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Molecular phylogenetic relationship of Thiaridean genus *Tarebia lineate* (Gastropoda: Cerithioidea) as determined by partial *COI* sequences

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Abstract
An attempt was made to investigate phylogenetic affinities of the genus *Tarebia lineata* sampled from the Indian subcontinent using partial mitochondrial COI gene sequence. The amplified partial mt-COI gene sequence using universal primers, LCO1490 and HCO2198 resulted into ~700 base pair DNA fragment. The obtained nucleotide sequence of partial COI gene of *T. lineata* was submitted to BLAST analysis and 36 close relative sequences of the chosen genera, Cerithioidea were derived. Maximum likelihood (ML) algorithm in-biuilt in RAxML software tool was used to estimate phylogenetic their affinities. The present analysis revealed that a single assemblage of the family Thiaridae supported by a bootstrap value of 96% is earmarked at the base of the derived cladogram as a cluster and emerged as a sister group with another four Cerithioideans. Our dataset brought add-on value to the current taxonomy of Thiaridae of the clade Sorbeconcha by clustering them as sister and non-sister groups indicating the virtual relations. Out of seven genera, *Tarebia* and *Melanoides* formed as primary and secondary clusters within the Thiaridae. The monophyly of Thiaridae and its conspecifics were depicted in the cladogram.

Keywords: Gastropoda, COI, Monophyly, Thiaridae, *Tarebia lineata*, RAxML

1. Introduction
Thiaridaens are freshwater gastropods, have a place within the super family Cerithioidea [1]. Thiaridae [2] is a group basically circum-tropical among various incredibly expansive species inferable from their "tramp" abilities [3]. They are wealthiest gastropod segments of freshwater families and taxonomic position stays unverifiable [4]. As early as 1898, Moore elevated the thought regarding the polyphyly of Thiaridae considering morphological features. Furthermore, it is shown that the family Thiaridae is formerly included under Pachychilidae [5] and found to be polyphyletic reported to consist of a minimum three independent evolutionary lineages. The species grouped under Thiaridae are characterized with a regular brood pouch in the neckline vicinity, a profound twisting caecum and an immense accessory pad [6, 7, 4]. The pattern of a "Gondwanian group" in case of Thiaridae is bolstered by cutting edge molecular studies that shed light on its phylogenetic relationships within the super family Cerithioidea [8]. Thiaridae are monophyletic in almost all analyses reported by Strong [9]. Therefore, in the present article it is aimed at to analyze the phylogenetic affinities of the genus *Tarebia lineata* using partial sequence of COI gene in order to improve our understanding of kinship among Asian freshwater snails.

2. Materials and methods
2.1 Sample collection and species identification
In particular, *Tarebia lineata* is a dominant freshwater snail widespread across India [10]. In the present examination, these freshwater snails were sampled in their respective habitats throughout a monsoon rainy season (June to September, 2016) and a post-monsoon period (October to November, 2016) from suburbs of Bhubaneswar, Odisha, India (Fig. 1), situated at Lat.20 85 'N and Long.86 33 'E. They were kept in plastic trough having 20 L freshwater with an aerator and fed *ad libitum* with *Hydrilla*, *Pistia*, and so forth, acclimatized for a week before utilized for experimental studies. *Tarebia lineata* was identified by Zoological Survey of India, Kolkata, India.
The diagnostic characteristics and morphological markers to identify *T.lineata* inhabiting at Bhubaneswar, India are as follows: It has a dextrally coiled, extended cone shaped shell, with 8-12 whorls The apex of the spire is typically dissolved and the sides are curved in layout.

The shell is sculptured with prominent nodes overlapping the suture between whorls and forming crenulations, the base of which is marked with prominent spiral ridges. Adult shell height ranges from 6 to 40 mm and normally reach 20-35 mm.

Fig 1: Conical dextral shells of *Tarebia lineate* showing three sutures with a prominent body whorl with a wide aperture and a tapering terminal whorl with an apex. The shell is laden with a series of knob-like projections called tubercles.

2.2 Tissue isolation and genomic DNA extraction

Isolated live foot muscle tissues from the snail were kept in the petridish separately and washed them in distilled water. 0.5 g muscle tissue was weighed for DNA extraction. Total genomic DNA was extracted employing the phenol-chloroform method described by Sokolov [11]. The yield of genomic DNA was found to be 2835 ng/µl and A260/A280 ratio was 1.76 [12].

