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Dactylogyrus Kolodynensis sp. n. (Platyhelminthes: Monogenea) Infecting Gills of *Osteobrama Cotio* (Hamilton, 1822) (Cypriniformes: Cyprinidae) from India

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Abstract

Dactylogyrus kolodynensis, a new monogenean parasite species, is described and illustrated from *Osteobrama cotio* (Cyprinidae) collected from Lawngtlai (Mizoram) and Lucknow (Uttar Pradesh), India using morphological examination and sequencing of partial 28S rRNA gene. The new species is morphologically characterized and distinguished from all congeners by a combination of the following characters: copulatory tube a loose coil of one complete clockwise ring, jaw-shaped accessory piece comprising variable sheathes enclosing and guiding the copulatory tube, and sclerotized vaginal tube, with a terminal flower-bud-shaped vaginal pore. The molecular analyses of specimens of *D. kolodynensis* collected from the two different localities using 28S rRNA gene showed identical genotype that did not match any of the known sequences in GenBank, confirming our initial morphological identification. *Dactylogyrus cotius*, a sympatric species on the gills of *O. cotio*, is regarded as a *species inquirenda* because of its poor description. This is the first report of a monogenean species from Mizoram, in northeast India, bringing the total number of *Dactylogyrus* species in India to 57.

Introduction

Osteobrama cotio (Hamilton 1822) is an Asian freshwater benthopelagic fish species inhabiting the rivers, lakes, ponds, and ditches throughout Pakistan, India, Nepal, and Bangladesh (Froese and Pauly 2021). In India, *O. cotio* is widely distributed in states of Assam, Manipur, Mizoram (Kar and Sen 2007), West Bengal, Madhya Pradesh, Punjab, and Uttar Pradesh (Vishwanath and Shantakumar 2007). This small indigenous fish provides a nutritional supplement to a large section of the economically backward population (Kumar and Goswami 2013). Two monogenean species have previously been described from the gills of *O. cotio*: *Dactylogyrus cotius* (Jain 1957) Gusev, 1973 and *Dactylogyroides osteobramii* Agrawal, Pandey and Tripathi, 2002.

During a recent parasitological investigation of the cyprinid fishes, several specimens of monogeneans were found on the gills of *O. cotio* collected from the Kolodyne river in Mizoram and Gomti river in Uttar Pradesh. A morpho-molecular examination revealed that these specimens represent a new species of *Dactylogyrus*, which is described and illustrated in this paper.

Materials And Methods

Fish sampling

Between August and December 2021, thirty-five specimens of *O. cotio* [(total weight: 8.71-10.91 gm, length: 7-11 cm)] were collected from the Kolodyne river in Lawngtlai, Mizoram, northeast India (11 specimens) and the Gomti river in Lucknow, Uttar Pradesh, north India (25 specimens). They were dissected, and their gills, along with the flatworms, were fixed and preserved separately in 5% formalin and 95% ethanol for morphological and molecular analysis. The scientific name, including taxonomic authority and date, of fish followed Fishbase (Froese and Pauly 2021).

Parasite Sampling

Morphological methods

Formalin-preserved monogeneans were slightly flattened under a coverslip, mounted unstained in Hoyer's medium and glycerine for examination of the sclerotised structures. Some of them were stained with Horen's trichrome, dehydrated in ascending series of ethanol, cleared in xylene, and mounted in DPX (Dibutylphthalate Polystyrene Xylene) for permanent preservation. The mounted specimens were examined, photographed, and measured using a light microscope (Leica DM4B) equipped with phase-contrast and Differential Interference Contrast (DIC) optics, a digital camera (Leica DFC7000 T), and image analysis software (LAS X; Leica Microsystems Ltd.). The measurement techniques (straight–line distances between two extreme points), terminologies, and identification of the flatworms were adapted from Gusev (1976), with the following modifications; the term "anchor length" was used instead of 'dorso-apical length', and the terms 'thumb'" and 'shank' were used in place of 'heel' and 'handle', respectively. The copulatory tube, on the other hand, measured as the total distance along the median line of the coiled tube and the direction of its coil (clockwise vs counterclockwise) was determined using the procedure suggested by Kritsky et al. (1985). All measurements are expressed in micrometres and presented as the mean with the range and number (n) of specimens measured in parentheses. Numbering and distribution of hooks followed Kulwiec (1927). An illustration plate was prepared using a drawing tube attachment fixed to the microscope. Prevalence and mean intensity of infection were calculated according to Bush et al. (1997). The type specimens were deposited with the Zoological survey of India, Kolkata, India.

