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MOLECULAR CHARACTERIZATION OF *DACTYLOGYROIDES TRIPATHII* (MONOGENEA, DACTYLOGYRIDAE) USING LONG SUBUNIT rDNA FROM NORTH EAST REGION OF INDIA

H. R. Chiary, A. Chaudhary, H. S. Singh

Molecular Taxonomy Laboratory, Department Of Zoology,
Chaudhary Charan Singh University, Meerut (*U. P.*), *250004 India*
E-mail: anshu8282@rediffmail.com

Molecular Characterization of *Dactylogyroides tripathii* (Monogenea, Dactylogyridae) Using Long Subunit rDNA from North East Region of India. Chiary H. R., Chaudhary A., Singh H. S. — The present study describes the molecular characterization of the monogenea *Dactylogyroides tripathii* (Tripathi, 1959) Gussev, 1973 infecting the gill filaments of fish, *Puntius ticto* from River Brahmaputra, Guwahati, Assam, India. This study shows the *D. tripathii* species identification resulted from the use of molecular data, particularly the 28S rDNA gene. We compared the 28S partial rDNA sequence of *D. tripathii* with same gene region of the other species of monogeneans available in GenBank. With this comparison, we determined that the sequence had a similarity with one available species of the genus *Dactylogyroides* Gussev, 1963 i. e., *D. longicirrus* and also with the species of *Dactylogyrus* from which this genus was distinguished.

Key words: *Dactylogyroides tripathii,* 28S rDNA, Assam, India.

Молекулярная характеристика *Dactylogyroides tripathii* (Monogenea, Dactylogyridae) с использованием длинных субъединиц рДНК из Северо-восточного региона Индии. Чиари Х. Р., Чаудари А., Сингх Х. С. — Представлена молекулярная характеристика моногенеи *Dactylogyroides tripathii* (Tripathi, 1959) Gussev, 1973 инфицирующую жаберные волоски рыбы *Puntius ticto* из реки Брахмапутра, Гувахати, Ассам, Индия. Это исследование показывает идентификацию вида *D. tripathii* с помощью использования молекулярных данных, в частности гена 28S рДНК. Мы сравнили 28S частичной рДНК последовательности вида *D. tripathii* с той же областью гена другого вида моногеней, доступного в GenBank. С помощью этого сравнения мы определили, что последовательность имела сходство с одним из доступных видов рода *Dactylogyroides* Gussev, 1963, то есть с видом *D. Longicirrus*, а также с видами *Dactylogyrus*, от которых отличается этот род.

Ключевые слова: *Dactylogyroides tripathii*, 28S рДНК, Ассам, Индия.

Introduction

Dactylogyroides was proposed by Gussev, 1963 for the worms previously described under the genus *Dactylogyrus* Diesing, 1850 viz., *Dactylogyrus tripathii* (Tripathi, 1959) Gussev, 1973 from *Puntius ticto* and *P. stigma* at Lucknow, India. These are the parasites of freshwater cyprinids. Gussev (1963) differentiated the genus from *Dactylogyrus* in having anchors with their points directed towards each other, the dorsal bar usually double or single with a different degree of separation into two parts. On this basis *Dactylogyrus tripathii* Tripathi, 1959 was transferred to the new genus *Dactylogyroides* Gussev, 1963.

The use of molecular tools for the identification of parasites has become commonplace. The nuclear rDNA gene regions have also been used extensively in the study of phylogeny at several different taxonomic levels. So far, in platyhelminth systematics, rDNA genes, have been used successfully (Šimková et al., 2006; Lee et al., 2007; Chiary et al., 2013) with 28S rDNA, in particular, to estimate the relationships existing among the Platyhelminthes (Olson et al., 2003). Monogenean sequences of partial 28S rDNA have been used successfully to study phylogenetic relationships (Mollaret et al., 2000 a; 2000 b; Justine et al., 2002; Olson, Littlewood, 2002; Whittington et al., 2004; Wu et al., 2008; Šimková et al., 2006; Lee et al., 2007; Chaudhary, Singh, 2012; Verma et al., 2012).

The aim of the present study is to characterized *Dactylogyroides tripathii* found infecting the fish *P. ticto* from River Brahmaputra, Guwahati, Assam, India using partial sequence of the 28S rDNA.

Material and methods

Monogeneans were collected from the gills of *Puntius ticto* Hamilton, from River Brahmaputra at the site Guwahati (26°11′ N and 91°44′ E). After the fish identification, they were killed by a sharp blow on the top of the head and dissected. Methods of collection, extraction, amplification and sequencing of monogeneans were followed from Singh and Chaudhary (2010) using specifically designed primer (forward, 5′−TCTAGTAACGGCGAGTGAACG−3′) and (reverse, 5′−GGTGGAAGGTCTACCTCAGC−3′). The specimens of *D. tripathii* have been deposited in the Museum, Department of Zoology (voucher number HS/monogenea/2012/11), Chaudhary Charan Singh University, Meerut (U. P.), India. The obtained sequence that included the partial 28S sequence was submitted to GenBank under accession number JX993982.

