

EXECUTIVE SUMMARY

of

**UGC Major Research Project
entitled**

**Molecular Taxonomy of Aquatic Insects from various
Water bodies of Maharashtra (India) by Employing DNA
Barcoding**

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Executive Summary of the Research Project

Taxonomic study of aquatic insects is a challenging task due to known reasons. Very little attention is given to this group of organisms in the Indian subcontinent, therefore necessitating a reliable, universal and effective method for identifying different life stages. 682 aquatic insect specimens were collected from various water bodies across Maharashtra State (India) between July 2012 and June 2015. DNA barcode based molecular method was applied for taxonomic assignment of the aquatic insects.

Collected insects were digitally photographed and efforts were made for primary taxonomic assignment of the specimen using different taxonomic keys. Little tissue in the form of single leg, leg clip or small piece of muscle was used for DNA isolation using standard protocol. Isolated DNA were quantified, quality checked and used for further downstream applications. Amplification of cytochrome oxidase-I gene was done using universal primer pair (LepFol - F & R) and PCR amplicons were bidirectionally sequenced in each case. High quality COI sequences (658 bp) obtained were subjected to further analysis. Overall nucleotide composition of all aquatic insects have been computed and the average composition observed was A, 31.2%; T, 34.4%; G, 16.8% and C, 17.6%.

In almost all cases, the insect samples were taxonomically differentiated. Few cases where some ambiguity existed in the morphological and DNA barcode based identification of specimen, the same were assigned to lower taxonomic rank. Based on the degree of genetic differentiation within and between clusters, barcode sequences were grouped into operational taxonomic units (OTU) on the basis of ABGD tool (>3%). Further, in the entire library of 120 specimens, there were 30 singletons and the highest number of individuals in an OTU was 8. The data retrieved from Barcode of Life Data Systems search engine, consistently fit into the cluster groupings delimited by ABGD and NJ clustering. Within-group K2P genetic distance was in the range of 0.00% to 2.77% (<3) and among-group genetic distance was in the range of 5.54% to 36.75% (>3), where "group" is a clade or unresolved taxa of ABGD and NJ tree. No overlap was observed between successive taxonomic ranks.

Thus, the exploration under the present study confirmed the identification of aquatic insects at molecular level, whereby they were categorised into fifty six separate groups showing large COI divergence (13.85%), which is a clear indication of no conflict existing in the selected approach. However, in the absence of data on specimens in triplicate, the interspecific COI divergences could not be examined. The outcome of the data analysis promotes the application of DNA barcoding as an efficient means for biodiversity assessment, specifically of demanding group like aquatic insects.

Key words: aquatic insects, DNA barcoding, taxonomy, genetic diversity, M.S. India