Molecular Methods

Genomic DNA was extracted from two 95% ethanol-fixed monogenean parasites (one individual randomly selected from each locality) with Extracta DNA Prep for PCR–Tissue (Quantabio, Beverly, US), according to the manufacturer's instructions. The 28S ribosomal RNA gene was amplified in 20 µl volume using the universal primers c1 and d2 (Hassouna et al. 1984) and the thermo-cycle profile of Simkova et al. (2006). Purification and Sanger sequencing were performed in both forward and reverse directions by a commercial facility (Eurofins Genomics India Pvt. Ltd.), using the identical primers that generated the PCR products. The resulting sequences were analysed with SnapGene version v.5.3 (http://www.snapgean.com) and a consensus sequence was obtained using BioEdit Program (Hall 1999). To achieve species-rank identification based on 28S rRNA gene, the consensus sequence was aligned with all sequences from related species as retrieved from the NCBI GenBank database using BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) (see accession numbers in Table 1).

Results

Class Monogenea Van Beneden, 1858

Order Dactylogyridea Bychowsky, 1937

Family Dactylogyridae Bychowsky, 1933

Genus *Dactylogyrus* Diesing, 1850

Dactylogyrus kolodynensis sp. n.

Description

Morphological characterisation (Figures 1 and 2)

[Based on 15 specimens]: With characters of the genus as defined by Diesing (1850). Body fusiform 307 (290-312; n=5) long; greatest width 90 (85-95; n=5) near midlength. Single pairs of (dorsal) anchors 27 (24-32; n=8) long with elongated inner root 11 (9-13; n=9) long; short outer root 4 (2-6; n=8) long, curved shaft 20 (17-25; n=8) long, recurved point 11 (9-13; n=8) long and extending the past level of the tip of inner root. Single (dorsal) bar 20 (17-23; n=10) long, 5 (3-7; n=10) wide, rod-shaped with a short posteromedial expansion, and rounded lateral ends posteriorly directed. Seven pairs of hooks, similar in shape but dissimilar in size, each with a delicate point, depressed thumb (except 6th, which has upright thumb), shank comprised of 2 subunits (proximally expanded subunit), a pair of needles located near the hooks of pair 6, HF loop extending to near union of shank subunits; hook lengths: pair I, 19 (16-22; n=5) long, pair II, 18 (16-21; n=5) long, pair III, 20 (17-22;n=5) long, pair IV, 10 (7-12; n=5) long, pair V, 18 (16-21;n=5) long, pair VI, 19 (17-21; n=5) long, pair VII, 20 (17-22; n=5) long. Male copulatory organ comprised of a copulatory tube articulated to the base of a complex accessory piece by a thick ligament. Copulatory tube 85 (77-88; n=8) long, with a loose coil following a circular path of one complete clockwise ring, narrowing to delicate termination. Accessory piece 29 (26-32; n=10) long, complex, with lower and upper variable sheathes curving towards each other like claws; upper sheath longitudinally grooved to guide the distal end of copulatory tube. Vagina 35 (32-38; n=10) long, a sclerotized tube with a terminal flower-bud-shaped vaginal pore. Egg 66 (n=1) long, 46 (n=1) wide, operculate,

one short polar filament on adopercular end.

Molecular Characterisation

Sequencing of partial 28S rRNA gene of *D. kolodynensis* resulted in amplicons of the same length size (508 bp) and showed no intraspecific nucleotide variations between different individuals collected from two different localities examined here. The sequence of *D. kolodynensis* sp. n. was deposited in GenBank database (http://www.ncbi.nlm.nih.gov), accession number OL964059. A BLAST search revealed that this sequence did not match 100% to any of the known any available sequences in GenBank, confirming our initial morphological identification (Table 1).

