



A preliminary study on Molecular phylogenetic analysis of aquatic beetles

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Abstract

The current study investigated molecular phylogenetic relationship among insects. We have collected *Sternolophus* sp. from freshwater lake. We have used specific marker COI gene as a tool for amplification. PCR amplified product was purified and sequenced. Phylogenetic tree was constructed using maximum likelihood method, tree showed that all four examples of *Sternolophus* belong to same species.

Keywords : Aquatic coleopteran, DNA extraction and phylogenetic tree

The aquatic coleopterans are one of the most diverse groups in the freshwater environment (Bouchard *et al.*, 2009). It has been classified into 13 families spreading all over the India. Hydrophilidae, Dytiscidae and Gyrinidae are the dominant families (Mukhopadhyay and Ghosh, 2003). There are 117 species belonging to family Dytiscidae, 30 species to Gyrinidae and 66 to Hydrophilidae (Vazirani, 1970, 1984; Biswas and Mukhopadhyay, 1995; Mukhopadhyaya and Ghosh, 2007; Jach and Balke, 2008). Generally, the aquatic coleopterans were identified based on their morphological variations. These variations could not be considered as genetic identity and it will not be possible to identify the phylogenetic tree. But the genotypic variations can give the interrelationship between the species and their phylogenesis. The present work on the aquatic coleopterans of India is mainly based on the taxonomy and DNA Barcoding to conduct a phylogenetic and molecular evolutionary analysis.

Interestingly, we have collected and identified *Sternolophus* species of aquatic coleopterans from Laxmipur Cheruvu (17.03'N

and 77.43'E), Shameerpet lake (17.60'N and 78.56'E), localities belonging to Hydrophilidae family. The genomic DNA was isolated and purified by using a Nucleospin Tissues kit according to the manufacturer protocol. The quality of the genomic DNA was checked using 0.8% Agarose. Polymerase chain reaction (PCR) amplifications were undertaken using the primers LCO1490 and HCO2198. The amplified DNA was purified and sequenced by Chromous biotech using the same set of primers as used for amplification. The forward and reverse DNA sequences were aligned and edited manually in sequence analysis software. A Phylogenetic tree analysis was made through MEGA 6 software.

The sequences of *Sternolophus* sp. (Fig- 1) of aquatic coleopteran insects are shown in (Table - 1). The maximum likelihood phylogeny tree of *Sternolophus* sp. KJ848329, KP406967, KM262661 and KP406968 are being identical with one another (Fig-2). The blast results gave *Sternolophus* sp. KJ848329, KP406967, KM262661 are 100% identical and KP406968 is 99% identical with all the above three species.



Fig.-1. *Sternolophus* sp. insect images ventral and dorsal view.

Table - 1. Nucleotide sequences of aquatic coleopteran and their Gen Bank accession number

S.No	Species, Gen Bank Accession No. & Sequence
1.	<p><i>Sternolophus</i> sp.(Fabricius, 1781) KJ848329</p> <p>aaatcataaagatattggtacattatattttatTTTTGGTGCTGAGCGGGAATAGTAGGTACATCTCTAAGAATTTAATTCGAGCTGAGTTA ggaaaccaggtacattaattggagatgaccaaattataatgttatcgtaacagccatgcattattataatTTTCTCATAGTAATACCA attataattggtgggTTGGTAATTGATTAGTACCCTGATGTTAGGTGCACCTGATATAGCATTCCCTCGAATGAATAATAAGATTTGAT tattacccttcattaactctactattaataagaagaatagtagaaagaggtgcaggtacaggtgaacagttaccctcattatcgca aatattgctcatgggggagcatctgttgatttagctattttcagattacacctagcaggtatcttcaatTTTAGGAGCTGTAATTTATTAC tacagttattaataacgatcaactaacttaacttacgaccgaatacctttatTTGTTGATCAGTAGCTATTACGACTTCTACTCTTATT ctctacgttagtagcaggggcaattactatactTTTAACAGATCGAAATCTTAATACTTCATTCTTGACCCTGCCGGTGGGGGTGATCCTAT tctttaccaacacctattctgattTTTTGGTCAACCCTGG</p>
2.	<p><i>Sternolophus</i> sp. (Solier,1834) KP406967</p> <p>cataaagatattggtacattatattttatTTTTGGTGCTGAGCGGGAATAGTAGGTACATCTCTAAGAATTTAATTCGAGCTGAGTTAGGAA acccaggtacattaattggagatgaccaaattataatgttatcgtaacagccatgcattattataatTTTCTCATAGTAATACCAATTAT aattggtgggTTGGTAATTGATTAGTACCCTGATGTTAGGTGCACCTGATATAGCATTCCCTCGAATGAATAATAAGATTTGATTATA ccaccttcattaactctactattaataagaagaatagtagaaagaggtgcaggtacaggtgaacagttaccctcattatcgcaaat tgctcatgggggagcatctgttgatttagctattttcagattacacctagcaggtatcttcaatTTTAGGAGCTGTAATTTATTACTACG ttattaataacgatcaactaacttaacttacgaccgaatacctttatTTGTTGATCAGTAGCTATTACGACTTCTACTCTTATTACTCTAC ctgtatttagcaggggcaattactatactTTTAACAGATCGAAATCTTAATACTTCATTCTTGACCCTGCCGGTGGGGGTGATCCTATTCTTA ccaacacctat tctgattTTTTGGT</p>
3.	<p><i>Sternolophus</i> sp. (Solier ,1834) KP406968</p> <p>ataaagatattggtacattatattttatTTTTGGTGCTGAGCGGGAATAGTAGGTACATCTCTAAGAATTTAATTCGAGCTGAGTTAGGAA acccaggtacattaattggagatgaccaaattataatgttatcgtaacagccatgcattattataatTTTCTCATAGTAATACCAATTATA attggtgggTTGGTAATTGATTAGTACCCTGATGTTAGGTGCACCTGATATAGCATTCCCTCGAATGAATAATAAGATTTGATTATTAC caccttcattaactctactattaataagaagaatagtagaaagaggtgcaggtacaggtgaacagttaccctcattatcatcaaatatt gctcatgggggagcatctgttgatttagctattttcagattacacctagcaggtatcttcaatTTTAGGAGCTTAAATTTATTACTACGTT attaataacgatcaactaacttaacttacgaccgaatacctttatTTGTTGATCAGTAGCTATTACGACTTCTACTCTTATTACTCTAC gtatttagcaggggcaattactatactTTTAACAGATCGAAATCTTAATACTTCATTCTTGACCCTGCCGGTGGGGGTGATCCTATTCTTACC aacacctat</p>

