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Occurrence, Morpho-Histopathological Characterization, and Infection Dynamics of *Posthodiplostomum* sp. (Strigeidida: Diplostomidae) in Cyprinid Fish of the Ganga River

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Original article

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A metacercarial infection of *Posthodiplostomum* sp. (Strigeidida: Diplostomidae) was reported in the cyprinid fish, *Labeo catla* and *Pethia conchonius* and was identified based on clinical signs, cyst morphometry and characteristic histopathological lesions. The parasite was oval-round in shape, encysted in musculocutaneous tissues, and well encapsulated. The cyst was 1.02±0.02 by 0.79±0.02 mm and 1.02±0.02 by 0.75±0.02 mm in *L. catla* and *P. conchonius,* respectively. Microscopically, the multifocal hyperpigmented areas in the musculocutaneous tissues showed pericystic melanization, focal necrosis, and an infiltration of mononuclear leukocytes. Out of the 5,820 freshwater fish examined, only 3 *L. catla* were found to be infected in October at the Balagarh and in August, 13 *P. conchonius* were found to be infected at the Farakka stretch of the Ganga River. The spatio-temporal prevalence of *Posthodiplostomum* sp. in *L. catla* and *P. conchonius* was <1%, indicating a lower infection rate. This is the first report of *Posthodiplostomum* sp. infection in *P. conchonius* and from the Ganga River. It is also the first report of *Posthodiplostomum* sp. infection in *L. catla* in the Ganga River.

Key words: *Labeo catla*, *Pethia conchonius*, black spot disease, Ganga River.

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Young and juvenile cyprinid fish species constitute the largest susceptible group to being the second intermediate host of *Posthodiplostomum* (JALALI 1998; ROLBIECKI 2004; ONDRAČKOVÁ *et al*. 2004a; RAKAUSKAS & BLAŽEVIČIUS 2009; KARIMIAN *et al*. 2013). Clinically, the infection is identified by the appearance of black spots in different organs such as the fins, skin, muscles, gills, operculum, and buccal cavity of affected fish. Infected fish also show signs of emaciation, spinal deformation, and degenerative changes in musculature, hepatic and renal dystrophy, anorexia, retarded growth, and pathological changes in the blood and even succumb to death in severe cases (ROLBIECKI 2004). Histologically, pinhead-sized black spots in the affected tissues show perifocal fibrous nodules bounded by a ring of melanin and an infiltration of inflammatory cells (TEIXEIRA-DE MELLO & EGUREN 2008; FERGUSON *et al*. 2010; FLORES-LOPES & THOMAZ 2011; NEGREA *et al*. 2015).

Freshwater digeneans often show seasonality in their infection pattern, which is related to cercarial emergence when water temperature increases (CHUBB 1979). The peak cercarial release coincides with the

breeding period of cyprinid fish therefore ensuring a chance for maximum infection in fish and hence parasite distribution by fish dispersal, as well as the maintenance of high parasite abundance (VLADIMIROV 1960). Further, metacercarial infection of juvenile fish provides the best chance of parasite transmission to the final piscivorous host (FISCHER & KELSO 1988). The infection pattern of *Posthodiplostomum cuticola* varies by fish species and season (ONDRAČKOVÁ *et al*. 2004a, b; IADRENKINA 2014; NEGREA *et al*. 2015), location on musculocutaneous tissue (NEGREA *et al*. 2015), type and zones of a water body, and their physico-chemical parameters (ONDRAČKOVÁ *et al*. 2004a; IADRENKINA 2014). The spatial, seasonal, and age-related variations in the prevalence of the diplostomid digenean, *Posthodiplostomum-Ornithodiplostomum* clade in valenciid fish have been reported (KALOGIANNI *et al*. 2017).

The Ganga River is the largest river in India and the fifth largest in the world. It flows through multiple agro-climatic zones and is home to 143 fish species with cyprinid fish constituting the major catch (53.47%) (SARKAR *et al*. 2011). Reports on fish diseases in feral populations are sporadic and scanty compared to those in aquaculture systems. Therefore, metacercarial infection of *Posthodiplostomum* in the fish of the Ganga River has been investigated to develop the status of infection in rivers.

