

## Occurrence of two *Tylodelphys* species (Trematoda: Diplostomidae) from the vitreous humour in Indian freshwater fish

Anshu Chaudhary✉, Shivi Gupta, Chandni Verma, Komal, Bindu Sharma, Hridaya Shanker Singh

Department of Zoology, Chaudhary Charan Singh University, Meerut 250004, India.

Correspondence: E-mail anshu8282@rediffmail.com

**Abstract.** Species of the genus *Tylodelphys* Diesing, 1850 are parasites of the cranial cavity of the brain and eyes of freshwater fishes with worldwide distribution. They are important pathogens of wild and cultured freshwater fishes. Recent investigations (from North America, Europe, Africa and a few from Asia and Australasia) have focused on metacercariae stages from piscine hosts. In the present study, two newly recorded metacercaria of *Tylodelphys* (metacercaria) were found in the hybrids of carp *Cirrhinus mrigala* × *Labeo rohita* from Meerut, Uttar Pradesh (U.P.), India. Both the species were distinguished from its congeners, *T. cerebralis* Chakrabarti, 1968; *T. chandrapali* Jain and Gupta, 1970; *T. darteri* Mehra, 1962; *T. duboisilla* Mehra, 1962; *T. excavata spinnata* Gupta, 1962; *T. rauschi* Singh, 1956; *Tylodelphys* sp. 1 and 2 from *Mystus vittatus*, *Tylodelphys* sp. CG from *Channa gachua* and *Tylodelphys* sp. PS1 and PS2 from *Puntius sophore* respectively, and from species described earlier under genus *Diplostomulum* Hughes, 1929 from India. *Tylodelphys* species were morphologically identified based on the light microscopy, SEM observations, compared with previously published data. The adults are not discovered yet, so we described them as distinct species of *Tylodelphys* and named as, *Tylodelphys* sp. IND/CL/VR-1 and *Tylodelphys* sp. IND/CL/VR-2. Furthermore, we have also briefly discussed the diversity of *Tylodelphys* species in India and their validity that is necessary to our knowledge regarding the taxonomy of larval diplostomids in fish.

**Keywords:** *Tylodelphys*; Metacercariae; Trematodes; Eye; Meerut; India.

**Apariția a două specii *Tylodelphys* (Trematoda: Diplostomidae) din umoarea apoasă la peștii de apă dulce din India**

**Rezumat.** Speciile din genul *Tylodelphys* Diesing, 1850 sunt paraziți ai cavității craniene și ochilor peștilor de apă dulce, cu distribuție mondială. Sunt agenți patogeni importanți ai peștilor sălbatici și de cultură de apă dulce. Investigațiile recente (din America de Nord, Europa, Africa și câteva din Asia și Australasia) s-au concentrat asupra etapelor metacercariene de la gazdele pești. În prezentul studiu, doi metacercari de *Tylodelphys* înregistrați de curând au fost găsiți în hibridii de crap *Cirrhinus mrigala* × *Labeo rohita* din Meerut, Uttar Pradesh (U.P.), India.

Ambele specii s-au distins de congenera, *T. cerebralis* Chakrabarti, 1968; *T. chandrapali* Jain și Gupta, 1970; *T. darteri* Mehra, 1962; *T. duboisilla* Mehra, 1962; *T. excavata spinnata* Gupta, 1962; *T. rauschi* Singh, 1956; *Tylodelphys* sp. 1 și 2 din *Mystus vittatus*, *Tylodelphys* sp. CG de la *Channa gachua* și *Tylodelphys* sp. PS1 și respectiv PS2 de la *Puntius sophore* și, respectiv, de specii descrise mai devreme în genul *Diplostomulum* Hughes, 1929 din India. Speciile *Tylodelphys* au fost identificate morfologic pe baza microscopiei optice, a observațiilor SEM, comparativ cu datele publicate anterior. Adulții nu sunt încă descoperiți, astfel încât i-am descris ca specii distincte de *Tylodelphys* și numite ca, *Tylodelphys* sp. IND/CL/VR-1 și *Tylodelphys* sp. IND/CL/VR-2. Mai mult decât atât, s-a discutat pe scurt diversitatea speciilor *Tylodelphys* în India și validitatea lor, care este necesară pentru cunoașterea referitoare la taxonomia diplostomidelor larvare la pești.

**Cuvinte cheie:** *Tylodelphys*; Metacercariae; Trematode; Ochi; Meerut; India.

Received 24.07.2020. Accepted 02.12.2020.

## Introduction

*Tylodelphys* Diesing, 1850 is a genus that widely distributed in freshwater systems (Chibwana et al., 2015). These helminthes have a complex life cycle, including lymnaeid snails and fish as intermediate hosts while the piscivorous birds are definitive hosts (Blasco-Costa et al., 2017). The diplostomid larval stage (metacercariae) is the important and frequently encountered pathogen that found unencysted in the cranial cavity of the brain and eyes of freshwater fishes (Blasco-Costa et al., 2014; Otachi et al., 2015; Chibwana et al., 2015; García-Varela et al., 2016; Blasco-Costa et al., 2017; Sereno-Uribe et al., 2018; Presswell and Blasco-Costa, 2020). Metacercaria stage of *Tylodelphys* sp. may exert serious impacts on wild and cultured fish populations, causing cataracts and disruption of the brain tissue that finally leads to the mortality in fish host (Chappell, 1995; Sandland and Goater, 2000).

