Dactylogyrus kolodynensis sp. n. (Platyhelminthes: Monogenea) infecting gills of Osteobrama cotio (Hamilton, 1822) (Cypriniformes: Cyprinidae) from India

Amit K. Trivedi · Sneha Prakash · Amit Tripathi

Abstract Dactylogyrus kolodynensis, a new monogenean parasite species, is described 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 closely related congeners by a combination of the following characters: copulatory tube a loose coil of one complete clockwise ring, jaw-shaped accessory piece comprising variable sheaths 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 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.

Keywords Dactylogyrus kolodynensis sp. n. · India · Mizoram · Monogenea · Osteobrama cotio · 28S rRNA gene

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

Thirty-five specimens of O. cotio [total weight: 8.71-10.91 gm, length: 7–11 cm] were collected using gillnets between August and December 2021 from the Kolodyne river in Lawngtlai, Mizoram, northeast India (11 specimens) and the Gomti river in Lucknow, Uttar Pradesh, north India (25 specimens). Fish were transported alive to
the laboratory in oxygenated containers filled with riverine water and dissected under a stereo zoom microscope according to standard parasitological procedures. 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**

**Collection and morphological identification**

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 studied, 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.). An illustration plate was prepared using a drawing tube attachment fixed to the microscope. The measurement (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, was 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 are presented as the mean with the range and number (n) of specimens measured in parentheses. Prevalence and mean intensity of infection were calculated according to Bush et al. (1997). Type specimens were deposited in the Helminthological collections of the Zoological survey of India, Kolkata, India.

**DNA extraction, PCR amplification and sequencing**

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. Partial fragment of 28S ribosomal RNA gene was amplified by employing the universal primers c1 forward and d2 reverse (Hassouna et al. 1984) and the thermo-cycle profile of Simkova et al. (2006). The PCR products were purified (on 1.5% agarose using the QIAquick PCR Purification Kit from Qiagen, USA) before being Sanger sequenced 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.snapgene.com) and a consensus sequence was obtained using BioEdit (Hall 1999). To achieve species-rank identification based on 28S rRNA gene, the consensus sequence was compared 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 Dactylogyrida Bychowsky, 1937  
Family Dactylogyridae Bychowsky, 1933  
Genus Dactylogyrus Diesing, 1850  
*Dactylogyrus kolodynensis* sp. n. (Figs. 1, 2)  
*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 (26.85°N; 80°95 E)  
*Infection parameters:* 72.72% (eight out of 11 fishes) were infected from Lawngtlai (Mizoram) with intensity

| 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      |
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.

Period of sampling: August 2021 to October 2021

Type specimens: Holotype (ZSI/W10929/1) and 4 Paratypes (ZSI/W10930/1–33/1) in Zoological Survey of India, Kolkata, India; Other paratypes (PL/SP/2022/1–5) 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 urn:lsid:zoobank.org:pub:3F3B6373-A588-4061-8873-C4F2B956AB1B.

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

Morphological characterisation

Description (based on 10 adult 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, dextral, comprising a sclerotized tube with a terminal flower-bud-shaped vaginal pore. Egg 66 X 46 (n = 1) long, 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).

Differential diagnosis

*Dactylogyrus kolodynensis* sp. n. is differentiated from its closely related congeners by having 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.

Discussion

*Dactylogyrus* Diesing, 1850 is a genus with the most species in the Monogenea (Platyhelminthes), with over 900 nominal species worldwide (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, particularly those under intensive culture/captive conditions (Paperna 1963; Ramadan et al. 1995; Kritsky and Heckmann 2002; Lu et al. 2012).

*Dactylogyrus kolodynensis* sp. n. can be easily confused with *D. bucinus* Gussev, 1976 from *Barbus dorsalis* (Jerdon, 1849) (now *Puntius dorsalis*) and *D. parvianchoris* Gussev, 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. Although 56 Dactylogyrus species are known from India, only four have molecular sequences: D. catlaius (Thapar, 1948) Monaco & Mizelle, 1955; D. labei Musselius and Gusev, 1976; D. longiacus Gusev, 1976; and D. subtilis Gusev, 1976. Due to this paucity of publicly available comparative molecular data as yet, we have not attempted to create a phylogeny including the new species, but have submitted the 28S gene sequence to GenBank for future comparison.

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 Dactylogyrus 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.

Acknowledgements SP gratefully acknowledges financial support received from the Department of Science and Technology, Government of India [No. DST/INSPIRE Fellowship/2019/IF190017]. AT utilised the lab facilities created under the research grant received from Science and Engineering Research Board (SERB), Government of India (SERB–EMR/2017/003232). We also thank two anonymous reviewers for their insightful comments. This paper is a part of initial draft of Ph.D. Thesis of SP.

Author contributions AKT performed the molecular experiments and authored the initial draft of the paper. SP collected the fish and parasite samples, and prepared mounts and figures. AT analysed the morpho-molecular data, and authored and approved the final draft.

Funding Funding was provide by Department of Science and Technology, India (Grant No. DST/INSPIRE Fellowship/2019/IF190017) and Science and Engineering Research Board, India (Grant No. SERB–EMR/2017/003232).

Declarations

Competing interests The authors have no competing interests to disclose.

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

References

Barman AS, Singh M, Singh SK et al (2018) DNA barcoding of freshwater fishes of indo-myanmar biodiversity. Hotspot Sci Rep 8:8579. https://doi.org/10.1038/s41598-018-26976-3
Bush AO, Lafferty KD, Lotz JM, Shostak AW (1997) Parasitology meets ecology on its own terms: Margolis et al revisited. J Parasitol 83:575–583