Materials and Methods

Study area and fish samples

Regular sampling surveys were conducted at four stations, viz., Farakka (24°47′38.478′′N, 87°55′26.413′′E), Jangipur (24°27′58.16′′N, 88°03′57.05′′E), Rejinagar (23°50′10.64′′N, 88°13′55.60′′E), and Balagarh (23°07′44.05′′N, 88°27′58.04′′E) (Fig. 1) in the lower stretch of the Ganga River, West Bengal, India. Fish samples were caught using gill nets (mesh 22-120 mm), cast nets (12-14 mm), and indigenous traps set along the shallow (depth: 0.5-2 m) and vegetated areas of the river and were identified following the keys described by JAYARAM (1999). The total length (TL) and weight (W) of the fish were measured to the nearest 1 mm with a digital calliper and 0.01 g on an electronic scale, respectively. The sampled fish were examined carefully for the presence of black spots that deviated from the natural color pattern of the fish. Live fish were euthanized with excess doses of clove oil before examination. All of the visibly in-

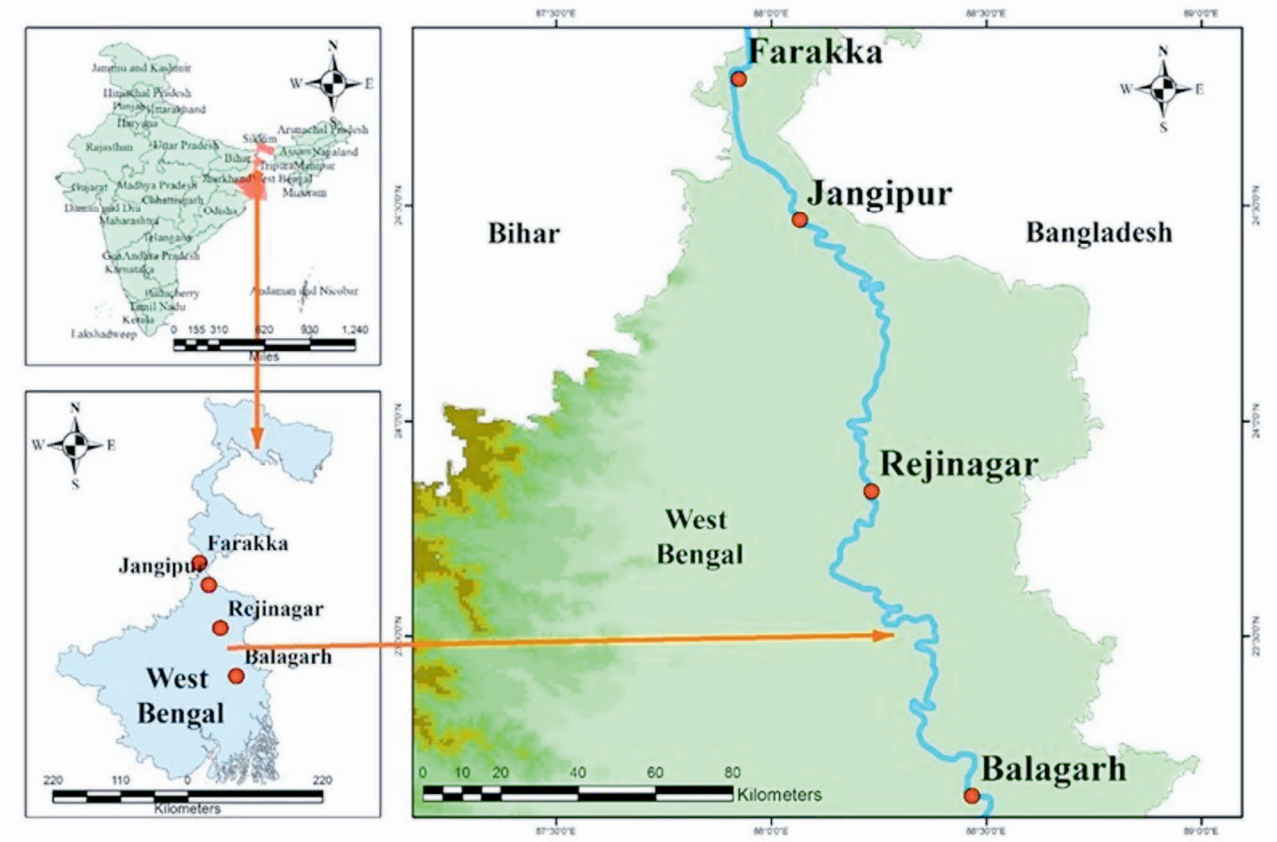

Fig. 1. Sampling sites along the lower stretch of the Ganga River.

fected and some of the non-infected fish samples were preserved in 10% neutral buffered formalin.

Parasitological examination

In the laboratory, infected fishes were examined thoroughly and the number of black spots in various body parts of individual fish was recorded. The encysted metacercariae were removed carefully using a pair of fine forceps from infected body parts, viz., the trunk muscles close to the head, the middle and caudal portions of a fish separately. Isolated cysts were washed vigorously in 0.85% physiological saline. Forty metacercarial cysts were picked randomly from the *Labeo catla* and *Pethia conchonius* specimens for morphometric characterization using a microscope equipped with a digital camera. Measurements (mm) of fresh cysts were taken with a calibrated eye-piece micrometer.

Histopathological analyses

The musculocutaneous tissues collected from infected fish were fixed in 10% neutral buffered formalin for histopathological examination. The fixed tissues were processed for histological examination using standard techniques, embedded in paraffin wax, sliced into 5 µm thick sections, mounted, and then stained with haematoxylin and eosin following standard protocol (PRESNELL & SCHREIBMAN 1997).