There are many species of *Tylodelphys* (= *Diplostomum*) metacercaria, described within India under genus *Diplostomulum* Hughes, 1929. Some of them have already been synonymized with *Tylodelphys* by earlier workers (Blasco-Costa et al., 2014) and many are still questioned for their validity and need to be re-described (Blasco-Costa et al., 2014; Pandey and Agrawal, 2013; Chaudhary et al., 2017a). Moreover, as far as data on the metacercarial species of diplostomids from

India is concerned, it is still obscure due to limited studies on their taxonomy and poor morphological data. Many Indian species of diplostomids described in the past on the basis of adults from birds are well described but in case of metacercaria no proper morphological descriptions are available, some lacking informative illustrations, no type specimens deposited to Museums, so hard to compare them with the new material and mostly described in local journals without thorough peer-review of the manuscript (Chakrabarti, 1968; Pandey, 1970; Sudarikov, 1974; Pandey and Tewari, 1984; Jain and Gupta, 1970; Gupta, 1962, Mehra, 1962a, Dubois, 1964; Mehra, 1962b; Yamaguti, 1971; Singh, 1956; Odening, 1970; Pandey and Agrawal, 2013).

Economically important major carps of India includes: rohu (*Labeo rohita*), catla (*Catla catla*) and mrigal (*Cirrhinus mrigala*). Hybrids of major carp's results due to mixed seed in the spawning pools of carp hatcheries. Investigators with the aim to look up the proper identification of piscine diplostomids in the Indian region started the present investigation. An attempt have also been made to provide a list of fish diplostomids in India reported previously. During the course of study here, two *Tylodelphys* sp. metacercaria i.e., *Tylodelphys* sp. IND/CL/VR-1 and *Tylodelphys* sp. IND/CL/VR-2 found in India is described after comparing them morphologically with their previously reported congeners.

## Materials and methods

A total of 32 hybrids of carp *Cirrhinus mrigala* × *Labeo rohita* (Cypriniformes: Cyprinidae) were collected from the local fish market of Meerut (29°01' N, 77°45' E), Uttar Pradesh, India during the months from April to July 2016. Fish were kept alive and transported to the laboratory and identified with the help of Ichthyologists. They were then euthanized by clove oil, sacrificed, immediately examined and unencysted metacercariae were collected from the vitreous humour of the eyes. Collected larval trematodes were fixed in 4% formalin for light morphology and scanning electron microscopic (SEM) study. For light microscopy, unflatten worms were stained using acetocarmine, dehydrated through ascending grades of ethanol series, cleared in xylene and mounted in Canada balsam.

All the specimens were examined using an Olympus microscope (CH30) and measurements were taken using the microscope software. All the measurements are in micrometers unless otherwise stated with the range followed by the mean in parenthesis. Drawings were made with the help of a drawing tube attached to the microscope. For SEM, parasites were dehydrated through a graded ethanol series and then dried using critical point-drier (K850 Quorum, U.K.). The specimens were mounted on metal stubs, coated with a thin layer of gold (2nm) and examined with a JOEL Neoscope JCM5000 SEM at an accelerating voltage of 10 kV. Voucher specimens were deposited in the Museum of the Department of Zoology, Chaudhary Charan Singh University, U.P., India and the Museum d'Histoire Naturelle (MHNG), Geneva, Switzerland.

## Results

*Family Diplostomidae Poirier, 1886*  
*Genus Tylodelphys Diesing, 1850*

- *Tylodelphys* sp. IND/CL/VR-1 (Metacercaria) (figures 1A and 2A-2C)

**Host.** Hybrid of *Cirrhinus mrigala* × *Labeo rohita* (Cypriniformes: Cyprinidae). Locality. Meerut, Uttar Pradesh, India.

**Site of infection and Prevalence.** The vitreous humor of eye, 72% (23 infected out of 32 hosts; 65 parasites were found from eye of 32 infected hosts).

**Material deposited.** The holotype slides were deposited in the Museum d'Histoire Naturelle, Geneva, Switzerland (MHNG-PLAT-94127) and paratype in the Museum, Department of Zoology, Chaudhary Charan Singh University, U.P., India under the voucher number HS-TR/2016/09.

**Description** (based on measurements of sixteen stained specimens and two specimens from SEM study). Metacercariae were found unencysted in the vitreous humor of the hybrid fish host eye. The metacercaria appeared to be elongated (figures 1A and 2A-2C). Body pyriform and distinct into large fore body and small hind body, filled with a large amount of excretory corpuscles in the reserve excretory system, comprises an irregular ridge, ventrally concave, 860-948 (904) long, 160-204 (182) wide (figures 1A and 2A-2C). Oral sucker oval in outline, 51-77 (64) long, 46-70 (58) wide. At the level of oral sucker borders, two bilaterally situated well-developed pseudosuckers present, each measuring, 31-47 (39) long, 52-64 (58) wide. Ventral sucker situated above holdfast organ measures 47-59 (53) long, 32-50 (41) wide. Prepharynx not observed and pharynx shape elongate oval extends into oesophagus. Intestine bifurcated into two elongated intestinal caeca, terminate posteriorly at about half the length of holdfast organ. Holdfast organ muscular, oval, elongated, situated between ventral sucker and posterior end of body measuring, 88-126 (107) long, 32-50 (41) wide. Hindbody, 78-118 (98) of metacercaria, small and slightly pointed, smooth surface with excretory pore present below holdfast organ. Excretory system was not observed due to the abundant amount of excretory corpuscles. Distance between oral sucker-ventral sucker, 372-454 (413), and ventral sucker-holdfast organ 82-112 (97). Tegument around the oral sucker was smooth without non-ciliated papillae (figures 2A-2C). Hindbody tegument was relatively smooth with non-ciliated papillae as compared to forebody that has unevenly ridged (figures 2A-2C). Reproductive system poorly developed.

