Conclusion

Ceriagrion coromandelianum is one of the dominating species in paddy fields due to its high predation on pest species (Das Bidyut *et al.,* 2015). The presence of this species is valuable information for assessing the ecological status of wetland ecosystem. Results confirmed that species authentication of this species is strictly correlated with the classical taxonomy. Phylogenetic analysis showed that it is a Coenagrionidae member because of its closer relationship with other Coenagrionidae members. Thus the present study confirmed the taxonomic status of this species and also concluded that it is taxonomically more related to *C. nipponicum* and also it is strictly a damselfly because of its closer relationship with other Coenagrionidae members. The nucleotide divergence is mainly due to the transitional mutation. The COI DNA barcode developed in this study can be used for the accurate identification and also its taxonomic relationship with other damselflies.

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