The ecological aspects of infection dynamics, viz., mean prevalence, mean abundance, mean intensity, and range of infection were estimated following MARGOLIS *et al*. (1982) and BUSH *et al*. (1997). The standard statistical computation was carried out using Microsoft Excel (Office 2016).

Results and Discussion

Morphometric characterization of the parasite

Posthodiplostomum cuticola and *Apophallus* sp. (HOOLE *et al*. 2001), *Uvulifer ambloplitis* and *Crassiphiala bulboglossa* (WOO 2006) have been known to infect the early stages of a wide range of fish species and causing black spot disease. *U. ambloplitis* and *C. bulboglossa* are mainly known from temperate inland waters in the Northern hemisphere (PAPERNA & DZIKOWSKI 2006), *Apophallus brevis* is limited to the continent of North America, infecting only Yellow perch, *Perca flavescens* (SINCLAIR 1972), and *P. cuticola* is globally distributed (DÖNGES 1964; ONDRAČKOVÁ *et al*. 2004b; ZRNČIĆ *et al*. 2009; NGUYEN *et al*. 2012; KARIMIAN *et al*. 2013; ATHOKPAM & TANDON 2014).

Five thousand eight hundred and twenty (5,820) fish samples belonging to 30 families (Cyprinidae, Channidae, Schilbeidae, Bagridae, Ambassidae, Osphronemidae, Cobitidae, Heteropneustidae, Anabantidae, Gobiidae, Mastacembelidae, Sciaenidae, Engraulidae, Tetraodontidae, Siluridae, Clariidae, Belonidae, Mugilidae, Sisoridae, Nandidae, Notopteridae, Pangasidae, Synbranchidae, Chacidae, Badidae, Cynoglossidae, Soleidae, Clupeidae, Ailiidae, and Anguilidae) were examined from the studied stations in the lower stretch of the Ganga River. Among the several genera and species of the fish studied from the river, only two cyprinid species, namely *L. catla* and *P. conchonius,* were found to be parasitized by metacercariae of *Posthodiplostomum* sp. (Fig. 2a, 2b). Out of nearly six thousand fish samples examined, only sixteen (16) fish were found to be infected, in which the number of cysts varied widely, from 1-87 cysts, in each infected fish. The infected *L. catla* samples