Diagnostic Characters

The main diagnostic characters of the new species are: a copulatory tube that is a loose coil of one complete clockwise ring, a jaw-shaped accessory piece with variable sheathes enclosing and guiding the copulatory tube, and sclerotized vaginal tube with a terminal flower-bud-shaped vaginal pore.

Taxonomic Summary

Type host *Osteobrama cotio* (Hamilton, 1822) (Cypriniformes, Cyprinidae)

Type locality: River Kolodyne, Lawngtlai, Mizoram, India (22°35'15"N, 92°55'13"E)

Infection site: Gill lamellae

Additional locality: River Gomti, Lucknow, Uttar Pradesh, India (26°52'12" N; 8°55'20" E)

Infection parameters: 72.72% (eight out of 11 fishes) were infected from Lawngtlai (Mizoram) with intensity of 3–10 (mean = 5) parasites/ infected host; 70.83% (17 out of 24 fishes) were infected from Lucknow (Uttar Pradesh) with intensity of 4–9 (mean = 6) parasites/ infected host

Date of sampling: August 2021 to October 2021

Deposition of specimens: Holotype (xxxxxx) and Paratypes (xxxxxx) in Zoological Survey of India, Kolkata, India; Other paratypes (xxxxxx) in the helminthological collection of

the Department of Zoology, University of Lucknow, India.

Representative DNA sequence: 28S rRNA (508 bp), OL964059

ZooBank registration: The Life Science Identifier (LSID) for *Dactylogyrus kolodynensis* sp. n. is xxxxxxxxxxx.

Etymology: The species is named after the river “Kolodyne”, the type locality of the species, with the Latin suffix -ensis denoting a location.

Discussion

Dactylogyrus Diesing, 1850 is a genus with the most species in the Monogenea (Platyhelminthes), with over 900 nominal species world-wide (Gibson et al. 1996), including 56 species from India (Wangchu et al. 2017). These species are well-known for causing chronic debility, poor development and growth, impaired respiration, and finally mass mortality of infested host fish (Bauer 1951; Paperna 1963).

Dactylogyrus kolodynensis sp. n. can be easily confused with *D. bucinus* Gussev, 1976 from *Barbus dorsalis* (Jerdon, 1849) (now *Puntius dorsalis*) and *D. parvianchoris* Gusev, 1976 from *Chaila bacaila*

(Hamilton, 1822) (now *Salmostoa bacaila*) in the general morphology of the haptoral and reproductive hard parts. However, the new species differs from *D. bucinus* mainly in having smaller anchors (24-32 in *D. kolodynensis vs* 40-42 in *D. bucinus*), hooks (15-20 in *D. kolodynensis vs* 16-27 um in *D. bucinus*), and vaginal tube (32-38 in *D. kolodynensis vs* 50 in *D. bucinus*), as well as the claw-shaped distal end (*vs* absent in *D. bucinus*). In addition, *D. bucinus* is a parasite of *P. dorsalis* in south India, while the new species was collected from *O. cotio* in northeast and north India.

The new species can also be distinguished from *D. parvianchoris* in comparative morphometry of copulatory tube (77-88, one complete ring in *D. kolodynensis vs* 100-120, one complete ring or 8-like curved in *D. parvianchoris*), accessory piece (26-32, jaw-shaped in *D. kolodynensis vs* 33 um, solid plate with jagged edge in *D. parvianchoris*), and the vaginal pore (32-38, flower-bud-shaped in *D. kolodynensis* vs 48-55, funnel-like enlargement in *D. parvianchoris*).

It should be noted that no museum type specimens of *D. bucinus* and *D. parvianchoris* were available for examination. Inquiries to the Zoological Society of India, Kolkata and Zoological Institute, Russian Academy of Sciences, St Petersburg, where Gusev (1976) has deposited his specimens, were unsuccessful. Therefore, the comparison of *D. kolodynensis* sp. n. had to be based entirely on the published descriptions of *D. bucinus* and *D. parvianchoris*.

The most closely related species to *D. kolodynensis* sp. n., according to a BLAST results for our sequence OL964059, were two unpublished species from *Pethia ticto* (Hamilton, 1822) in India: *Dactylogyrus* sp. DD AT-2021 (MZ088043.1, 96.87%, 560 bp) which differed by 16 substitutions containing four gaps, and *Dactylogyrus* sp. AT-2021 (MZ227276.1, 96.09%, 508 bp) which differed by 5 substitutions containing three gaps. Given the close phylogenetic relationship between *P. ticto* and *O. cotio* (see, for example, Sobita et al. 2019), the molecular similarity between their parasites is not surprising.

