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Original paper

Redescription and new geographic distribution record of *Dactylogyrus pharyngocephalus* (Monogenea: Dactylogyridae) from tank goby *Glossogobius giuris* (Gobiidae, Teleostei) in Mizoram, northeast India

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ABSTRACT. *Dactylogyrus pharyngocephalus* is a dactylogyrid monogenean parasite originally described by Kulkarni, 1970 from tank goby *Glossogobius giuris* (Hamilton, 1822) in South India. The species has not been recorded since then. Fresh specimens of *D. pharyngocephalus* were collected from northeast India and examined morpho-molecularly. Temporary (glycerine mounted) and permanent (stained with Gomori's trichrome and DPX mounted) slides were made and photographed. Primer set C1 and D2 was employed to amplify a fragment of the 28S rRNA gene. The resulting nucleotide sequences, when examined by the BLAST tool, did not show 100% homology with any of the sequences deposited in GenBank. Based on new morpho-molecular data, the description of *D. pharyngocephalus* is revised, including the first description of its ventral bar.

Keywords: fish, parasite, northeast India, 28S rRNA gene

Introduction

Tank goby *Glossogobius giuris* (Hamilton, 1822) (Teleostei: Gobiidae) is a commercially important fish species [1,2] found widely distributed from Africa to Oceania [3]. To date, four species of monogenean parasites have been identified from *G. giuris*, all of which belong to *Dactylogyrus* Diesing, 1850. These are *D. sonii* Gusev, 1996, *D. lali* Gusev, 1973, *D. glossogobii* Jain, 1960, and *D. pharyngocephalus* Kulkarni, 1970.

Dactylogyrus pharyngocephalus was originally described by Kulkarni [4] from *G. giuris* in Lake Hussain Sagar at Hyderabad, South India. A parasitological investigation of *G. giuris* from Mizoram, northeast India, revealed the presence of *D. pharyngocephalus,* which showed some features not mentioned in the original description. This led us to redescribe the species for the first time by providing new morphological characteristics supported by photomicrographs. In addition, this study provided the first molecular characterisation (amplification and partial sequencing of 28S rRNA gene) of *D. pharyngocephalus*. The new data not only improves the distribution knowledge of this species, but also its diagnosis, which will help future workers in distinguishing congener populations of monogenean species from *G. giuris*.

Materials and Methods

Study area and collection of host samples

From March to June 2022, ten moribund specimens of *G. giuris* were captured using cast nets and gill nets from the local rivers in Serchhip District of Mizoram, northeast India. These samples were immediately fixed in 5% formalin and 95% ethanol. The identification and nomenclature of the fish followed by [5].

Microscopy

Monogeneans were collected from the fish gills using needles under a binocular microscope (Leica

Table 1. PCR reagents in the order and concentration they were added

Reagents	Concentration of stock solution	Volume	Final concentration
Distilled water	–	4 µl	–
Master Mix	2×	10 µl	1×
Forward Primer	10 µM=10 pmols/µl	1.0 µl	0.5 pmols
Reverse Primer	10 µM=10 pmols/µl	1.0 µl	0.5 pmols
Sample DNA	–	4 µl	20 ng/µl
Total (reaction) volume	–	20µl	–

Table 2. Standard 3–step thermocycling profile

Cycle step	Temperature	Time	Number of cycles
Initial Denaturation	95°C	3 minutes	1
Denaturation	94°C	1 minute	
Annealing	50°C	1 minute	35
Extension	72°C	2 minutes and 30 seconds	
Final Extension	72°C	7 minutes	1
Hold	4°C	∞	1

EZ4HD) following standard procedures [6]. The specimens were either mounted in glycerine or dehydrated through a graded series of ethanol and stained with Gomori's trichrome before being mounted in DPX (dibutyl phthalate polystyrene xylene). They were examined and photographed with a Leica DM4B upright microscope equipped with Phase Contrast and Differential Interference Contrast (DIC) optics and a Leica DFC7000T digital camera. Measurements representing straight lines between any two extreme points in micrometres were taken with LAS X image analysis software (Leica Microsystems Ltd., Germany) and have been presented in the text as mean followed by the range in parentheses.