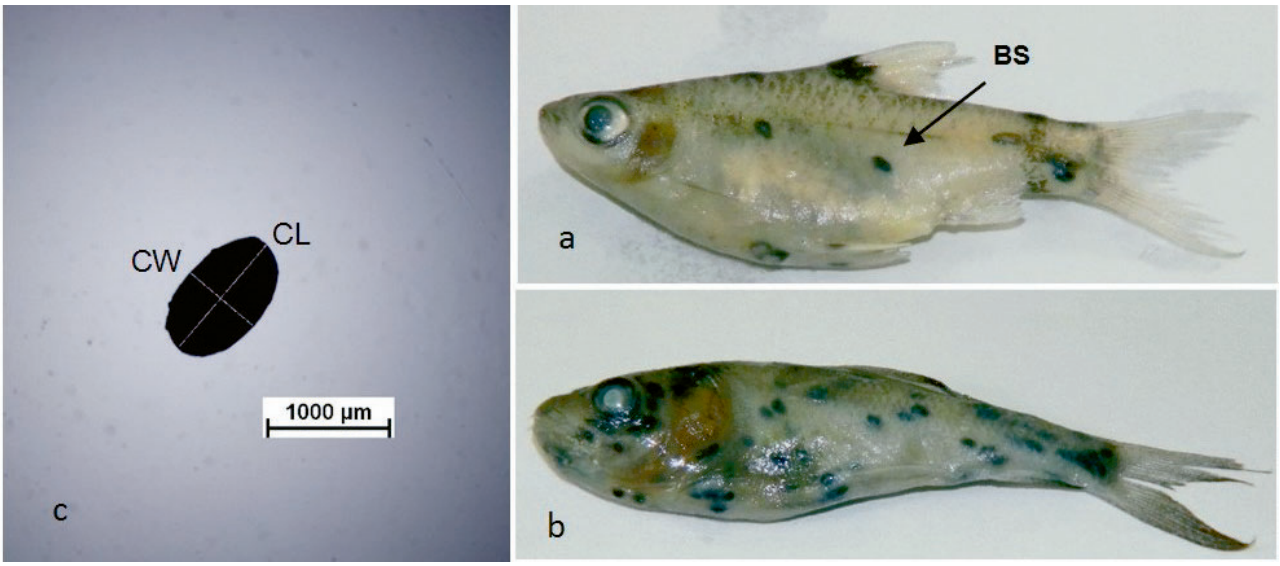

Fig. 2. Black spot disease in cyprinid fish from the lower stretch of the Ganga River: a – *P. conchonius*, b – *L. catla*., c – cyst, BS – black spot; CL – cyst length; CW – cyst width.

were juveniles with a mean length of 56.66 mm and mean weight of 2.52 g, while the mean length and weight of infected *P. conchonius* samples was 36.23 mm and 0.82 g, respectively.

In the present study, the metacercarial cysts were oval-round in shape (Fig. 2c) and 0.82-1.13 (1.02±0.02) by 0.63-1.05 (0.79±0.02) mm and 0.87-1.16 (1.02±0.02) by 0.6-0.91 (0.75±0.02) mm for *L. catla* and *P. conchonius,* respectively. For differential diagnosis, the morphometric comparison of the metacercariae of major *Posthodiplostomum* spp. that infect freshwater fish are presented in Table 1. A comparative description of the cyst dimensions of *Posthodiplostomum* sp. with morphologically similar parasitic species responsible for black spot disease in freshwater fish have also been tabulated (Table 2). The shape and dimensions of the cysts in the present investigation clearly indicated that the causative agent of black spot disease in *P. conchonius* and *L. catla* belonged to the genus *Posthodiplostomum* with a close resemblance to *P. cuticola*. Variations in the dimension of cysts may be correlated with the stage of development of the encysted digenetic trematodes as evidenced in case of *C. bulboglossa* (HOFFMAN 1956). However, similar studies on other digenetic trematodes are lacking.

