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RESEARCH ARTICLE

DNA BASED PHYLOGENETIC ANALYSIS OF AQUATIC BEETLE DYTISCUS MARGINALIS ISOLATED FROM NORTH KERALA, USING MITOCHONDRIAL COI MARKER

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ABSTRACT

Dytiscus marginalis are the diving beetles with their wings covered by hard wing cases known as elytra. It is the largest order including more species known to science than any other order not only in the class insect, but also in the entire animal kingdom. The order is huge and includes a wide variety of groups. In the present study we have isolated, PCR amplified and deciphered the partial nucleotide sequence of the mitochondrial cytochrome oxidase subunit I gene (COI) of *D. marginalis* from Kerala and its phylogenetic status. DNA sequence similarity searches of COI gene of *D. marginalis* (GenBankAcession: KM 230115) revealed that it is genetically identical to *Cybister ventralis* (DQ 813688) and Cybister cognatus (DQ 813672) isolated from USA. The results indicate slow evolution of the COI sequences among the morphologically distinct and geographically isolated *D. marginalis*.

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INTRODUCTION

Dytiscus marginalis, the diving beetles are insects belonging to the Order Coleoptera which means 'sheathed wings' as the wings are covered by hard wing elytra. The order is huge and includes a wide variety of groups, some of which have a close association with water, living on or in it for most of their adult life. It is frequently encountered in wet tropical and subtropical forests (Michael *et al*., 2004). Water beetles prefer shallower areas of water such as streams, ditches, river bottoms and margins, lake margins, ponds, pools, marshes and puddles. *D. marginalis* has a beautifully streamlined body shape and is dark brown to blackish in colour with yellow legs and a yellow border around both the head and the thorax. The elytra are ridged in females but smooth in males. Males can also be distinguished from females by the presence of suction pads on the front legs; two of which are very large. The border of the air supply closed in under the elytra gives the tip a silver seam. Like most water insects, the diving beetle needs to come up for a new supply of fresh air. This air is taken in by bringing the tip of the abdomen to the water surface and then lowering it. The routine identification of known species can be difficult, often requiring highly specialized knowledge and representing a limiting factor in ecological studies and biodiversity inventories.

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In response, recent proposals have called for a more prominent role of efficient DNA based methods in the delineation and identification of species (Blaxter, 2004; Floyed *et al*., 2002; Hebert *et al*., 2003). The objective of any method of species delineation, including DNA- based approaches, is to identify reproductively isolated groups of organisms that warrant classification as distinct species (Michael *et al*. 2005) Mitochondrial DNA is one of the most widely used and informative molecular markers due to their precise size and maternal inheritance (Avise, 2004). Mitochondrial genes have been used extensively in population genetic and phylogeographical analyses; in part due to a high rate of nucleotide substitution in animal mt DNA. DNA barcoding is the well adopted method for accurate taxonomic identification of animals. The main purpose attributed for DNA barcoding are the assignment of specimens to known species and discovery of new species. Identification by DNA barcoding is based on matching an unknown specimen's barcode sequence to one or more sequences from specimens that have been positively identified by other sequences. Molecular phylogeny analysis using mitochondrial COI gene sequences were extensively conducted in various insect groups ranging Odonata (Jisha *et al*., 2015), Hymenoptera (Rukhsana *et al*., 2014) Lepidoptera (Akhilesh *et al*., 2014), Heteroptera (Sreejith *et al*., 2014) and Diptera (Bindu *et al*., 2014; Priya *et al*., 2014). The present study reveals the partial DNA sequence of the mitochondrial cytochrome oxidase subunit I (COI) gene of the *D. marginalis* isolated from Kerala and its molecular phylogenetic status with related members.

MATERIALS AND METHODS

Genomic DNA from D. *marginalis was* isolated using genomic DNA extraction kit of NucleoSpin XS (Takara). About 2 ng of genomic DNA was amplified for mitochondrial COI gene using the forward primer (5'-GGTCAACAAATCATAAGATATTGG-3') and reverse primer (5'-TAAACTTCAGGGTGACCAAAAAATCA-3'). The PCR profile consisted of an initial denaturation step of 5 minutes at 95^{0}C followed by 30 cycles in 5 sec at 95^{0}C, 30 sec at 50^{0}C and 45 sec at 72^{0}C and ending with a final phase 72^{0}C for 3 minutes.