DNA extraction, PCR and sequencing

Total genomic DNA was extracted from a group of five ethanol-fixed monogeneans, using Extracta DNA Prep (Quantabio, Beverly, US) according to the manufacturer's instructions. Partial fragments of the 28S rRNA gene were amplified using the universal primers C1 (5′-ACCCGCTGAATTTAAG CA-3′) and D2 (5′-TGGTCCGTGTTTCAAGAC-3′) [7]. PCR reagents and their concentrations have been presented in table 1, while PCR cycling conditions have been presented in table 2. A standard 100 bp DNA ladder (HiMedia, Mumbai, India) was used to estimate the molecular weight of the amplified products. The amplified products were sent to a commercial sequencing facility (Future Biotech Pvt. Ltd, India) for purification and Sanger sequencing in both directions, employing the same primers as those used to generate the PCR products. The resultant sequences were edited, assembled and contigs were produced using the DNA Sequence Assembler v4 [8]. Sequence was deposited in GenBank (NCBI) under the accession number OR879966.

Ecological parameters

The prevalence (the percentage of infected hosts in a sample) and mean intensity (the average number of parasites per infected host in a sample) of infection were determined following the methodology outlined by Bush et al. [9].

Results

Class Monogenea van Beneden, 1858
Order Dactylogyridea Bychowsky, 1937
Family Dactylogyridae Bychowsky, 1933

Genus *Dactylogyrus* Diesing, 1850
Dactylogyrus pharyngocephalus Kulkarni, 1970 (Figs 1–3)
Type host: *Glossogobius giuris* (Hamilton, 1822)
Type locality: Hussain Sagar Lake, Hyderabad, South India
Present record and locality: *Glossogobius giuris* (Hamilton, 1822), Serchhip district, Mizoram
Infection site: gill lamellae
Infection parameters: prevalence 40% (4/10); mean intensity 4.5 ± 1.5 (18/4)
Voucher specimens: four specimens in the Helminthological Collection of Fish Parasitology Laboratory at the Department of Zoology, Lucknow, India

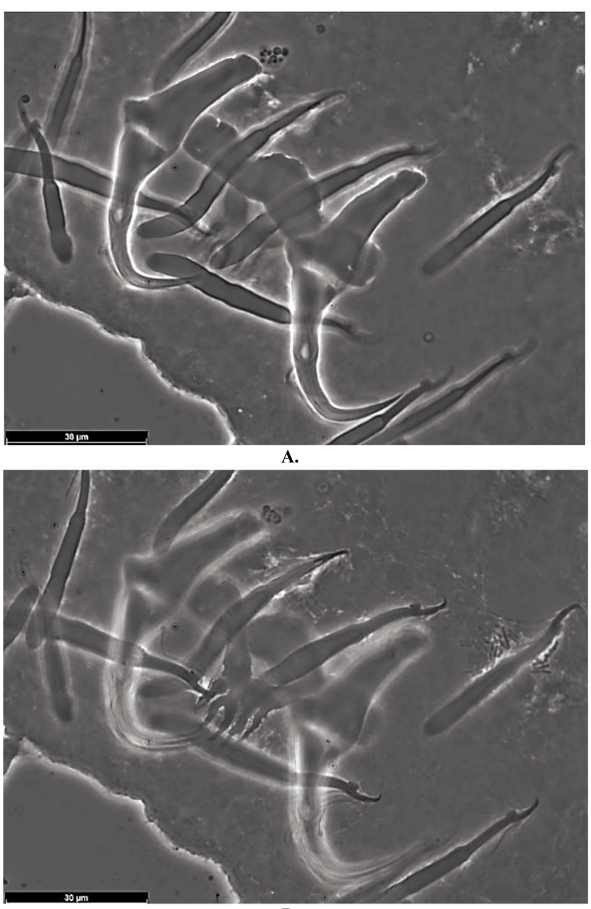

Figure 1. Phase contrast micrograph of dorsal (A) and ventral (B) view of haptoral hard parts (anchor-bar complex and hooks) of *Dactylogyrus pharyngocephalus* Kulkarni, 1970 from *G. giuris* (Hamilton, 1822). Scale bar = 30 µm

