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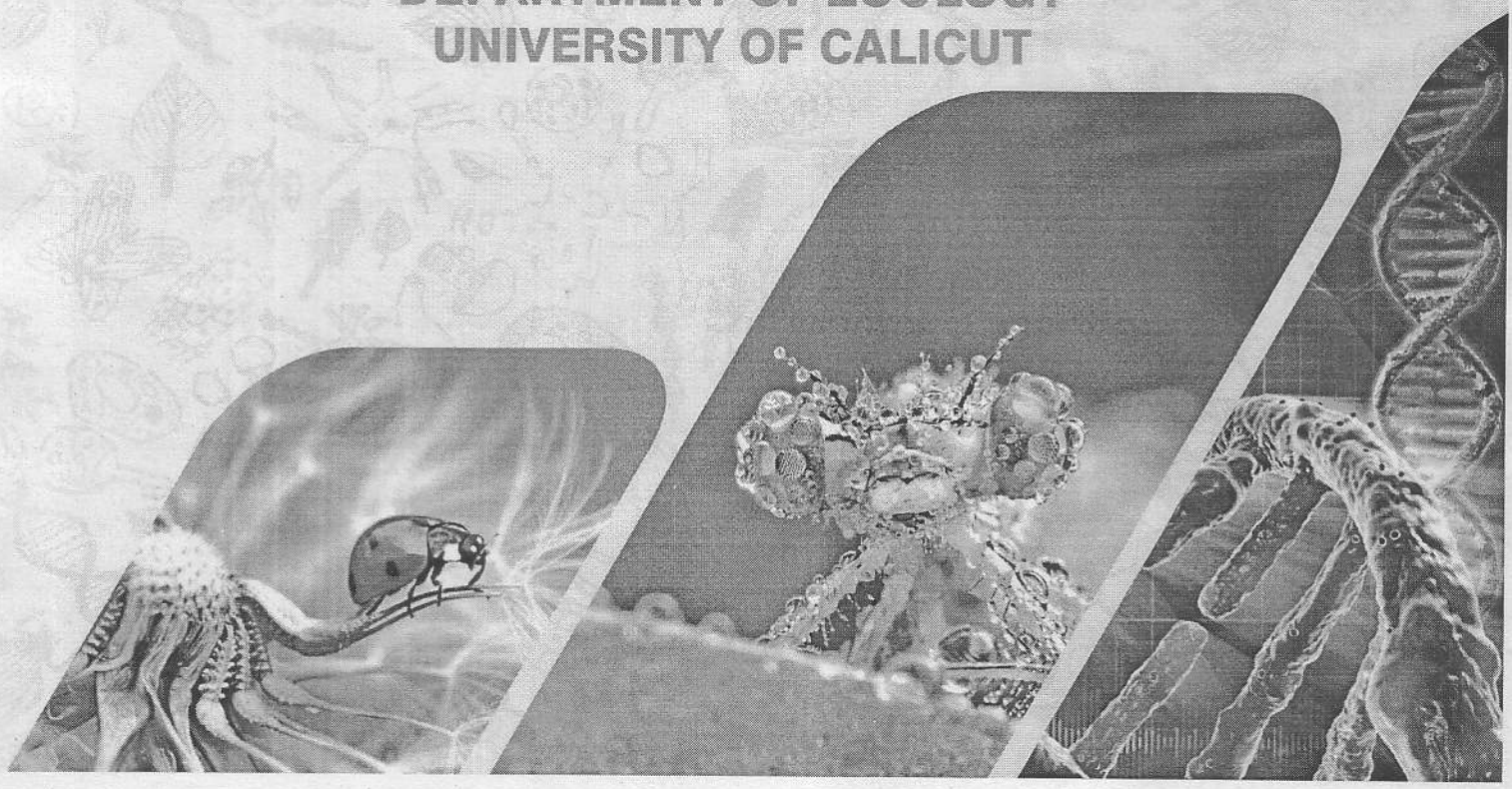

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DNA BARCODING AND PHYLOGENETIC ANALYSIS OF *VESTALIS APICALIS* (ZYGOPTERA: CALOPTERYGIDAE) USING CYTOCHROME OXIDASE I GENE