The PCR products were resolved on a 2% TAE agarose gel, stained with Ethidium bromide and photographed using a gel documentation system. After ascertaining the amplification of the corresponding COI fragment, the PCR product was column purified using Mo Bio Ultra PCR Clean-up Kit as per the manufacturer's instructions. The purified PCR product is sequenced from both ends using forward and reverse primers used for the PCR using the Sanger's sequencing method (Sanger, 1975). The forward and reverse sequences obtained were trimmed off the primer sequences and assembled by using Clustal W and was searched for its similarity using BLAST Programme of NCBI. A phylogenetic tree of *D. marginalis* was constructed using MEGA6 software (Tamura *et al*., 2013).

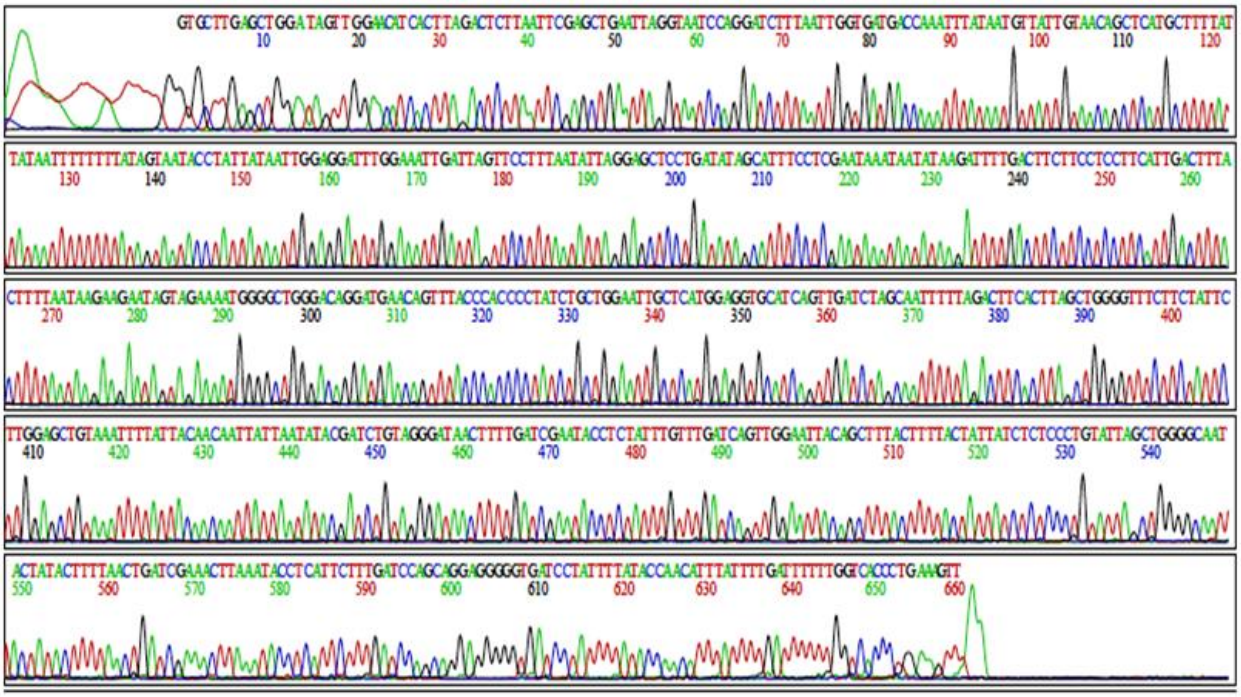

Figure 1. The chromatogram showing PCR amplified COI gene sequences of *D. marginalis* (Kerala), GenBank Accession: KM 230115