Redescription: Dorsal anchors 48 (40–50; n=7) long with elongated inner root 21 (15–25; n=7) long, moderately developed outer root 5 (4–6; n=7) long, shaft 30 (25–35; n=7) long, with a distinct

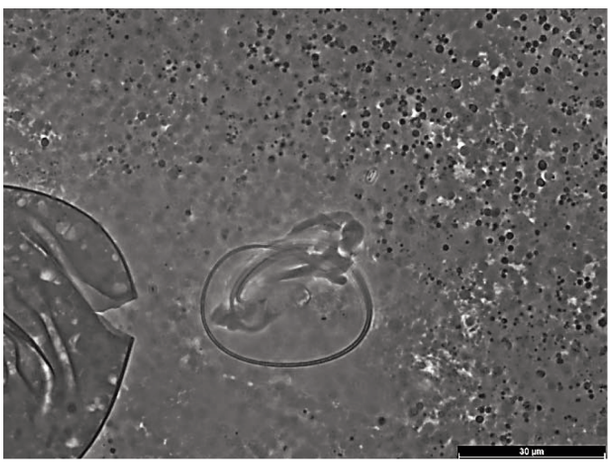

Figure 2. Phase contrast micrograph of male copulatory organ of *Dactylogyrus pharyngocephalus* Kulkarni, 1970 from *G. giuris* (Hamilton, 1822). Scale bar = 30 µm

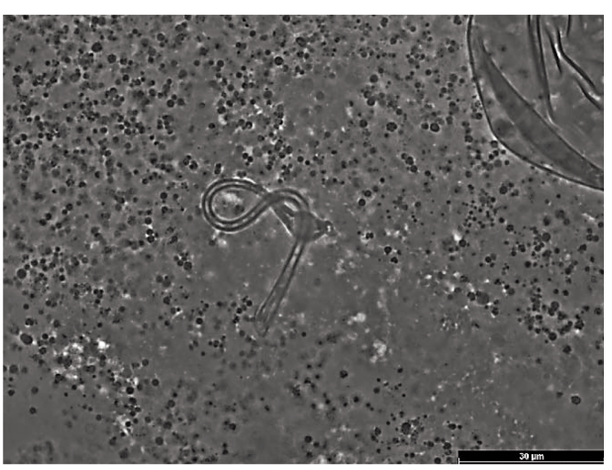

Figure 3. Phase contrast micrograph of vagina of *Dactylogyrus pharyngocephalus* Kulkarni, 1970 from *G. giuris* (Hamilton, 1822). Scale bar = 30 µm

small fenestration and slight inward bulge, point 14 (12–16; n=7) long, recurved. Dorsal bar 38 (30–40; n=7) long, 5 (3–6; n=7) wide, transverse, with slightly rounded ends, a medial dilation on the rough anterior margin, and a corresponding notch on the smooth posterior margin. Ventral bar 31 (30–35; n=7) long, 9 (7–10; n=7) wide, weekly sclerotised, with two horn-shaped projections on anterior margin and 5 unique filamentous processes (7–10 µm) long on posterior margin. Seven pairs of hooks (30–40; n=7) long, uniform in shape but diverse in size, each with a delicate point, depressed thumb, shank comprised of 2 subunits (proximal subunit significantly expanded subunit). The male copulatory organ comprised of a copulatory tube and a proximally articulating accessory piece. Copulatory tube 127 (120–130; n=8) long, a loose

Table 3. Top BLAST search matches of *Dactylogyrus pharyngocephalus* (OR879966) for 28S ribosomal RNA gene (841 bp) sequences on GenBank

Dactylogyrus spp.	Accession number	Host species	Locality	Query(%)
coverE value	% Identity			
D. anchoratus	MT997190	*Cyprinus carpio*	China	97%
0.0	85.47%			
D. sp. MG-2019	MK357774	*Carassius auratus*	China	94%
0.0	85.65%			
D. formosus	MT997191	*Carassius auratus*	China	95%
0.0	85.33%			
D. vastator	MK335463	*Carassius auratus*	China	95%
0.0	85.84%			