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Abstract

Vestalis apicalis is an emerald coloured damselfly species coming under the family Calopterygidae. This species is characterised by having dark brown eyes, green coloured head, thorax and abdomen, brown colored legs and amber coloured wings having black tip. They are commonly observed as groups in forest paths and are widely distributed throughout India, with their highest peak seen at August to September. Here we have PCR amplified the coding sequence of mitochondrial COI gene using suitable primer and which yielded a product having 561 bp length. The sequence was deposited in the Gen Bank having the accession number KU510326 for future references. The phylogenetic tree constructed by Neighbour joining method showed a sister clade relationship to *Vestalis grcilis* species, another Calopterygidae member. There were a total of 553 positions in the final dataset after eliminating all positions containing gaps and missing data. The close relative of this species is found to be *V. gracilis* by BLAST analysis. It also confirmed by the divergence table plotted by Maximum Likely hood method. There are a total of 14 nucleotide sequences were taken from the database in order to make the taxonomic comparison between sequences. They all provide a unique result that *V. apicalis* is more close to *V. gracilis* and then into *V. ambalis* with respective divergence of 0.21 and 0.22.All the Calopterygidae members were found to be originated from one clade showing monophyletic ancestry. We also confirmed that this species is taxonomically more close to dragonflies (*Anax speratus, Pantala flavescence* etc*)* than the Lepidopteran member (*Pappilio zalmoxis*) taken as an outgroup. Hence the present study provided a unique DNA barcode to this species and it is taxonomically more close to *Vestalis graciliis* than other Calopterygidae member.

Keywords: Zygoptera, *Vestalis apicalis*, Molecular barcoding, Cytochrome oxidase I gene

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Keywords: Zygoptera, *Vestalis apicalis*, Molecular barcoding, Cytochrome oxidase I gene

Introduction

Zygoptera represents one of the most ancient suborder of the Order Odonata commonly called as "Damselflies". They were known to be existed 250 milion years ago along with primitive proto odonates existed in the Mesozoic Era (Grimaldi and Engel, 2005). They are geographically distributed in all biological realms except Antartica and consists of 2942 extant species listed in 309 genera categorised in 28 families (Suhling, 2015).Most of the members can be easily diagonised by having widely separated eyes, fused thorax, a pair of tiny antennae, legs and long slender abdomen having 10 segments.Their wings are similar in size, coloured or uncoloured and supported by many cross veins filled with Haemolymph (Silsby, 2001).

Calopterygidae represents the 'broad winged' 1.5 to 2.5mm lengthed small damselfly family commonly observed in forest flora, streams and rivers. It is a well-distributed family both in temperate and tropical habitats and males are very active in sunny conditions. It has 16 genera with approximately 161 species globally (Cordoba, 2005).They characteristically have long antennae, long slender abdomen ,metallic blue blue coloured body with in males and brownish to green coloured wings in females. Their wings are heavenly veined consists of 18 or more antenodalveins. Both sexes can be easily diagnosed by having a white wing spot in female while it is absent in males. Most of the members can be easily recognised to all visual predators (Corbet, 1999). They generally feed upon Chironomidae and Culicidae (Higashi et al., 1976).

Vestalis apicalis is an emerald coloured Calopterygidae member generally has green colored head, thorax and abdomen, dark brown eyes, brown colored legs and amber coloured wings having black tip (Subramanian, 2009). Males have 49-55mm abdominal length,36-39mm hind wing length and females with 46-50 mm abdominal length and 38-40mm hind wing length respectively (Subramanian, 2009).They can be commonly observed in forest path in groups (Manoj, 2011). They are distributed throughout India, often seen in forest areas (Emiliyamma, 2005) with their highest peak seen at August to September (Manoj, 2011).

DNA barcoding is a novel system designed to provide rapid, accurate, and automatable species identification by combining taxonomy, genetics and computer science that automates the process of obtaining expert species

identification. Cytochrome oxidase I gene is used for bar-coding since it is the largest gene among the other 37 mitochondrial marker genes and widely used in phylogenetic studies due to it has high insertion deletion events. Hebert et al.,2003 Showed that a 608 bp region in the mt COI gene for animal bar-coding because it showed high efficiency for the identification of bird, fish, flies and other animals. The objective of the present study was to amplify the cytochrome oxidase I gene of *Vestalis apicalis* by PCR method inorder to provide a unique barcode and to interpret its phylogeny.