For phylogenetic analysis, GenBank was first queried to retrieve 28S sequences from monogeneans and then aligned using ClustalW implemented in MEGA 5.05 (Tamura et al., 2011). Phylogenetic trees were reconstructed using Neighbour Joining and Minimum Evolution methods by MEGA 5.05. In reconstructing the NJ tree, the Kimura two-parameter model was used to estimate the distances. Kimura's two-parameter model (1980) corrects for multiple hits, taking into account transitional and transversional substitution rates, while assuming that the four nucleotide frequencies are the same and that rates of substitution do not vary among sites. Robustness of the inferred phylogeny was assessed using a bootstrap procedure with 1,000 replications.

Results

A 733 bp fragment of the 28S rDNA sequence was amplified from the specimens of *D. tripathii*. Nucleotide frequencies (percent) were T = 215, C = 144, A = 167, and G = 207. Phylogenetic tree of long subunit sequences showed similar grouping using the different methods employed (fig. 1, 2). The phylogenetic reconstructions inferred from analyses of the 28S rDNA sequences showed great resolution for the species of the monogeneans. This species shows nucleotide identity of 90 % with the different species of genus *Dactylogyrus* from which it was originally differentiated by Gussev (1963) including one species of same genus viz., *Dactylogyroides longicirrus* (91 %).

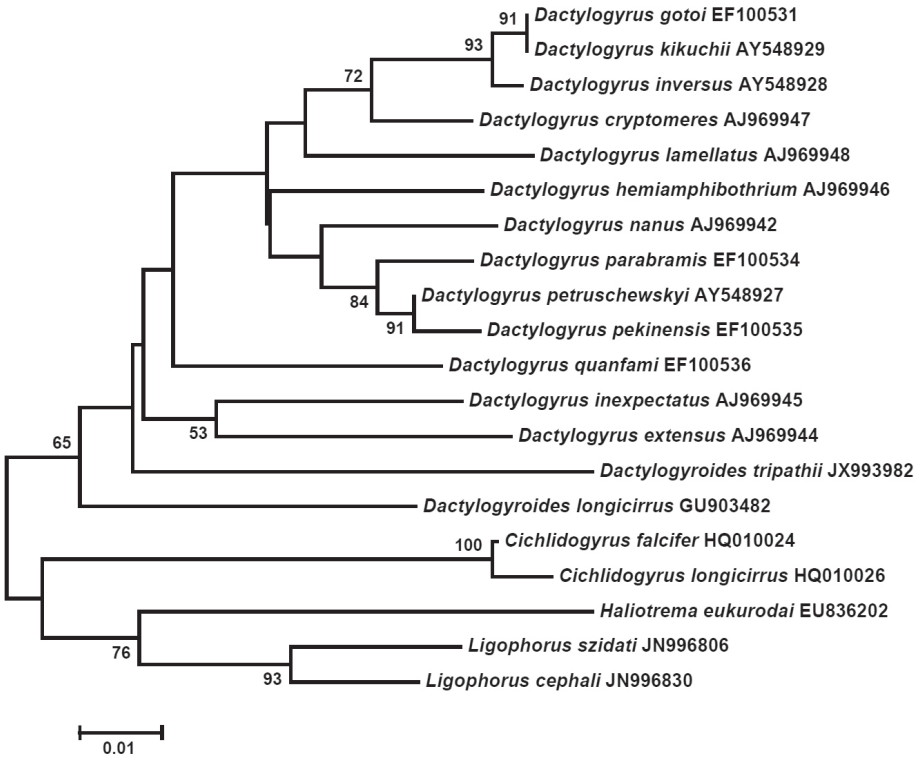

Fig. 1. Neighbor joining (NJ) tree of *Dactylogyroides tripathii* showed its phylogenetic relationship with other species of monogeneans; bootstrap values are indicated in the nodes.

Рис. 1. Дендрограмма по методу связывания ближайших соседей (NJ — метод) *Dactylogyroides tripathi*, отображающая отношения с другими видами моногеней; бутстрепные значения указаны в узлах.