Table 4. Comparative morphometric measurements (μm) of body parts of *Dactylogyrus pharyngocephalus* Kulkarni, 1970

Characters	Measurements	
	Kulkarni [4]	Present study
Haptoral armaments		
Dorsal anchor		
Total length	68–81	48 (40–50)
Outer root	–	5 (4–6)
Inner root	–	21 (152–5)
Point	–	14 (12–16)
Dorsal bar		
Length	33–38	38 (30–40)
Width	–	5 (3–6)
Ventral bar		
Length	–	31 (30–35)
Width	–	9 (7–10)
Hooks	28–36	30–40
Reproductive organs		
Male copulatory organ		
Copulatory tube	–	127 (120–130)
Accessory piece	–	42 (35–45)
Female Organ		
Vagina	–	67 (50–60)

Abbreviation: values indicated by (–) were not provided in the primary descriptions

coil of one complete clockwise ring, with a swollen base narrowing to the termination. Accessory piece 42 (35–45; n=7) long, with expanded proximal base and a complex of multi-layered distal sheath guiding the copulatory tube. Vagina 67 (50–60; n=7) long, tubular, with a bulbous proximal base and a distal opening.

Molecular characterisation

Amplicons of 841 base pairs were obtained for

the 28S rRNA gene of *D. pharyngocephalus*. They were compared with related sequences in the NCBI Database (BLASTN, https://blast.ncbi.nlm.nih.gov/Blast.cgi) to accomplish species-rank identification. A BLAST search indicated that the sequence of new material did not demonstrate 100% correspondence with any known GenBank sequences. Three most closely matched sequences, as determined by their highest BLAST scores, are presented in table 3.

Discussion

Morphological characteristics and measurements of *D. pharyngocephalus* specimens from Mizoram were very close to those described by Kulkarni [4]. The similarities included the general morphometry of haptoral structures (anchor and dorsal bar) and reproductive organs (male copulatory organ and vagina) (Tab. 4). The main difference between Hyderabad and Mizoram specimens was the absence of the ventral bar and the presence of comparatively larger dorsal anchors in Hyderabad specimen. Kulkarni [4] failed to observe a ventral bar, most likely due to its weekly sclerotised nature. Otherwise, ventral bar morphology with filamentous processes seems to be a distinctive feature of *D. pharyngocephalus*, as it has not been reported for any other monogenean species. The length of the dorsal anchor in the original description was stated to be 68–81, as opposed to 40–50 in our specimens. However, given the close similarities in other body structures and the fact that Hyderabad and Mizoram specimens share the same host, we consider this difference as intra-specific variation, insufficient for the description of a new species. Our phase-contrast microscopic observation also added new morphological data on the presence of a small fenestration and slight inward bulge on the shaft of the dorsal anchor, and the detailed structure of the dorsal bar, hooks, and the male copulatory organ. Furthermore, the present report extended the known geographic distribution of *D. pharyngocephalus* by a distance of more than 3000 km, from Hyderabad in South India to Mizoram in northeast India.

Family Gobiidae is one of the most diverse taxa of fish, with more than 200 genera and over 2200 species [10]. Because gobies are economically and ecologically important, there is a need to diagnose their parasitic fauna. They serve an important ecological function, first as secondary consumers and then as prey for larger fish in the food chain [11]. They also play an important role in commercial fishing [12], particularly in the aquarium hobby [13]. India has about 134 gobiid species, with two of them found in Mizoram: *Psammogobius biocellatus* (Valenciennes, 1837) and *Glossogobius giuris* (Hamilton, 1822) [14]. According to Whittington [15] conservative estimate of at least one monogenean species per fish host species worldwide, Indian gobiids are expected to support at least 134 monogenean species. However, only one gobiid species (*Glossogobius giuris*) has been screened in India thus far. Clearly, more intensified parasitological investigations are needed to map the diversity of monogenean parasites of gobiids in general, and that of Mizoram in particular.

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