2.3 PCR Amplification

Primers for the COI (Cytochrome oxidase subunit 1) gene specified by Folmer [13] were adopted in this study. PCR reaction was performed in a Thermal Cycler (Agilent). The components of PCR mixture contain template genomic DNA, gene specific primers, dNTPs, *Taq* buffer, MgCl2 buffer and *Taq* DNA polymerase. The reaction volume for all PCR reactions was set to 25µl. All reagents required for PCR were purchased from HiMedia, Mumbai, India. The PCR cycles were repeated for 35 times, subsequently a final extension for 10 min at 72 ºC. The reaction mixture devoid of template was run as a negative control. Amplified DNA fragment was separated by gel electrophoresis in 1.2 % agarose gel along with 2-log DNA ladder as marker and images were taken in Gel Doc system (Mediccare™, Chennai, India) (Fig. 2).

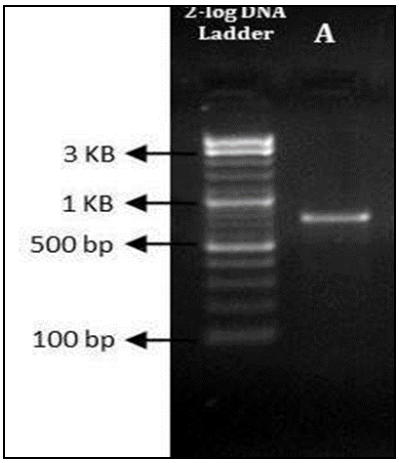

Fig 2: Amplified partial *COI* gene of *Tarebia lineate* (Lane A) shown along with DNA ladder run through 1.2% agarose gel.

2.4 Phylogenetic analyses

The DNA sequence alignment of Cerithiodiean species was performed with default parameters in MUSCLE [14] using the program MEGA v5.2 [15]. The resulted aligned sequences in FASTA format were converted to PHYLIP format. Maximum likelihood (ML) algorithm was used to estimate phylogenies adopting RAxML software tool [16]. For executing ML analysis, we selected 37 taxa along with two outgroups representing from Ampullaridae to build the phylogenetic affinities. RAxML increased the accuracy and ability to account for phylogenetic inferences. We used the GTR+ Γ DNA substitution model and analyzed independently in RAxML. The unpartitioned nucleotide sequences were subjected to ML phylogenetic analysis through raxmlIGUI v1.3 software package. The constructed phylogenetic tree nodal support values (Fig. 3) were obtained by performing 1000 bootstrap replications [17].

3. Results and Discussion

The eluted PCR products were sequenced bidirectionally with the same primers used for PCR amplification at Rajiv Gandhi Centre for Biotechnology, Kerala (India).The sequencing was performed by Big Dye Terminator 3.1 v sequencing kit. The annotated DNA sequences were checked further by ORF finder. The sequences obtained were submitted to NCBI and the given accession number was KY511306. In the present study, the taxonomic sampling was based on the closest sequences of species obtained in Basic Local Alignment Search Tool (BLAST) analysis in GenBank and the representative species of the families namely Cerithiidae, Melanopsidae, Turritellidae, Pachychilidae, Planaxidae, Potamididae, Pleuroceridae, Batillariidae, and Thiaridae were selected for phylogenetic analysis.

The resolution shown by our experimental results and *in silico* analyses was to clarify extent of phylogenetic affinities of the genus *T.lineata* (Thairidae) with related taxa. The maximum likelihood (ML) phylogenetic analysis included 37 species within the super family Cerithioidea (Fig. 3) and resulted cladogram was rooted on the taxa of outgroup namely *Pomacea maculate* and *Pomacea paludosa* [18]. In the present analysis, it was shown that single assemblage of the family Thiaridae group lied close to the base of the dendrogram in one cluster as a sister group of four Cerithioideans families namely Planaxidae, Potamididae, Pleuroceridae and Batillariidae. Further, this single cluster comprising of five families including Thiaridae must have shared their affinities with the three remaining non-sister Cerithioideans families namely Melanopsidae, Turritellidae and Pachychilidae. It is shown that formerly a few species of Thiaridae are included under Pachychilidae [5] and the present analysis revealed that Pachychilidae being non-sister taxa of Thiaridae (Fig. 3), the rearrangement shown by Guideo and Sheila [5] is found justifiable. This molecular phylogenetic analysis advocates that these types of phylogenetic studies are more significant aspects in enriching the taxonomy with phylogenetic relations within the Super family Cerithiodea and its phylogeographical distribution for the comprehension of the monophyletic nature of its families. The partial nucleotide sequence of COI gene of *T. lineata* Odisha complied its phylogenetic affinities with the taxa of cerithoidean families as found evaluated with all the chosen taxa (Fig. 3) and further as depicted in the gastropodan classification [1,5].