This study presented us with a unique problem involving *D. cotius* Jain (1957), one of the two monogenean species, previously described from the gills of *O. cotio*, along with *D. osteobrami*. *Dactylogyrus cotius* was originally described by Jain (1957) as *Neodactylogyrus cotius* from the gills of *Rohtee cotio* (now *D. cotio*) from Lucknow. Gusev (1973) transferred the species to the genus *Dactylogyrus*. While we consistently found *D. kolodynensis* sp. n. and *D. osteobrami* associated with *O. cotio* in both Mizoram and Uttar Pradesh, we never found *D. cotius* in either location. This absence of *D. cotius*, especially in material collected from Lucknow–the same host and locality as the previous one (Jain, 1957) took us completely by surprise. This could be due to *O. cotio* being an atypical host for *D. cotius*, or it could be due to Jain (1957) misidentifying his host specimens as *O. cotio* and instead describing *D. cotius* from a different host species. A major problem with *D. cotius* is that its morphological description is mostly incomplete and its illustrations are highly diagrammatic, which means they do not correspond to each other. Additionally, Jain (1957) did not specify the location of his type specimens, indicating that they unlikely to have been deposited in a national or international museum, and thus be available for comparison. As a result, we believe it necessary to place *D. cotius* as a *species inquirenda* until the species is redescribed using specimens collected from the type host and locality.

The validity of *D. kolodynensis* sp. n. and its placement within the genus *Dactylogyrus* was thus supported both by morphological and molecular comparisons among related species. This is the first report of a monogenean parasite from the Mizoram, bringing the total number of nominal *Dactylogurus* species known from India to 57. Given that Mizoram is a biodiversity hotspot (Barman et al. 2018), with no fewer than 156 fish species, including approximately 78 cyprinids distributed in its diverse hilly terrain (Goswami et al. 2012), we anticipate a high species richness of monogenean parasites from this region.

Declarations

Competing interests

The authors have no competing interests to disclose.

Author contributions

Amit Kumar Trivedi performed the molecular experiments and authored the initial draft of the paper. Sneha Prakash collected the fish and parasite samples, and prepared mounts and figures. Amit Tripathi analysed the morpho-molecular data, and authored and approved the final draft.

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Ethical approval

Experiments were conducted in accordance with institutional guidelines for animal care.

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Tables

Table 1. Top BLAST search matches of *Dactylogyrus kolodynensis* n. sp. (OL964059) for 28S ribosomal RNA gene sequences on GenBank.

***Dactylogyrus* spp.**	**Accession number**	**Host species**	**Locality**	**Query cover**	**E value**	**% identity**
Dactylogyrus sp. DD AT-2021	MZ088043.1	*Pethia ticto*	India	99%	0.0	96.87%
Dactylogyrus sp. AT-2021	MZ227276.1	*Pethia ticto*	India	94%	0.0	96.09%
Dactylogyrus sp. LXF-2019	MH790264.1	*Sikukia flavicaudata*	China	100%	0.0	92.94%
Dactylogyrus mascomai	MN338215.1	*Luciobarbus graellsii*	Spain	98%	0.0	89.90%

Figures

Figure 1

Line drawings of haptoral and reproductive hard parts of *Dactylogyrus kolodynensis* sp. n. from *Osteobrama cotio* (Hamilton, 1822) collected from Lawngtlai (Mizoram) and Lucknow (Uttar Pradesh). A. Anchor. B. Dorsal bar. C. Hook (pairs I–VII). D. Male copulatory organ. E. Vagina. Scale bar = 50 µm

Figure 2

Montage of *Dactylogyrus kolodynensis* sp. n. from *Osteobrama cotio* (Hamilton, 1822). Phase contrast and DIC microscopy images of haptoral and reproductive hard parts. A. anchor-bar complex and hooks. B. Male copulatory organ. C. Vagina. D. Egg. E. Hook no. 6 with erect thumb and needle. F. Hook no. 4 with depressed thumb.