Materials and Methods

Sample Collection and Preservation

The Calopterygidae member *Vestalis apicalis* was collected from Palakkad district of Kerala by hand sweep netting and random field sampling method was used to cover the entire study area. Identification was done by observing wing venation, colour pattern and genitalia, described in available keys/identification guides. Additional information regarding date of collection, locality etc., about each specimen was also recorded. Each specimen was then placed in a separate collecting bottle, assigned a code number and stored in 70% ethanol until further use. One or more legs were removed for DNA isolation and kept in ethanol until further use.

DNA extraction, amplification and sequencing

DNA from selected dragonflies was extracted from leg using 'Origin DNA Extraction kit'. The obtained DNA was confirmed using 1% agarose gel. About 2ng of DNA was PCR amplified for mitochondrial cytochrome oxidase subunit I (COI) gene using forward primer (5'-GATATTGGAACCCTTTACCTG-3') and reverse primer (5' –GTTGATAAAGGATTGGCAGGGTGACC-3') in Takara PCR thermocycler. The thermocycler conditions were slightly modified as follows; 1 initial cycle of 5 minute at 95° C followed by 30 cycles of 95° C for 10 seconds and 50° C for 1 minute, 72° C for 45 seconds. This is followed by a final step of 72° C for 3 minutes. The obtained PCR product was checked using 2% agarose gel electrophoresis and were sequenced with both the forward and reverse primers using an automated sequencer ABI 3730XL by Sanger's

method. Phylogenetic analysis was done by MEGA software (Tamura et al., 2013).

Data Analysis

Mitochondrial COI sequence data for *Vestalis apicalis* was sequenced and submitted in GenBank (KM 510326). The aligned sequences were used for species identification using NCBI BLAST. The sequences from GenBank were retrieved and sequences of each species generated from this study were compared and aligned using ClustalW.

Results

This species has an emerald coloured body with green colored head, thorax and abdomen, dark brown eyes, brown coloured legs and amber coloured wings having black tip (Fig. 1).

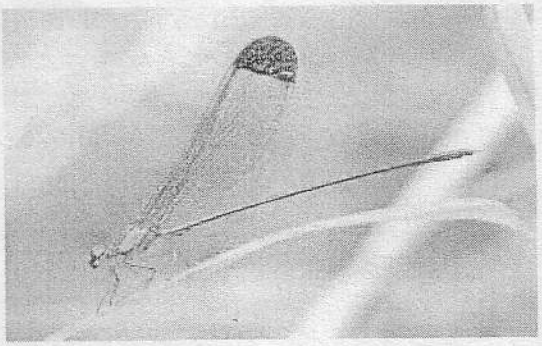

Figure 1: *Vestalis apicalis*

The PCR amplification of partial COI sequence of *Vestalis apicalis* isolated from Kerala, India (Fig. 1) yielded a product having 561bp. The molecular phylogenetic tree and evolutionary divergence table are presented in the figures respectively (Fig. 2 and Table 1).

The partial amplified product of CO I gene of *Vestalis apicalis* produced 561bp sequenced DNA and it gets deposited in to the GenBank with Accession number (KU 510326) for future reference.

The evolutionary history was inferred using Neighbour joining method. The optimal tree with the sum of branch length =0.990.The percentage of replicate trees inwhich the associated taxa clustered together in the bootstrap test are shown here. The number of base substitution per site from between sequences are shown. The analyses were made using Maximum composite Likelyhood method.