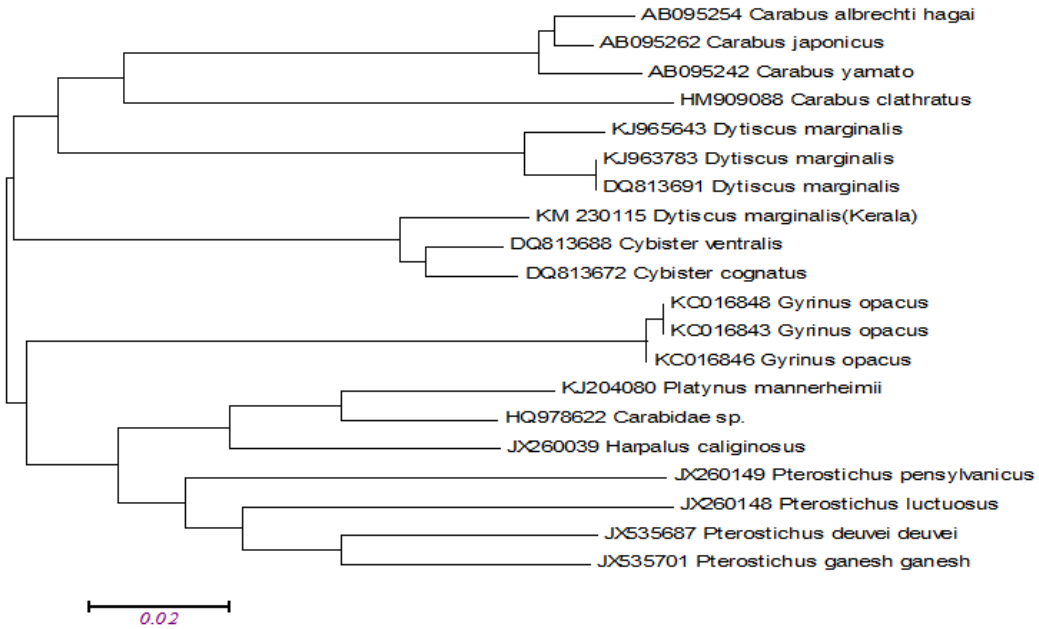

Figure 3. The phylogenetic tree plotted for *D. marginalis* was inferred using cytochrome oxidase subunit I (COI) gene partial sequence by Neighbor joining method.

RESULTS AND DISCUSSION

The PCR amplified sequences of mitochondrial cytochrome oxidase subunit I gene fragment of *D. marginalis* yielded a single product of 626 bp. The sequence has been deposited in the NCBI GenBank with Acession No. KM 230115 (Figure 1). The phylogenetic tree plotted using neighbor joining method in rectangle format presented in Figure 2.

Table 1. Percentage of evolutionary divergence between *D. marginalis* (Kerala) and other sequences from GenBank

Sl. No.	GenBank Accession No.	Species Name	% of Divergence
	KM 230115	*Dytiscus marginalis*	0%
	DQ 813688	*Cybister ventralis*	3%
	DQ 813672	*Cybister cognatus*	3%
	DQ 813691	*Dytiscus* sp.	12%
	KJ 963783	*Dytiscus* sp.	13%
	JX 535701	*Pterostichus ganesh_ganesh*	15%
	JX 260148	*Pterostichus luctuosus*	16%
	JX 260039	*Harpalus caliginosus*	16%
	AB 095262	*Carabus japonicus*	16%
	KJ 204080	*Platynus m*	16%
	HQ 978622	*Carabidae* sp.	16%
	KC 016848	*Gyrinus opacus*	17%
	KC 016846	*Gyrinus opacus*	17%
	AB 095242	*Carabus yamato*	17%
	HM 909088	*Carabus clathratus*	17%
	KC 016843	*Gyrinus opacus*	17%
	JX 260149	*Pterostichus* sp.	17%

Genetic diversity is central to the breeding success of most populations. Reduced genetic variation can greatly impair a population growth and can jeopardize the recovery of endangered species. The DNA sequences in organisms are maintained from generation to generation with very little change. Although such genetic stability is crucial for the survival of individuals, the survival of organisms may depend on genetic variation through which they can adapt to a changing environment. DNA sequence based identification technique has revealed the morphological and ecological traits of many species during larval stages (Rukhsana *et al*., 2014). Thus an important property of the DNA in cells is its ability to undergo rearrangements that can vary the particular combination of genes present in any individual genome as well as the timing and the level of expression of these genes. Cytochrome oxidase subunit 1 is the most widely used gene for molecular barcoding and phylogeny analysis of organisms especially higher eukaryotes for its high level of sequence variations compared to the other region of mitochondrial DNA. The partial DNA sequence of cytochrome oxidase subunit I gene of *D. marginalis* collected from Kerala is genetically more similar to *Cybister ventralis* (DQ 813688) and *Cybister cognatus* (DQ 813672) isolated from USA. *D. marginalis* isolated from Finland having 12% and 13% sequence divergence against *D. marginalis* isolated from Kerala. *D. marginalis* isolated from USA having 13% sequence divergence against *D. marginalis* isolated from Kerala. The present study finds clear genetic breaks and deep divergence between many con-generic species, which is likely to have resulted from taxonomic sampling across a comparatively broad range of evolutionary divergence, and geographic regions. This study represents important progress towards understanding the evolution and biodiversity of aquatic beetles and provides a foundation for similar future work. The *D. marginalis* provides an excellent study system as the order is huge and includes a wide variety of groups, some of which have a close association with aquatic habitat, living in or on for most of their lifespan.

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