4.	<p><i>Sternolophu sp.</i> (Fabricius, 1781) KM262661</p> <p>Gttttggtgcttgagcgggaatagtaggtacatctctaagaattttaattcgagctgagttaggaaaccaggtacattaattggagatgacaaatttataatggtatcgtaacagcccatgcattattataattttctcatagtaataccaattataattgggtgggttgtaattgattagtacccttgatgtaggtgcacctgatatagcattccctcgaatgaataatataagattttgattattaccaccttcattaactctactattaataagaagaatagtagaaagaggtgcaggtacaggtgaacagttaccctccattatcgtaaatattgctcatgggggagcatctgttgattagctattttcagattacacctagcaggtatcttcaatttttaggagctgtaattttattactacagttattaatatacgatcaactaacttaacttacgaccgaatacctttattgtttgatcagtagctattacagcacttctactctattatctctacctgtattagcaggggcaattactat acttttaacagatcgaaatctaataacttcattctttgaccctgccggtgggggtgatccttat</p>
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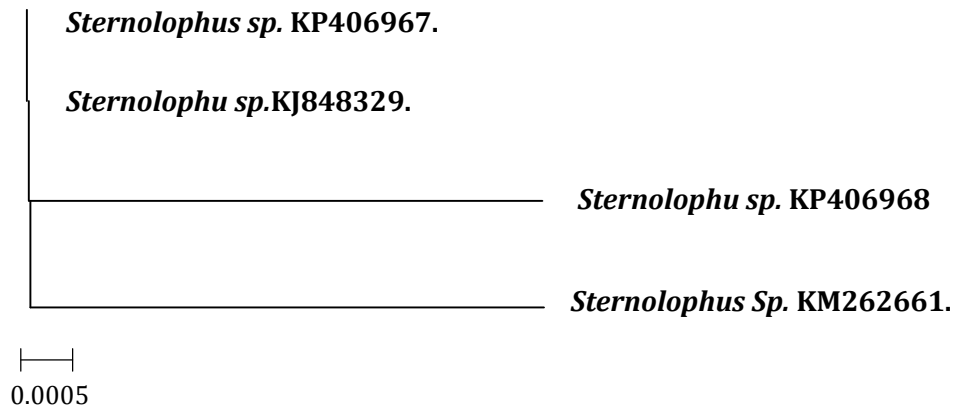


Fig. -2. Maximum likelyhood phylogenetic tree of *Sternolophus* aquatic coleopterans

According to Miller *et al.* (2005) DNA Barcoding and phylogenetic analysis would help to identify the unknown species and even the larval stage of the species. According to Baselga *et al.* (2012) Barcoding helps in identifying the community similarity at all levels. Further molecular taxonomy and phylogeny studies on the Coleopteran insects will be rewarding to assess the evolutionary interrelationship. It would also help in finding the close relationship of insects and geographic distance.

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