Histopathologic characterization of the parasite

The metacercariae-infected musculocutaneous tissues of *L. catla* and *P. conchonius* showed a proliferation of melanocytes just beneath the epidermis and a melanization of the fibrous capsule surrounding the metacercariae (Figs 3a-3c) which were visible macroscopically as multifocal hyperpigmented areas (Fig. 2a) along with slightly raised nodular structures, seen in the case of *L. catla* (Fig. 2b). The surrounding muscle tissue suffered from medium to high levels of pressure atrophy, and marked degeneration as well as focal necrosis (Figs 3a-3c). An infiltration of numerous immune cells (mononuclear leukocytes) (Fig. 3d) could be observed in the fibrous capsule encircling the parasite. A melanization in the fibrous capsule surrounding the parasite could be seen (Figs 3a-3c*).* Because of the thick capsule, the thick-walled metacercariae of digenetic trematodes are able to evade host immune responses (GRACZYK 1997). The metacercarial cyst of *A. brevis* also has a bony component (SINCLAIR 1972) and a distinctive spiny tegument unique to *Apophallus* spp. (KENT *et al*. 2004). Further, encysted metacercariae of *Apophallus* sp. (Heterophyidae Leiper, 1909) can be distinguished from other black spot forming digenetic trematodes by their cyst bi-layer (a thin inner layer of parasitic origin and thick outer layer of host origin), fibrous outer layer, and occasional calcification (GIBSON 1996). This substantiates that the histological changes observed in the present study were not due to the metacercariae of heterophyid digenetic trematodes, mainly becaue the melanized capsule around the cyst was thinner. *Crassiphiala bulboglossa* (Crassiphialinae Sudarikov, 1960) has a loosely fitted, spacious and thinner cyst wall, mainly infects skin, and is occasionally found between myotomes and on gill arches (GIBSON 1996). The present study has recorded the infection in musculocutaneous tissue, but not on the gill arches of *P. conchonius* and *L. catla.* Metacercariae of *Uvulife*r Yamaguti, 1934 are fitted relatively

Table 1

Morphometric comparison of *Posthodiplostomum* spp. metacercarial cyst dimensions from freshwater fish

Host species	Parasitic species	Site of infection	Shape	Cyst dimensions (mm)	Reference
L. catla	*Posthodiplostomum* sp.	Muscle	oval-round	0.82-1.13 (1.02±0.02) by 0.63-1.05 (0.79±0.02)	Present study
P. conchonius	*Posthodiplostomum* sp.	Muscle	oval-round	0.87-1.16 (1.02±0.02) by 0.6-0.91 (0.75±0.02)	Present study
Channa punctata	*Posthodiplostomum* sp.	Muscle	oval-round	0.64-0.67 (0.65±0.01) by 0.53-0.55 (0.51±0.06)	ATHOKPAM & TANDON 2014
Channa argus	*Posthodiplostomum* sp.	Trunk muscle	oval-round	0.79-1.40 (1.04±0.18) by 0.54-0.94 (0.69±0.14)	NGUYEN *et al*. 2012
Ctenopharyngodon idella	*P. cuticola*	Skin, fins, oral cavity	round	0.900-0.990 (0.946±0.025)	MAJA *et al.* 2012
Aristichthys nobilis	*P. cuticola*	Skin, fins, oral cavity	round	0.900-0.980 (0.944±0.023)	MAJA *et al.* 2012
Carassius gibelio	*P. cuticola*	Skin, Musculature	spherical-oval	0.734-0.918 by 0.286-0.306	SUKHEROVA *et al*. 2005
Phoxinus laevis	*P. cuticola*	Muscle, skin, fins	oval-round	0.80-0.96 (0.89±0.05) by 0.69-0.88 (0.81±0.05)	DÖNGES 1964 (cited in NGUYEN *et al.* 2012)

