*Vestnik zoologii*, **48**(5): 451–456, 2014
DOI 10.2478/vzoo-2014-0053



DE GRUYTER OPEN

UDC 595.122:597.55(540)

# MORPHOLOGICAL REDESCRIPTION AND MOLECULAR CHARACTERIZATION OF *DACTYLOGYRUS LABEI* (MONOGENEA, DACTYLOGYRIDAE) FROM *CATLA CATLA*: A NEW HOST RECORD IN INDIA

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**Morphological Redescription and Molecular Characterization of *Dactylogyrus labei* (Monogenea, Dactylogyridae) from *Catla catla*: a New Host Record in India. Chiary, H. R., Chaudhary, A., Singh, H. S.** — *Dactylogyrus labei* Musselius et Gusev, 1976 (atypical form) specimens were collected from host *Catla catla* from the river Brahmaputra, Guwahati, Assam, India. The *Dactylogyrus* samples were examined by morphological and molecular methods. This parasite was originally described from *Labeo rohita* from fish farm Kalyani, West Bengal, India, but the morphological study of sclerotized parts showed the existence of *D. labei* on *Catla catla*. This is the first record of *D. labei* from *Catla catla* in India.

Key words: Monogenea, Phylogeny, 28S, ribosomal DNA, Assam, India.

**Морфологическое переописание и молекулярные параметры *Dactylogyrus labei* (Monogenea, Dactylogyridae) от *Catla catla*: находка нового хозяина в Индии. Чиари Х. З., Чаудхари А., Сингх Х. С.** — Экземпляры атипичной формы *Dactylogyrus labei* Musselius et Gusev, 1976 были собраны с индийского карпа *Catla catla* из реки Брахмапутра (Гувахати, Ассам, Индия) и исследованы с помощью морфологических и молекулярных методов. Этот паразит был впервые описан на *Labeo rohita* с рыбной фермы Кальяни, Западная Бенгалия, Индия, но морфологическое исследование склеротизированных частей показало, что *D. labei* паразитирует и на *Catla catla*. Это первая находка *D. labei* на *Catla catla* в Индии.

Ключевые слова: Monogenea, филогения, 28S, рибосомальная ДНК, Ассам, Индия.

## Introduction

*Dactylogyrus* established by Diesing in 1850 includes more than 900 nominal species (Gibson et al., 1996). The high diversity of this genus can be explained by their cyprinid host diversity, which represents the most diverse family of freshwater fish (Helfman et al., 1997). The species of *Dactylogyrus* are host-specific although in this genus, a high number of congeneric species coexist on the same host (Rohde, 1989; Kennedy, Bush, 1992; Šimková et al., 2000, 2002, 2004). During the course of this study of monogeneans of freshwater fishes of the river Brahmaputra, which remained unexplored for this group of parasite, authors came across specimens of *Catla catla*. The host was found infected with atypical form of *Dactylogyrus labei* Musselius et Gusev, 1976 as this species was originally described from the gills of another host, *Labeo rohita* from fish farm Kalyani, West Bengal in India as atypical form by Musselius and Gusev (Gusev, 1976). Moreover, it was also reported from the same host at Bac Ninh, Mekong River Delta, Vietnam by Musselius and Gussev (Gussev, 1976) as atypical form.

The parasite was originally differentiated on morphological basis and has now been supplemented with molecular biology to validate it. This report provides morphological evidence and the first molecular confirmation of the presence of the *D. labei* atypical form in two hosts, *C. catla* and *L. rohita*, from India.

**Material and methods**

River Brahmaputra enters India from state of Arunachal Pradesh and commences its journey to the Bay of Bengal through Bangladesh. During the study, the specimens of *Catla catla* (Cypriniformes: Cyprinidae) were caught from the river Brahmaputra at the site Guwahati (26°11' N and 91°44' E). After confirmation their identification by keys (Day, 1958; Misra, 1959; Srivastava, 1986) and ichthyologists, the fish were killed by a sharp blow on the top of the head and dissected. The monogeneans were collected from the gill filaments and processed for morphological examination according to the method suggested by Malmberg (1970). The specimens of *D. labei* have been deposited in the museum of the Department of Zoology, Chaudhary Charan Singh University, Meerut, U. P., India (voucher number HS/Monogenea/2012/05). All the measurements were taken in μm and number of hooks from I to VII were considered as per Mizelle (1936).