- *Tylodelphys* sp. IND/CL/VR-2 (Metacercaria) (figures 1B and 2D-2G)

**Host.** Hybrid of *Cirrhinus mrigala* × *Labeo rohita* (Cypriniformes: Cyprinidae).

**Locality.** Meerut, Uttar Pradesh, India.

**Site of infection and Prevalence.** The vitreous humor of eye, 56.25% (18 infected out of 32 hosts; 59 parasites were found from eye from 32 infected hosts).

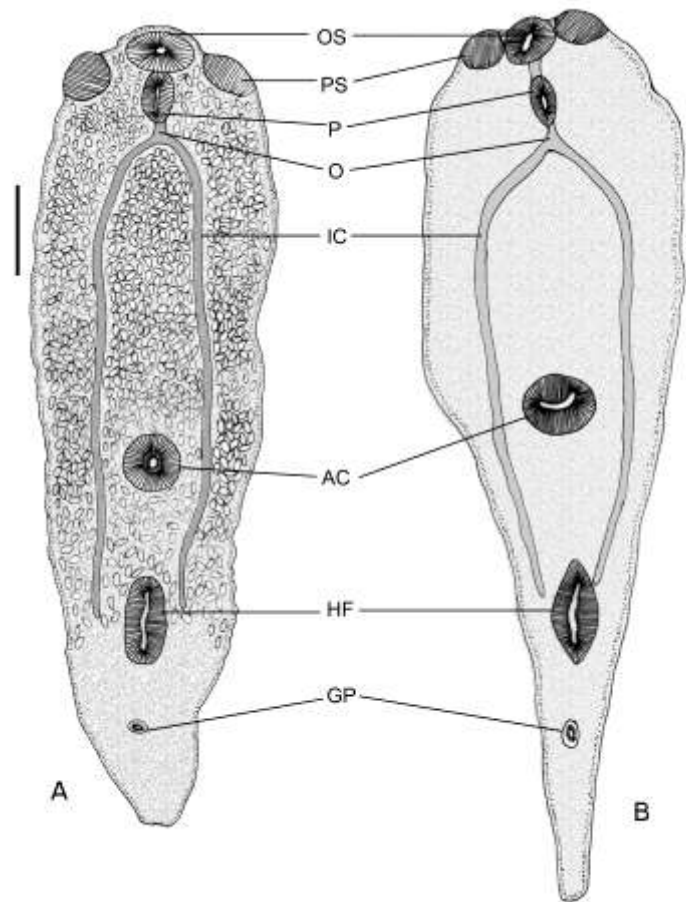
**Material deposited.** The slides were deposited in the Museum, Department of Zoology, Chaudhary Charan Singh University, U.P., India under the voucher number HS-TR/2016/10 and in the Museum d'Histoire Naturelle, Geneva, Switzerland (MHNG-PLAT-94128).

**Description** (Based on measurements of fourteen stained and mounted specimens and two specimens for SEM). Metacercariae were found unencysted in the vitreous humor of eye of hybrid host (*Cirrhinus mrigala* × *Labeo rohita*). Larvae are with an obovoid body anteriorly and pointed posteriorly. Body measures 639-727 (683) long, 137-181 (159) wide. Body tegument devoid of spines, but papillae present (figures 1B and 2D-2G). Hindbody from mid of the body reduce to pointing tail like. Body comprised of a well-developed oral sucker, almost rounded, 10-36 (23) long, 11-35 (23) wide. Lateral pseudosuckers present, flanked oral sucker, 24-32 (28) long, 25-31 (28) wide. Ventral sucker muscular, present almost the middle of the body, 38-50 (44) long, 31-49 (40) wide. Prepharynx absent, pharynx oval, muscular, leads to oesophagus. Intestine bifurcated into two elongated intestinal caeca reaching up to level of anterior part of a holdfast organ (figures 1B and 2D-2G). Holdfast organ broad, present between ventral sucker and excretory pore, situated along two-thirds of the body length, 65-87 (76) long, 21-35 (28) wide. Hindbody pointed and long measuring 94-134 (114). Distance between oral sucker-ventral sucker, 203-245 (224), ventral sucker-holdfast organ, 81-109 (95) (figures 1B and 2D-2G). Excretory pore presents at the posterior end of the hindbody Reproductive system poorly developed.