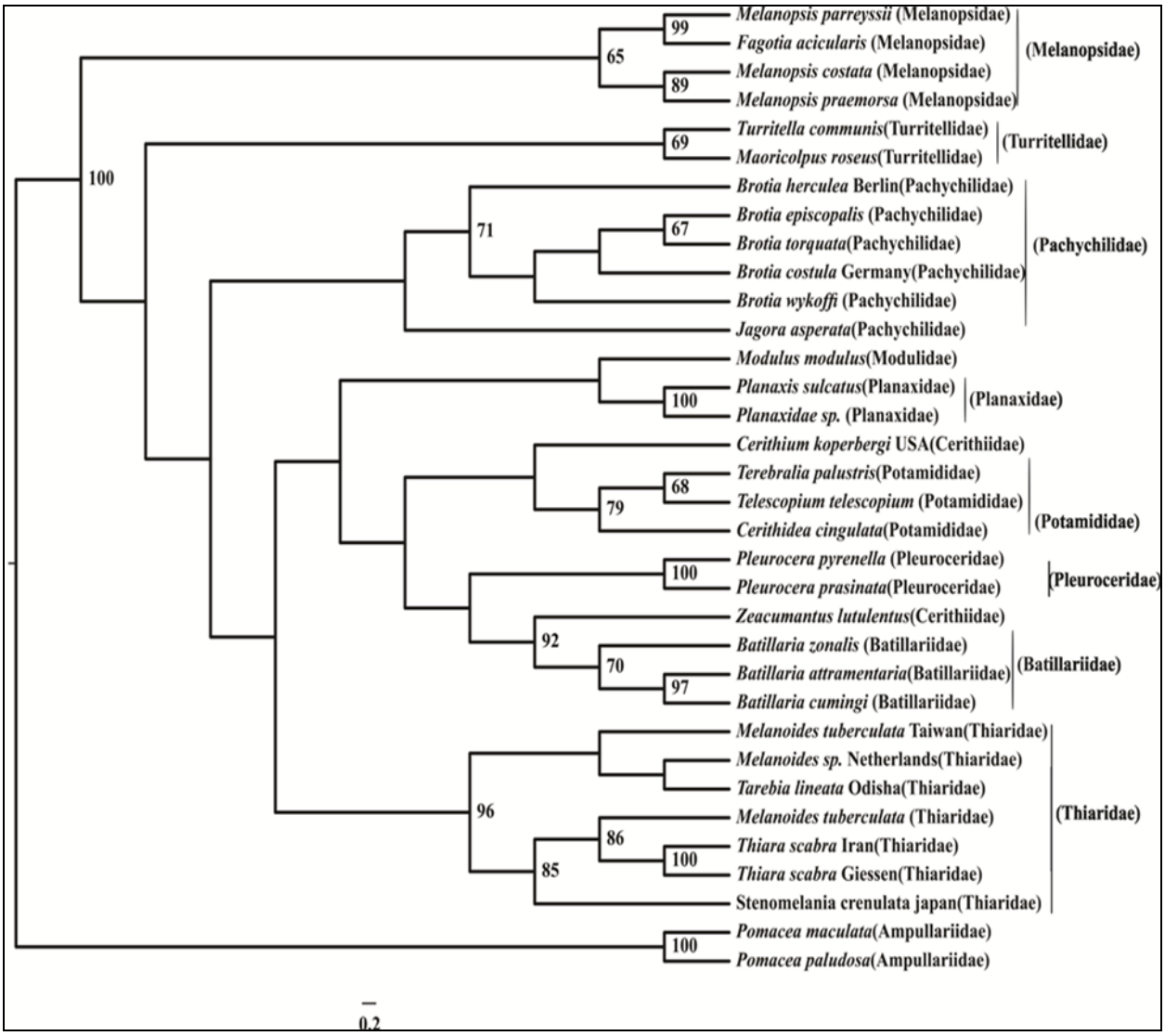

Fig 3: Maximum-likelihood cladogram based on RAxML analysis of the full concatenated data set of partial gene *COI* of the chosen genera of Cerithioidea belong to the class Gastropoda. The bootstrap values above 50% are shown.

4. Conclusion

The present analysis concluded that *T. lineata* Odisha is more closely related to its conspecifics across continents and our results demonstrated a strong support to the monophyly of Thiaridae taxa amongst its sister taxa with our focal sample nested.

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