For molecular study, each monogenean was examined with the help of light microscope under magnification 40X and 100X and then subjected to the process of DNA extraction. Extraction, amplification and sequencing of the parasite were performed as described by Chaudhary and Singh (2012), by using the specific primers: forward 5'-TCTAGTAACGGCGAGTGAACG-3', and reverse 5'-GGTGGAAGGTCTACCTCAGC-3'. The obtained nucleotide sequence is available in the GenBank under accession number JX566720. GenBank was first queried to retrieve 28S sequences from monogeneans and then aligned using ClustalW. Subsequent phylogenetic analysis of these sequences was performed using MEGA 5 (Tamura et al., 2011) by neighbour-joining (NJ) and maximum-parsimony (MP) methods. In reconstructing the NJ tree, the Kimura two-parameter model (Kimura, 1980) was used to estimate the distances. For constructing the MP tree, only sites at which there were at least two different kinds of nucleotides or amino acids, each represented at least twice, were used (parsimony-informative sites). Robustness of the inferred phylogeny was assessed using a bootstrap procedure with 1,000 replications.

## Results

Morphological examination revealed that the structures of male copulatory organ and haptoral parts were similar to *D. labei* reported by Musselius and Gusev (Gusev 1976) as atypical form (fig. 1). Measurements of various body parts are: body 440 (435–445; n = 5) long, greatest width 94 (92–96; n = 5) usually near middle. Four eye-spots, with one terminal and two bilateral well developed cephalic lobes are present. Pharynx spherical, diameter 60 (56–64; n = 5). Testis and vas deferens were not observed, copulatory complex 75 (73–77; n = = 4) is composed of a tube and accessory piece. Tube curved, with bubble-like inflated initial part of diameter 5 (4–6; n = 4), and 1 μm in the medial part. Vitelline follicles co-extensive with intestinal caeca and confluent posteriorly. Ovary and oviduct not observed, egg wide in the middle, pointed towards base, 40 (n = 1) long and 30 (n = 1) wide, without any polar filament. Haptor 78 (76–80; n = 5) long, 63 (60–66; n = 5) wide. Single pair of dorsal anchors, total length 56 (52–60; n = 5), outer root shorter than inner root, 8 (7–9; n = 5), inner root elongated wide, 14 (13–15; n = 5) long, shaft strong, almost straight, 32 (30–34; n = 5), points recurved, 13 (10–16; n = 5) long. Anchor filaments prominent. Hooks 7 pairs, equal in shape and unequal in size, hook lengths (n = 5): pair I, II and VII = 12 (11–13), pair III, IV and V = = 18 (17–19), and VI = 15 (14–16) with projecting "heel" of hooklet. Two connecting bars: ventral bar 'T'-shaped with pointed long end, total length 26 (25–27; n = 4), dorsal bar slightly bowed with laterally expanded ends, total length 24 (23–25; n = 6), width 5 (4–6; n = 6).

Sequence of the partial 28S rDNA (741 bp) confirmed that the isolates belongs to the genus *Dactylogyrus* as homology searching showed high similarity with the sequences of the species of *Dactylogyrus* available on database of GenBank. *Dactylogyrus labei* was found to be closely related with *D. quanfami* (EF100536) as both NJ and MP analyses inferred from 28S rDNA sequences gave similar topology with high bootstrap values (fig. 2). Branches corresponding to partitions reproduced in less than 50 % bootstrap replicates were collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown above the branches. Maximum support is seen in the phylogenetic elaborations and congruent tree topologies that showed the existence of a well supported clade formed by *D. labei* in both analyses (fig. 2). Since this clade exhibited high level of bootstrap support, *D. labei* and *D. quanfami* appear to be sister species which validates