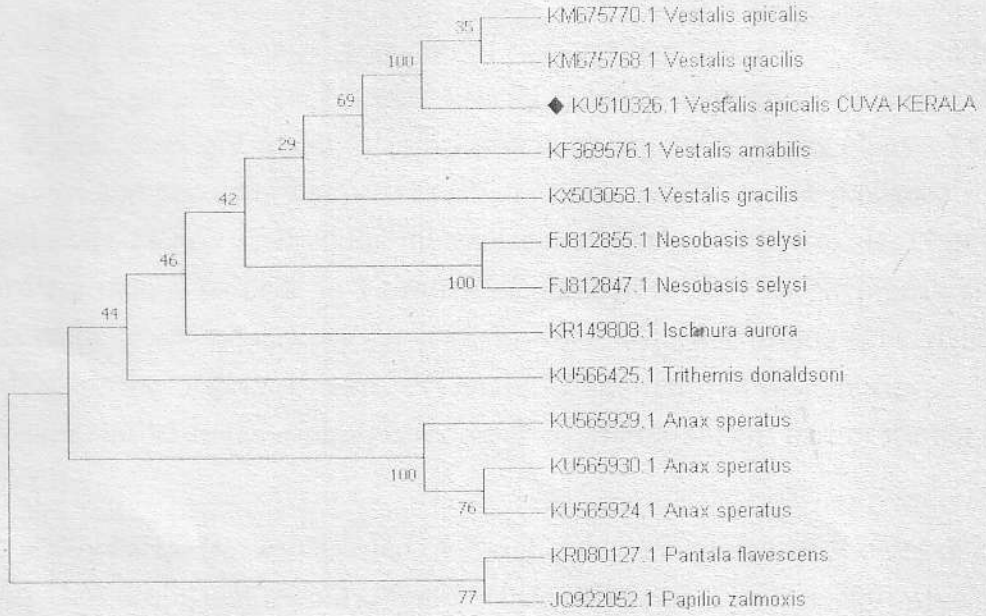

Figure 2: Phylogenetic tree of *Vestalis apicalis* inferred by Neighbour joining method

Table 1: Estimate of evolutionary divergence between sequences plotted using Maximum Likelyhood method

Species name	Percentage of divergence
KM510326 *Vestalis apicalis* (Kerala)	0
KM675770 *Vestalis apicalis*	0
KM675768 *Vestalis apicalis*	0
KX503058 *Vestalis gracilis*	0.21
KF369576 *Vestalis ambalis*	0.22
FJ812855 *Nesobasis selysi*	0.20
FJ812847 *Nesobasis selysi*	0.21
KU565929 *Anax speratus*	0.21
KU566425 *Trithemis donaldson*	0.21
KU565930 *Anax speratus*	0.21
KR149808 *Ischura aurora*	0.21
KR080127 *Pantala flavescence*	0.23
JQ922052 *Pappilio zalmoxis* (Lepidoptera)	0.4

Discussion

There have been made alot of studies to resolve the phylogeny in Odonata based on morphological features (Fraser, 1957; Trueman, 1996; Bechley, 2002). Most of the characters are mainly based upon wing venation, morphology of flight apparatus and copulatory structures (Pfau, 1991), which do not produced robust conclusions. Hence we used the molecular marker cytochrome oxidase I gene to provide a unique barcode to *Vestalis apicalis* and to infer its phylogeny on the basis of its DNA sequences. The partial coding sequence of this gene yielded a product having 561bp. The BLAST analysis showed that this species is 100% sequence similar to the same species reported from Southern part of Kerala (KM 675770).

The phylogenetic tree constructed by Neighbour joining method showed that this species has originated from a common clade (Boot strap value = 100) which gets splitted into one clade contains *Vestalis apicalis* and the other with *Vestalis gracilis* and *V. apicalis* together, indicating *V. apicalis* is taxonomically more close to *V. gracilis* (Fig. 3). The above result also confirmed by the divergence table plotted by Maximum Likely hood method (Table 1). There are a total of 14 nucleotide sequences were taken from the database inorder to make the taxonomic comparison between sequences. They all provide a unique result that *V. apicalis* is more close to *V. gracilis* and then into *V. ambalis* with respective divergence of 0.21 and 0.22. All the Calopterygidae members were found to be originated from one clade showing monophyletic ancestry. This result has supported the works of Rehn (2003) and Bybee (2008) which supported monophyletic ancestry in Calopterygidae members. We also confirmed that this species is taxonomically more close to dragonflies (*Anax speratus, Pantala flavescence* etc) than the Lepidopteran member (*Pappilio zalmoxis*) taken as an outgroup member. Thus the above result supported the view of monophyletic ancestry by all the previous taxonomic works done in the fied of Odonatology (Bechly, 2002; Rehn, 2003; Bybee, 2008)

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