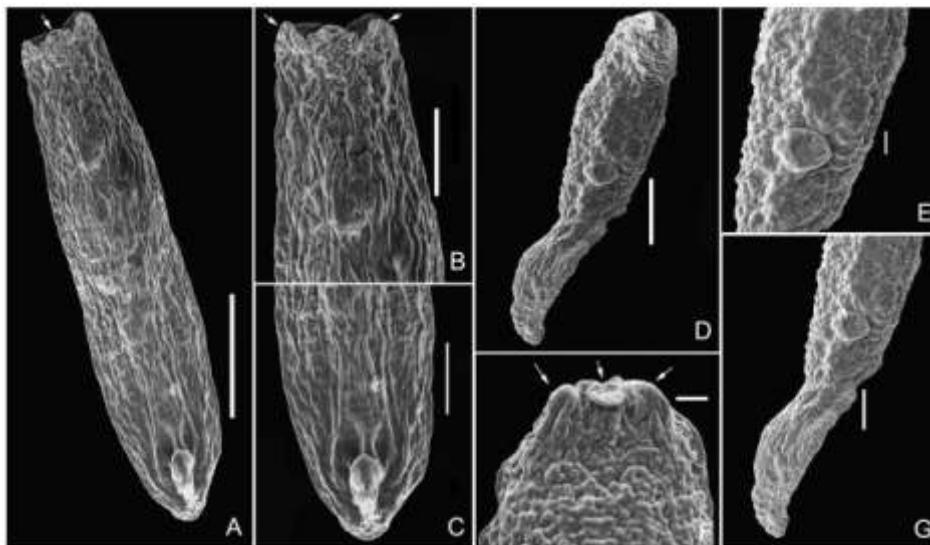
**Remarks.** The metacercaria of two newly recorded *Tylodelphys* metacercaria species, parasitize the vitreous humour of hybrid fish (*Cirrhinus mrigala* × *Labeo rohita*) showed a clear difference in the morphology and morphological data. In India, previously few *Tylodelphys* species were described mainly during 1950s to 1980s under the genus *Diplostomulum* Hughes, 1929 (table 1). The earlier authors possibly ignored the genus *Tylodelphys* Diesing, 1850 though this genus has already been established before *Diplostomulum* Hughes, 1929. During that period, the *Tylodelphys* species were misinterpreted/incorrectly described under the *Diplostomulum* instead of *Tylodelphys*.

The above described two novel metacercaria *Tylodelphys* sp. IND/CL/VR-1 and *Tylodelphys* sp. IND/CL/VR-2 morphologically resemble with *Tylodelphys* species described earlier of *Tylodelphys* (= *Diplostomulum*) mostly based on metacercaria and few with adult stages (table 1). The species, *T. cerebralis*, an unknown *Diplostomulum* sp. by Rai and Pande (1969), *Tylodelphys* sp. CG, *Tylodelphys* sp. PS1, *Tylodelphys* sp. PS2 and *Tylodelphys* sp.1 populate the eye of the fish host (table 1). *Tylodelphys* sp. IND/CL/VR-1 and *Tylodelphys* sp. IND/CL/VR-2 both shows variations with an unknown *Diplostomulum* sp. by Rai and Pande (1969) as it is smaller in all metrics with the present species. Besides this, *Tylodelphys* sp. IND/CL/VR-1 is larger in body size from other reported species i.e., *Tylodelphys* sp. CG, *Tylodelphys* sp. PS1, *Tylodelphys* sp. PS2, *Tylodelphys* sp.1, *Tylodelphys* sp. 2 (table 2).

*Tylodelphys* sp. IND/CL/VR-1 can be further differentiated from the above five species by body shape as specimens at the author's disposal gradually much convex from midbody to the posterior part in comparison to others (table 2). Although *Tylodelphys* sp. IND/CL/VR-1 is morphologically close to *T. cerebralis* but differ in the distance between holdfast organ and ventral sucker that is greater in *Tylodelphys* sp. IND/CL/VR-1 as compared to *T. cerebralis*. Pseudosuckers are much developed and body is more tapered in *Tylodelphys* sp. IND/CL/VR-1 as compared to *T. cerebralis* (table 2).



**Figure 1.** Morphology of (A) *Tyloodelphys* sp. IND/CL/VR-1. (B) *Tyloodelphys* sp. IND/CL/VR-2. Scale bars (A, B) 100 µm. OS, oral sucker; PS, pseudosucker; P, pharynx; O, oesophagus; IC, intestinal caeca; AC, acetabulum; HF, holdfast organ; GP, genital primordium



**Figure 2.** Scanning electron microscopic (SEM) views of the *Tyloodelphys* species. (A) Whole body of *Tyloodelphys* sp. IND/CL/VR-1 with highlight to the oral sucker. (B) Anterior portion bearing the pseudosuckers (highlighted) and oral sucker of *Tyloodelphys* sp. IND/CL/VR-1. (C) Posterior end of *Tyloodelphys* sp. IND/CL/VR-1. (D) Whole body of *Tyloodelphys* sp. IND/CL/VR-2. (E) Showing an enlarged view of acetabulum of *Tyloodelphys* sp. IND/CL/VR-2. (F) Showing oral sucker and pseudosuckers (highlighted) of *Tyloodelphys* sp. IND/CL/VR-2. (G) Showing posterior portion having tail of *Tyloodelphys* sp. IND/CL/VR-2 with the opening of the holdfast organ. Scale bars (A, D) 100 µm; (B, C, G) 50 µm; (E, F) 20 µm.