Table 2

Comparative description of the metacercarial cyst dimensions of *Posthodiplostomum* species with morphologically similar parasitic species responsible for black spot disease in freshwater fish

Causative agent	Host/family	Site of infection	Cyst dimensions (mm)	Reference
Posthodiplostomum sp.	*L. catla*	Muscle	0.82-1.13 (1.02±0.02) by 0.63-1.05 (0.79±0.02)	Present study
Posthodiplostomum sp.	*P. conchonius*	Muscle	0.87-1.16 (1.02±0.02) by 0.6-0.91 (0.75±0.02)	Present study
P. cuticola	*Phoxinus laevis*	Muscle, skin, fins	0.80-0.96 (0.89±0.05) by 0.69-0.88 (0.81±0.05)	DÖNGES 1964
P. cuticola	Cyprinidae	Skin	0.69-0.99 (Dia.)	HOOLE *et al.* 2001
Apophallus sp.	Cyprinidae	Skin	0.14-0.28 (Dia.)	HOOLE *et al.* 2001
U. ambloplitis	Pike, Minnows, Small-mouth bass, Bluegill, Sunfish, Western mosquitofish, and Bluegill sunfish	Muscle	0.377 by 0.2	HICKNER 2011
C. bulboglossa	Ecosidae, Cyprinidae, Cyprinodontidae, Etheostomidae, Percidae, and Umbridae	Muscle	0.31-0.4 by 0.18-0.215	HOFFMAN 1956