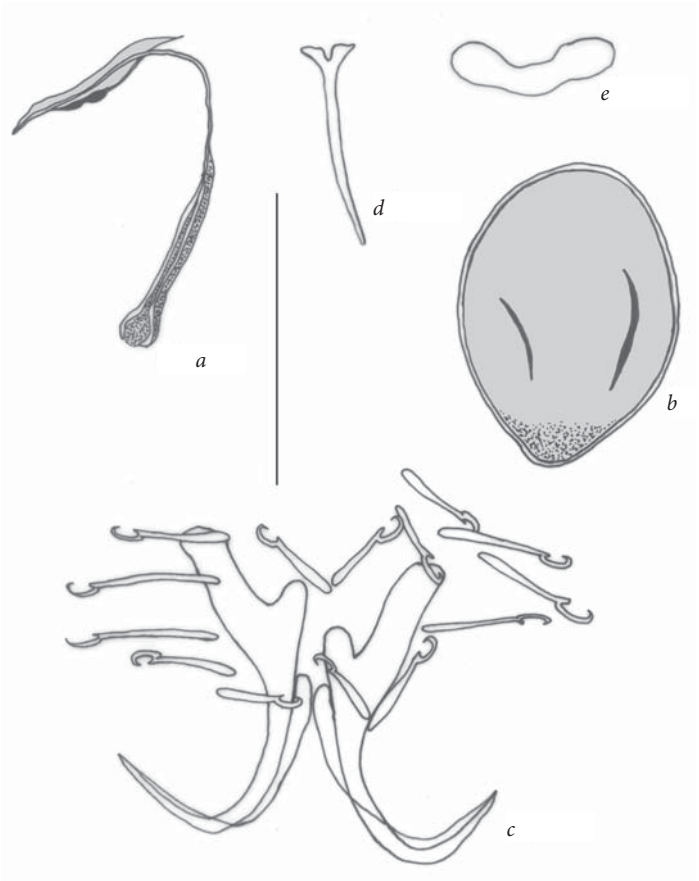


Fig. 1. *Dactylogyrus labei*: *a* — copulatory complex; *b* — egg; *c* — dorsal anchors and hooks I–VII; *d* — ventral bar; *e* — dorsal bar. Scale bar 40 μm.

Рис. 1. *Dactylogyrus labei: a* — копуляторный комплекс; *b* — яйца; *c* — дорсальные якоря и крючья I–VII; *d* — вентральная пластинка; *e* — дорсальная пластинка. Масштабная линейка 40 мкм.

the existence of *D. labei*. Unfortunately, we do not have the data on *D. labei* reported from *L. rohita* for comparison with the present sequence, but this work would help in future study to reveal the molecular characteristics of *D. labei*.

## Discussion

Among parasitic groups, host specificity differs widely (Sasal et al., 1998) and in comparison to other parasitic groups of fishes, monogeneans are considered to be highly host specific (Poulin, 1992). It was also hypothesized by Noble et al. (1989), that parasites like monogeneans with simple life-cycles are more host specific than parasites with complex life-cycles such as digeneans. The present study is the first report of *D. labei* from *C. catla*, with molecular characterization. With phylogenetic analysis, as a general rule, if the bootstrap value for a given interior branch of a phylogenetic tree is 70 % or higher, then the topology at that branch is considered reliable. The present findings show the bootstrap value to be > 70 % for the tree obtained and the 28S sequence of *D. labei* species, genetically well distinct from the other species of *Dactylogyrus* previously recognized with the same ribosomal markers. The tree topologies derived from the phylogenetic analysis are in agreement where they depicted *D. labei* and *D. quanfami* as genetically closely related sister species and a high bootstrap value was obtained for the clade formed by these sibling species.