**Table 1.** List of Tyloodelphys species described from India under the genus Diplostomulum. M, metacercaria; A, adult

Species	Host	Developmental stage	Infected organ	Reference
<i>Diplostomulum</i> sp.	<i>Catla catla</i>	M	Fins, muscles	Ganapati and Rao, 1955
<i>D. pigmentata</i>	<i>Catla catla</i> , <i>Labeo rohita</i> , <i>Cirrhinus mrigala</i>	M	Scales, base of caudal fin	Singh, 1956
<i>T. Chandrapali</i> syn. <i>Tyloodelphis onkari</i> Jain and Gupta, 1970	<i>Anastomus oscitans</i>	A	Intestine	Jain and Gupta, 1970
<i>T. darteri</i> syn. <i>T. indiana</i> Gupta, 1962; <i>T. indica</i> Mehra, 1962	<i>Anhinga melanogaster</i>	A	Intestine	Mehra, 1962a
<i>T. duboisilla</i> syn. <i>Glossodiplostomum duboisilla</i> Mehra, 1962	<i>Anhinga melanogaster</i>	A	Intestine	Mehra, 1962b
<i>T. excavata spinnata</i> (Gupta, 1962) syn. <i>T. spinnata</i> Gupta, 1962	<i>Anastomus oscitans</i>	A	Intestine	Gupta, 1962
<i>T. rauschi</i> (Singh, 1956) syn. <i>Diplostomum rauschi</i> Singh, 1956	<i>Dissoura episcopus episcopus</i>	A	Intestine	Singh, 1956
<i>D. singhi</i>	<i>Heteropneustes fossilis</i>	M	Cyst of intestine and body cavity	Pande et al., 1964
<i>D. nurius</i>	<i>Fries of Nuria daurica</i>	M	Body cavity	Thapar, 1968

**Table 1.** List of Tylodephys species described from India under the genus Diplostomulum. M, metacercaria; A, adult (continuation)

Species	Host	Developmental stage	Infected organ	Reference
<i>Tylodephys cerebralis</i> syn. <i>Diplostomulum cerebrales</i> Chakrabarti, 1968, <i>D. ophthalmi</i> Pandey, 1970; <i>D. tulsipurensis</i> Chakrabarti and Baugh, 1973; <i>D. lucknowensis</i> Chakrabarti and Baugh, 1973; <i>D. ellipticus</i> Chakrabarti and Baugh, 1973	<i>Channa punctata</i>	M	Cranial cavity, Eye	Chakrabarti, 1968
<i>Diplostomulum</i> sp.	<i>Catla catla</i>	M	Eye	Rai and Pande, 1969
<i>D. batrachi</i>	<i>Clarias batrachus</i>	M	Muscles	Rai and Pande, 1969
<i>D. carpi</i>	<i>Catla catla</i> , <i>Labeo rohita</i> , <i>L. gonius</i> , <i>Cirrhinus mrigala</i>	M	Body muscles, below scales mostly near caudal fin	Rai and Pande, 1969
<i>D. minutum</i>	<i>Puntius</i> sp.	M	Skin and caudal region	Pandey, 1970
<i>Diplostomulum</i> type I	<i>Rana cyanophlyctis</i>	M	Muscles of thigh	Nath, 1973
<i>Diplostomulum</i> type II	<i>Rana cyanophlyctis</i>	M	Muscles of leg	Nath, 1973
<i>D. dutti</i>	<i>Heteropneustes fossilis</i>	M	Encysted in muscles	Agrawal and Khan, 1982
<i>D. chauhani</i>	<i>Heteropneustes fossilis</i>	M	Cyst of intestine and body cavity	Pande et al., 1964
<i>D. nurius</i>	<i>Silonia silondia</i>	M	Cyst on gonads	Mukherjee and Srivastava, 1989

**Table 1.** List of Tyloodelphys species described from India under the genus Diplostomulum. M, metacercaria; A, adult (continuation)

Species	Host	Developmental stage	Infected organ	Reference
<i>D. tetrai</i>	<i>Schizothorax richardsonii</i>	M	Fins, gills, operculum and spine	Chopra, Kumar and Singh, 1983
<i>Tyloodelphys</i> sp. CG	<i>Channa gachua</i>	M	vitreous humour of eye	Chaudhary et al., 2017b
<i>Tyloodelphys</i> sp. PS1	<i>Puntius sophore</i>	M	vitreous humour of eye	Chaudhary et al., 2017b
<i>Tyloodelphys</i> sp. PS2	<i>Puntius sophore</i>	M	vitreous humour of eye	Chaudhary et al., 2017b
<i>Tyloodelphys</i> sp.1	<i>Mystus tengara</i>	M	vitreous humour of eye	Chaudhary et al., 2017c
<i>Tyloodelphys</i> sp.2	<i>Mystus tengara</i>	M	Brain	Chaudhary et al., 2017c



**Table 2.** Comparative measurements shown as range (mean) of *Tylodelphys* species collected in the present study with previously described morphologically similar species from vitreous humor of the eye and brain. TBL=Total Body Length; TBW=Total Body Width; OL=Oral sucker Length; OW=Oral sucker Width; PSL= Pseudosucker Length; PSW= Pseudosucker Width; VSL= Ventral sucker Length; VSW= Ventral sucker Width; HOL= Holdfast Organ Length; HOW= Holdfast Organ Width; HBL=Hind body Length; OSVS= Distance between oral sucker-ventral sucker; VSHO= ventral sucker-holdfast organ. Species collected in this study are in bold, na=data not available. Asterisk shows the species measurements are in millimeters