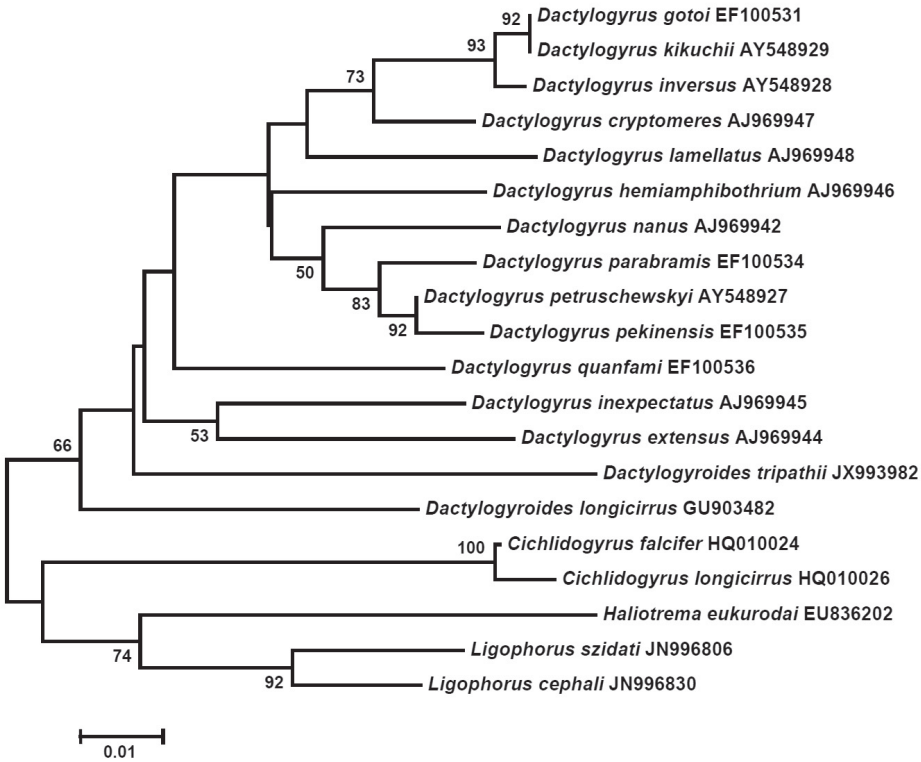

Fig. 2. A phylogenetic tree constructed for *Dactylogyroides tripathii* by minimum evolution (ME) method for 28S region with different species showed similar topology as Neighbor joining tree.

Рис. 2. Филогенетическое древо, построенное для *Dactylogyroides tripathii* по методу минимальной эволюции (МЭ) для области 28S с различными видами, показавшее сходную топологию, как и NJ-дендрограмма.

Discussion

Delimiting species of *Dactylogyroides* monogeneans is often difficult, owing to their limited morphological characters, and it may have resulted in a gross estimation of the true number of species. About fourteen species of this genus have been described from India on the basis of morphological analysis and from these till now only one species viz., *D. longicirrus* (Tripathi, 1959) Gussev, 1973 has been characterized at molecular level (Singh, Chaudhary, 2010).

Gussev (1973) recorded four related monogeneans of genus *Dactylogyroides* from the different piscine hosts *Puntius stigma*, *Barbus mahecola* and *B. dorsalis*. But the original description of these specimens was not completed and the author (Gussev, 1973) was not sure about the taxonomic status of these worms. So, the taxonomic status of these species viz., *Dactylogyroides tripathii f. dorsalis*, *D. tripathii f. filamentosi*, *D. tripathii f. mahecoli* and *D. tripathii f. stigma* suffers from serious lapses.

Moreover, Dubey et al. (1997) redescribed *Dactylogyroides tripathii* (Tripathi, 1959) Gussev, 1973 from *Puntius sophore* at Raipur. Agrawal et al., (2002) made a comprehensive review of Indian species of *Dactylogyroides* Gussev, 1963. The evaluation of morphological criteria for phylogenetic and taxonomic studies of the monogeneans seems to be the most controversial area (Desdevises, 2001; Wu et al., 2006, 2007; Chaudhary, Singh, 2012). 28S region analyses in this study revealed that this gene is a good phylogenetic marker for inferring relationship between closely related species. DNA based identification used during this study has enabled the molecular characterization of *D. tripathii*.

In the present study, *D. tripathii* shows the close similarity with another species of same genus viz., *D. longicirrus* (Tripathi, 1959) Gussev, 1973 and after that with various species of genus *Dactylogyrus* from which it was differentiated. The tree topologies derived from the phylogenetic analysis inferred from 28S rDNA data depicted that both the genus *Dactylogyroides* and *Dactylogyrus*, as genetically, closely related sister taxa as they formed closely related clade (fig. 1, 2). Therefore, based on our molecular analysis results by different methods, we propose that the species *D. tripathii* was correctly accommodated in the genus *Dactylogyroides* by Gussev, 1973. This study further confirmed that 28S rDNA is useful marker for distinguishing sister genera or species and helpful in discriminating species especially when morphological differences are often difficult to determine.

In conclusion, the present identification of the *D. tripathii* species with 28S sequence analysis is consistent with investigations made using only traditional approaches, i. e., by morphology. The molecular study with 28S sequence is a promising tool for monogenean identification at species level. We believe that such taxonomic revisions based on molecular biology will continue with the increasing number of *Dactylogyroides* species for comparison and being used for molecular phylogenetic investigations in the future.

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