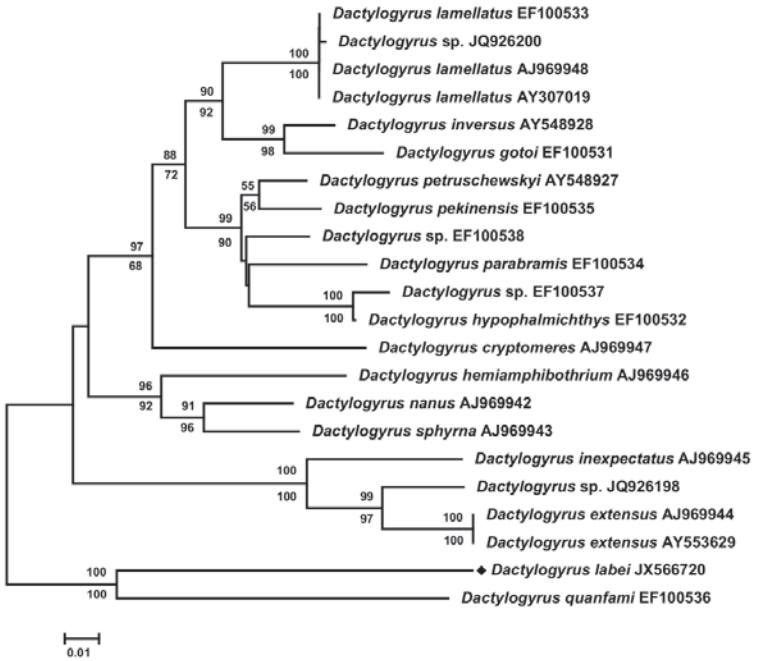


Fig. 2. Phylogenetic position of the present *Dactylogyrus* species based on 28S rDNA sequences. Distances were estimated using Kimura two-parameter model. The tree was constructed using the neighbor-joining method. The tree was identical to that obtained using maximum-parsimony and the numbers along branches represent bootstrap values given as above branch NJ and lower branch MP. Bootstrap support (> 50 % for 1,000 replicates) is shown at each node.

Рис. 2. Филогенетическое положение данного вида *Dactylogyrus*, основанное на анализе последовательностей гена 28S рДНК. Дистанции определены с использованием двухпараметрической модели Кимуры. Древо построено с использованием метода «ближайшего связывания» (neighbor-joining). Древо было идентично полученному с использованием максимальной парсимонии (maximum-parsimony); бутстреп-значения приведены для NJ (над ветвями) и MP (под ветвями). Бутстреп-поддержка (> 50 % для 1000 итераций) показана для каждой ноды.

Musselius and Gusev (Gusev 1976) determined two forms of *D. labei*, typical and atypical form. The atypical forms described in present manuscript were similar morphologically on the basis of haptoral parts and male copulatory organ to those of atypical form of Musselius and Gusev (Gusev 1976). Both the forms (typical and atypical) can be differentiated on the basis of hard parts like male copulatory organ and haptoral armature. Copulatory organ in atypical form is supplemented with a free lying chitinoid piece on the front side that is not present in typical form. In the typical form, dorsal bar contains a posterior tongue-shaped process that is not found in the atypical form. Similarly, the ventral bar shows the differentiation regarding having 5 rayes in the typical form that are absent in the atypical forms as also showed by its name. These above morphological characteristics separate both the forms from each other.

In wild conditions, most species of *Dactylogyrus* have been found on a single host species but in case of *D. labei* it was also reported from different host, considered that strict host specificity does not follow a regular pattern within this species. This study suggests that the speciation of *D. labei* via host switching from *L. rohita* to *C. catla* might be caused by the fact, that the two fish hosts are closely related, belong to same order, family and subfamily i. e. cypriniformes, cyprinidae and cyprininae respectively and it is easy for parasite to diversify within the family cyprinidae via host switching by sympatric intra host speciation. It has been demonstrated that in Europe, *Dactylogyrus* species from cyprinids, showed certain duplication events which gave rise to generalist species (Šimková et al.,

2004). In general, monogenean parasites are found to be ideal candidates for speciation by host switching (Zietara, Lumme, 2002) and this specificity to the host might at measure of ecological adaptation.

This study hypothesized that when the parasites jump onto new hosts due to closely relatedness, it might be adapted to at least to some degree to the environment within the host. The parasites start exploring the new host as alternative resources which may provide these with, additional breeding opportunities and infesting two species of hosts that helps perpetuating the parasite species. Host switching during aquaculture practices can be possible due to the fact that different species of fish were kept together in a tank. But occurrence of these processes in wild conditions, hypothesizes that the low specificity in *Dactylogyrus* during adverse environmental conditions, where hosts are dead or rare, causes accidental switching to available host. We could expect that the switching of *D. labei* and its establishment in new host were successful because the hosts are closely related. Further study of the host switching and adaptation of parasites in new host is required in order to improve our understanding of this mechanism.

We are thankful to the Head of the Department of Zoology, Chaudhary Charan Singh University, Meerut, India for providing laboratory facilities. The identification of the hosts was kindly confirmed (from specimens) by Prof. Umesh C. Goswami, Department of Zoology, Guwahati University, Guwahati, Assam, India. Funding for this study was provided by the UGC (University Grants Commission), India, under the Junior Research Fellowship (RGF) to HRC.

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Received 16 December 2013
Accepted 24 September 2014