Species	Host	Infection site	Locality	TBLxTBW	OL xOW	PSL xPSW	VSL xVSW	HOL xHOW	HBL	OSVS	VSHO	Reference
<i>Tylodelphys</i> sp. IND/CL/VR-1	Hybrids of <i>Cirrhinus mrigala</i> × <i>Labeo rohita</i>	Vitreous humor	Meerut (U.P.), India	860-948(904)x 160-204(182)	51-77(64) x 46-70(58)	31-47(39)x 52-64(58)	47-59(53) x32-50(41)	88-126(107) 32-50(41)	78-118(98)	372-454(413)	82-112(97)	Present study
<i>Tylodelphys</i> sp. IND/CL/VR-2	Hybrids of <i>Cirrhinus mrigala</i> × <i>Labeo rohita</i>	Vitreous humor	Meerut (U.P.), India	639-727(683) x 137-181(159)	10-36(23) x 11-35(23)	24-32(28)x 25-31(28)	38-50(44)x 31-49(40)	65-87(76)x 21-35(28)	94-134(114)	230-245(224)	81-109(95)	Present study
<i>T. cerebralis</i> (=D. <i>cerebralis</i> )*	<i>Channa punctata</i>	Cranial cavity	Lucknow (U.P.), India	0.86-1.23x0.18-0.33	0.051-0.073x 0.035-0.042	na	0.042-0.056	0.12-0.17x0.05-0.08	na	na	na	Chakrabarti 1968
<i>Diplostomulum</i> sp.*	<i>Catla catla</i>	Eye	Mathura (U.P.), India	0.55x0.40	0.058	0.08x0.05	0.058-0.091	na	na	na	na	Rai and Pandey, 1969
<i>T. cerebralis</i> (=D. <i>cerebralis</i> )*	<i>Channa punctata</i>	Vitreous humor	Meerut (U.P.), India	0.55-0.61(0.58) x0.11-0.13(0.12)	0.018-0.034(0.026) x0.019-0.036(0.026)	0.021-0.034(0.027) x0.014-0.022(0.018)	0.016-0.028(0.022)x 0.01-0.02(0.02)	0.06-0.07(0.06)x 0.02-0.04(0.03)	0.05-0.06(0.06)	0.28-0.34(0.31)	0.05-0.07(0.06)	Chaudhary et al., 2017a

**Table 2.** Comparative measurements shown as range (mean) of *Tylodelphys* species collected in the present study with previously described morphologically similar species from vitreous humor of the eye and brain (continuation)

Species	Host	Infection site	Locality	TBL xTBW	OL xOW	PSL xPSW	VSL xVSW	HOL xHOW	HBL	OSVS	VSHO	Reference
<i>T. cerebralis</i> (= <i>D. cerebralis</i> )*	<i>Channa punctata</i>	Brain	Meerut (U.P.), India	1.07-1.24 (1.16)x 0.11-0.16 (0.14)	0.062- 0.081 (0.071)x 0.058- 0.086 (0.072)	0.054- 0.095 (0.080)x 0.030- 0.046 (0.032)	0.024- 0.043 (0.033)x 0.02-0.04 (0.03)	0.18-0.23 (0.21)x 0.06-0.08 (0.07)	0.06- 0.09 (0.07)	0.36 (0.30- 0.42)	0.13- 0.22 (0.17)	Chaudhary et al., 2017a
<i>Tylodelphys</i> sp. CG	<i>Channa gachua</i>	Vitreous humor	Bijnor, (U.P.), India	505- 593(549) x140- 184(162)	8-34(21) x24-48 (36)	16- 22(19) x13- 17(15)	25-37 (31) x17- 35(26)	35-57 (46)x 19- 33(26)	30-70 (50)	263- 321 (292)	55-77 (66)	Chaudhary et al., 2017b
<i>Tylodelphys</i> sp. PS1	<i>Puntius sophore</i>	Vitreous humor	Meerut (U.P.), India	140- 228(184) x64- 104(86)	43- 69(56)x 38- 62(50)	34- 45x24-30	47- 59(53) x44-62 (53)	77- 98(88)x 39- 65(52)	72-112 (92)	335- 417 (376)	47-77 (62)	Chaudhary et al., 2017b
<i>Tylodelphys</i> sp. PS2	<i>Puntius sophore</i>	Vitreous humor	Meerut (U.P.), India	717- 805(761) x176- 220(198)	42- 68(55)x 39- 63(51)	33- 39x42-45	43- 55(49)x 35- 53(44)	86-124 (105)x 34-52 (43)	72-116 (96)	291- 369 (330)	55-85 (70)	Chaudhary et al., 2017b
<i>Tylodelphys</i> sp. 1	<i>Mystus tengara</i>	Vitreous humor	Hastinapur (U.P.), India	552-648 (602.4)x 104-148 (126.8)	26- 50(37.4) x31- 57(44.4)	30- 48(38.8)x 14- 26(20.4)	31- 45(38.8) x29- 47(38)	67- 87(75.2)x 44-66 (54.4)	81-123 (104.4)	255- 345 (305.2)	91-133 (114.4)	Chaudhary et al., 2017c
<i>Tylodelphys</i> sp. 2	<i>Mystus tengara</i>	Brain	Hastinapur (U.P.), India	429-527 (476.8)x 178-218 (196.8)	33- 57(45.4)x 32-58(45)	41- 63(52.6)x28 -40(34.4)	32- 42(37)x 21-33 (27.4)	34-72 (55.6)x32 -54(43.4)	36-76 (56.8)	91-173 (135.2)	93-13 (112.2)	Chaudhary et al., 2017c