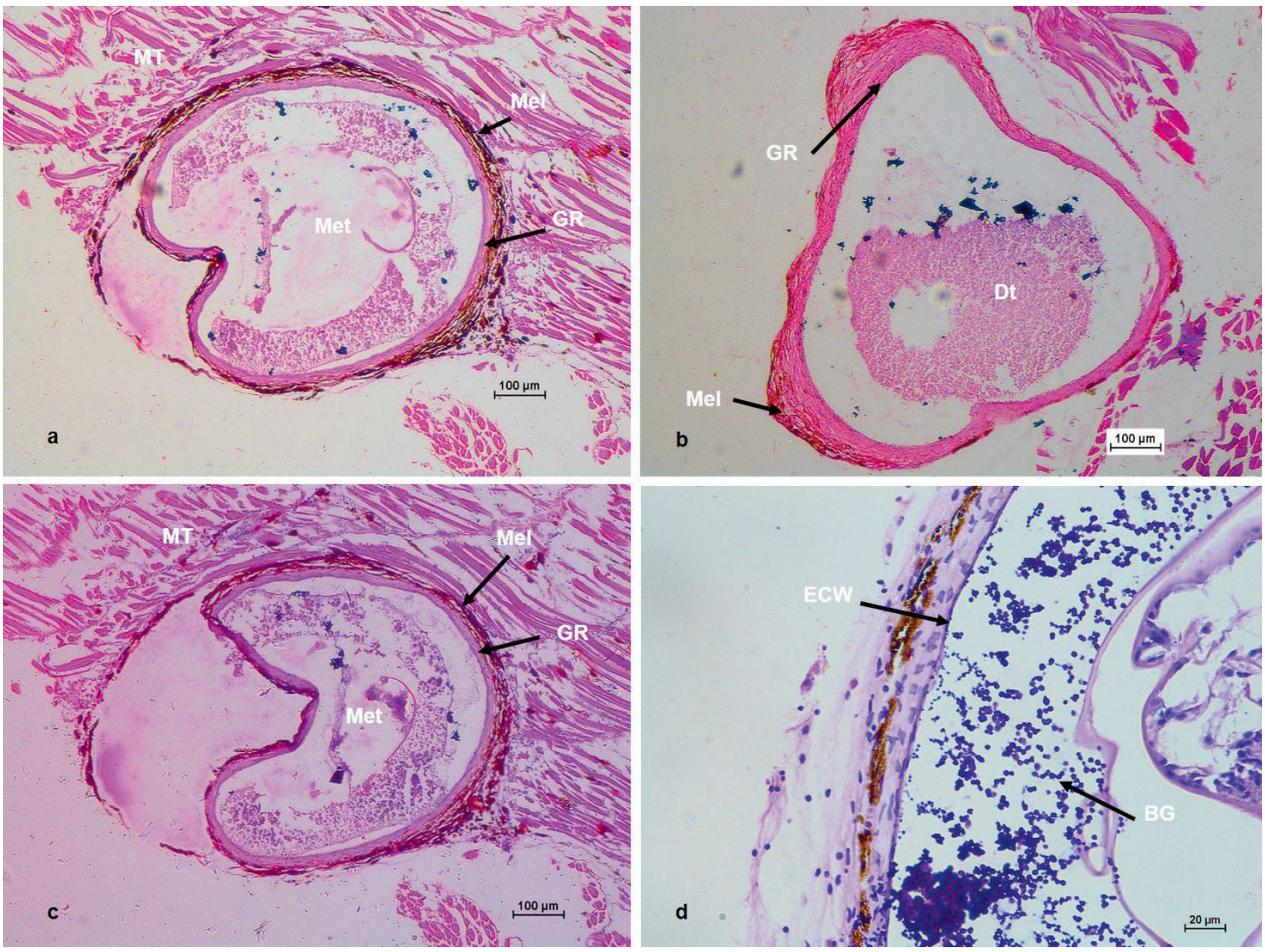

Fig. 3. Light micrographs of the metacercariae of *Posthodiplostomum* sp. in the musculocutaneous tissue: a-b – *P. conchonius* and c-d – *L. catla* fingerling, MT – musculocutaneous tissue, Mel – melanocytes, BG – basophilic granula, ECW – eosinophilic cyst wall; Met – metacercariae; GR – granulomatous reaction; Dt – degenerating metacercariae in distorted section. H&E staining.

tightly in cysts and the cysts are oval-pyriform and infect the fins, skin, and muscles of host fish. Thus, based on the location of the cysts on the body, the characteristics and histopathological lesions, the metacercariae infecting *P. conchonius* and *L. catla* could be *Posthodiplostomum* sp.

Infection pattern

Among fish species belonging to 30 families investigated in the present study, the occurrence of metacercarial *Posthodiplostomum* sp. was observed only in juveniles of *P. conchonius* and *L. catla*. The cysts of *Posthodiplostomum* sp. infected the epidermal layer of the musculocutaneous tissue. The present study is well supported by earlier observations that young and juvenile cyprinid fish species constitute the largest susceptible group to being the second intermediate host *of Posthodiplostomum* sp. (ROLBIECKI 2004; ONDRAČKOVÁ *et al*. 2004a; RAKAUSKAS & BLAŽEVIČIUS 2009; ZRNČIĆ *et al*. 2009; KARIMIAN *et al*. 2013).

The sampling period and station-wise prevalence, mean intensity, mean abundance, and range of infection are given in Table 3, and the same have been calculated at species level within family (Table 4). Table 3

Table 3

Sampling period and station-wise infection pattern of *Posthodiplostomum* sp. in the lower stretch of the Ganga River

Fish species	Items	Sampling periods						Sampling stations			
		Aug-16	Oct-16	Dec-16	Feb-17	Apr-17	Jun-17	Farakka	Jangipur	Rejinagar	Balagarh
L. catla	**N**	**1455**	**902**	**650**	**960**	**730**	**1123**	**2530**	**695**	**1150**	**1445**
	n	–	3	–	–	–	–	–	–	–	3
	Q	–	214	–	–	–	–	–	–	–	214
	P	–	0.33	–	–	–	–	–	–	–	0.2
	MI	–	71.33	–	–	–	–	–	–	–	71.33
	MA	–	0.23	–	–	–	–	–	–	–	0.14
	R	–	62-87	–	–	–	–	–	–	–	62-87
P. conchonius	**N**	**1455**	**902**	**650**	**960**	**730**	**1123**	**2530**	**695**	**1150**	**1445**
	n	13	–	–	–	–	–	13	–	–	–
	Q	67	–	–	–	–	–	67	–	–	–
	P	0.89	–	–	–	–	–	0.51	–	–	–
	MI	5.15	–	–	–	–	–	5.15	–	–	–
	MA	0.04	–	–	–	–	–	0.02	–	–	–
	R	1-10	–	–	–	–	–	1-10	–	–	–