The other species *Tylodelphys* sp. IND/CL/VR-2 can be easily differentiated from previously described species from India by having a more tapered hind body. However, *Tylodelphys* sp. IND/CL/VR-2 resembles *Tylodelphys* sp. PS2 and *Tylodelphys* sp.1 morphologically but the new species differs from *Tylodelphys* sp. PS2 in the distance between ventral sucker and holdfast organ and in the body shape (table 2) while from *Tylodelphys* sp. 1 in having larger pseudosuckers and having less distance between ventral sucker and holdfast organ and also in having a more pointed body from mid to posteriorly. Morphometric comparisons of the new species *Tylodelphys* sp. IND/CL/VR-1 and *Tylodelphys* sp. IND/CL/VR-2 with congeneric species from India that illustrate both the new species shows their specific characters (table 2).

Two newly recorded metacercariae species reported here in the present study are described as *Tylodelphys* sp. IND/CL/VR-1 and *Tylodelphys* sp. IND/CL/VR-2 and their naming will be completed in the future after the full and proper description of the adults worked out either by experimental methods or will be discovered from definitive hosts. Till then, this represents the first record for *Tylodelphys* sp. IND/CL/VR-1 and *Tylodelphys* sp. IND/CL/VR-2 as species of *Tylodelphys* and members of the Diplostomidae in India.

## Discussion

Parasites impact on their host and its population is known to have broad ramifications for the entire biological system functioning (Poulin, 1999). In all sensory systems, vision is one of the most important for fish (Bowmaker and Loew, 2008). Vision of fish species adapt to different environments for vital functions such as prey capture, mate selection and predator identification. Fish eyes diseases affecting the vision and could epitomize an immune privileged structure (Caspi, 2013), so that the parasites can easily escape from the responses generated by the hosts to decrease the negative impacts by parasites. Trematodes represent most frequent parasites amongst the diseases that caused fish eyes (Chappell, 1967; Brady, 1989; Locke et al., 2010; Chibwana et al., 2013;

Blasco-Costa et al., 2014; Faltýnková et al., 2014; Locke et al., 2015; Sereno-Uribe et al., 2018; Padrósa et al., 2018).

Diplostomids diversity infecting Indian fish is poorly known, as compared to other geographical regions (Musiba and Nkwengulila, 2006; Chibwana et al., 2013; Otachi et al., 2014; Locke et al., 2015; García-Varela et al., 2016; Sereno-Uribe et al., 2018; Presswell and Blasco-Costa, 2020; Selbach et al., 2020). In the initial observation of the two species of metacercaria *Tylodelphys* sp. IND/CL/VR-1 and *Tylodelphys* sp. IND/CL/VR-2, we suspect primarily that they are one species but after thorough examination of morphological, morphometrical and scanning electron microscopy it was clear that they are distinct from each other. Both the species clearly differ in morphometry with other published data (table 2), although some interspecific morphometric overlapping was seen in case of *Tylodelphys* sp. IND/CL/VR-1 with *T. cerebralis* species from the cranial cavity of *C. punctata* by Chakrabarti (1968). The two unidentified *Tylodelphys* spp. viz., *Tylodelphys* sp. IND/CL/VR-1 and *Tylodelphys* sp. IND/CL/VR-2, are described only using metacercaria stage that is still incomplete till to the date they will be linked to their adult stages and also exhibited the necessity for further research for this group of parasites. Till then, as the adult stages will be recognized, these metacercaria species *Tylodelphys* sp. IND/CL/VR-1 and *Tylodelphys* sp. IND/CL/VR-2, awaits for formal description for naming. To date, in all diplostomid species reported in India (table 1) only five have been described with adult worms, some were originally described under *Diplostomulum* which also warrants being correctly identified with proper morphological redescription (Pandey and Agrawal, 2013). In this study, a detailed view is provided with the SEM examinations of the two species *Tylodelphys* sp. IND/CL/VR-1 and *Tylodelphys* sp. IND/CL/VR-2. *Tylodelphys* sp. IND/CL/VR-1 can be separated from all Indian species described under *Tylodelphys/Diplostomulum* from vitreous humour/cranial cavity/brain principally by having a larger body size from *Tylodelphys* sp. CG, *Tylodelphys* sp. PS1, *Tylodelphys* sp. PS2,

*Tylodelphys* sp.1, *Tylodelphys* sp.2 and from *T. cerebralis* in greater distance between holdfast organ and ventral sucker, in much developed pseudosuckers and tapered body (Chaudhary et al., 2017a, 2017b, 2017c). In addition, *Tylodelphys* sp. IND/CL/VR-2 can be distinguished from all the above mentioned species (Chaudhary et al., 2017a, 2017b, 2017c) by the presence of more tapered hindbody, in the distance between ventral sucker and holdfast organ and in the body shape.