Number of fish examined at each station and sampling period (N), number of infected fish specimens (n), number of metacercariae recovered (Q), prevalence (P), mean intensity (MI), mean abundance (MA), and range of infection (R)

Table 4

Intra-familial infection pattern of *Posthodiplostomum* sp. in the lower stretch of the Ganga River

Parasite	Samples	Fish species							
		Labeo rohita	*Labeo calbasu*	*Labeo catla*	*Cirrhinus mrigala*	*Puntius ticto*	*Puntius sarana*	*Puntius sophore*	*Pethia conchonius*
	N	**93**	**170**	**66**	**55**	**759**	**285**	**1955**	**554**
Posthodiplostomum sp.	n	–	–	3	–	–	–	–	13
	Q	–	–	214	–	–	–	–	67
	P	–	–	4.54	–	–	–	–	2.3
	MI	–	–	71.33	–	–	–	–	5.15
	MA	–	–	3.24	–	–	–	–	0.12
	R	–	–	62-67	–	–	–	–	1-10

Number of fish examined at each station and sampling period (N), number of infected fish specimens (n), number of metacercariae recovered (Q), prevalence (P), mean intensity (MI), mean abundance (MA), and range of infection (R).

shows that *P. conchonius* at Farakka and *L. catla* at Balagarh were infected with the metacercarial stage of *Posthodiplostomum* sp. in August and October, respectively. Table 4 shows that *P. conchonius* and *L. catla* were the only species infected with the metacercarial stage of *Posthodiplostomum* sp. from among various cyprinid fish species. *Labeo catla* was infected more heavily than *P. conchonius*. The overall and intra-familial level of prevalence, mean intensity, mean abundance, and range of infection were higher for *L. catla* than *P. conchonius.* The period of August and October is monsoon and post-monsoon season, respectively on the east coast of India, which are also the periods of fish breeding and high foraging activity of younger fishes in shallow, vegetated areas of overflown river. This period also coincides with the breeding of aquatic snails and the aggregation of piscivorous birds in the same locality. The presence of all the intermediate and final host species of *Posthodiplostomum* favor completion of its life cycle (ONDRAČKOVÁ *et al*. 2004; TEIXEIRA-DE MELLO & EGUREN 2008; ZRNČIĆ *et al*. 2009; FLORES-LOPES & THOMAZ 2011).

Conclusion

Posthodiplostomum sp. (Strigeidida: Diplostomidae) infection was identified based on the clinical signs, comparative cyst morphometry, and the characteristic histopathological changes in the fish host. This is the first report of infection by the metacercariae of *Posthodiplostomum* sp. (Strigeidida: Diplostomidae) in *P. conchonius* and in the Ganga River. It is also the first report of infection in *L. catla* in the Ganga River. However, a less than 1% prevalence of *Posthodiplostomum* sp. in *L. catla* and *P.conchonius,* indicates a low infection rate.

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Author Contributions

Research concept and design: R.B., S.K.M., A.K.B., K.P.P., S.V.R., B.K.D.; Collection and/or assembly of data: R.B., S.K.K., N.D.; Data analysis and interpretation: R.B., S.K.M., S.K.K., N.D., A.K.B.; Writing the article: R.B.; Critical revision of the article: R.B., S.K.M., S.K.K., A.K.B., K.P.P., S.V.R., B.K.D.; Final approval of article: B.K.D.

Conflict of Interest

The authors declare no conflict of interest.

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