Although, the diversity of *Tylodelphys* in India is far from complete, may be the one-fourth of the known spp. diversity (Chakrabarti, 1968; Pandey, 1970; Sudarikov, 1974; Pandey and Tewari, 1984; Jain and Gupta, 1970; Gupta, 1962, Mehra, 1962a, Dubois, 1964; Mehra, 1962b; Yamaguti, 1971; Singh, 1956; Odening, 1970; Pandey and Agrawal, 2013; Chaudhary et al., 2017a, 2017b, 2017c). In comparison to India, a high diversity of *Tylodelphys* species is available from other continents viz., Africa, Europe and South and North America that exhibited a wide distribution of the species (Faltýnková, 2005; Moszczyńska et al., 2009; Chibwana et al., 2013; Otachi et al., 2015; García-Varela et al., 2016; Sereno-Uribe et al., 2019). The results of our study improve the knowledge on the *Tylodelphys* species diversity in India with the prediction that they have a much broader diversity is available that needs to be uncovered.

**Nomenclatural acts.** According to comply with the rules set out by the International Code of Zoological Nomenclature, details of the *Tylodelphys* species, *Tylodelphys* sp. IND/CL/VR-1 and *Tylodelphys* sp. IND/CL/VR-2 newly recorded have been submitted to ZooBank and their Life Science Identifier is urn:lsid:zoobank.org:pub: B09D47C8-2B7B-4527-8E8A-45AC93E98346.

**Acknowledgments.** We acknowledge the support by the Head, Department of Zoology, Chaudhary Charan Singh University, Meerut, India for providing laboratory facilities. The authors thank to Dr. Seema Jain and Dr. Manu Varma, Department of Zoology, R.G.P.G. College, Meerut (U.P.), India, for identification of fish species. The first author acknowledges

DST (Department of Science and Technology), Government of India, New Delhi for the Post-Doctoral Fellowship (SR/WOS-A/LS-382/2018) under WOS-A Scheme. The authors declare that they have no conflict of interests.

## References

- Agrawal N., Khan S. 1982. A new *Diplostomulum* Brandes, 1892 metacercaria from the fish *Heteropneustes fossilis*. Indian J. Parasitol. 6:81-83.
- Blasco-Costa I., Faltýnková A., Georgieva S., Skírnisson K., Scholz T., Kostadinova A. 2014. Fish pathogens near the Arctic Circle: molecular, morphological and ecological evidence for unexpected diversity of *Diplostomum* (Digenea: Diplostomidae) in Iceland. Int. J. Parasitol. 44:703-715.
- Blasco-Costa I., Poulin R., Presswell B. 2017. Morphological description and molecular analyses of *Tylodelphys* sp. (Trematoda: Diplostomidae) newly recorded from the freshwater fish *Gobiomorphus cotidianus* (common bully) in New Zealand. J. Helminthol. 91:332-345.
- Bowmaker J.K., Loew E.R. 2008. 1.04 – Vision in fish. In: Masland R.H., Albright, T.D., Albright, T.D., Masland, R.H., Dallos, P., Oertel, D., Firestein, S., Beauchamp, G.K., Catherine Bushnell, M., Basbaum, A.I., Kaas, J.H., Gardner, E.P. (Eds.). The Senses: a Comprehensive Reference. Academic Press, New York, pp. 53-76.
- Brady A. 1989. Studies on the Taxonomy and Biology of *Diplostomum* Species (Digenea). University of Stirling, Stirling, UK.
- Caspi R.R. 2013. In this issue: Immunology of the eye – inside and out. Int. Rev. Immunol. 32:1-3.
- Chakrabarti K. 1968. On a new strigeid metacercaria, *Diplostomulum cerebralis* n. sp. from an Indian freshwater fish. Zool. Anz. 181:303-306.
- Chappell L.H. 1995. The biology of diplostomatid eyefluks of fishes. J. Helminthol. 69:97-101.
- Chappell L.H. 1967. Ecological and Experimental Studies on the Parasites of Freshwater Fishes in Northern England. PhD Thesis. University of Leeds.
- Chaudhary A., Tripathi R., Gupta S., Singh H.S. 2017a. First report on molecular evidence of *Tylodelphys cerebralis* (= *Diplostomulum cerebralis*) Chakrabarti, 1968 (Digenea: Diplostomidae) from snakehead fish *Channa punctata*. Acta Parasitol. 62:386-392.
- Chaudhary A., Gupta S., Tripathi R., Singh H.S. 2017b. Morphological and molecular analyses of *Tylodelphys* spp. metacercaria (Trematoda:

- Diplostomidae) from the vitreous humour of two freshwater fish species, *Channa gachua* (Ham.) and *Puntius sophore* (Ham.). Vet. Parasitol. 244:64-70.
- Chaudhary A., Gupta S., Verma C., Tripathi R., Singh H.S. 2017c. Morphological and molecular characterization of metacercaria of *Tylodelphys* (Digenea: Diplostomidae) from the piscine host, *Mystus tengara* from India. J. Parasitol. 103:565-573.
- Chibwana F.D., Blasco-Costa I., Georgieva S., Hosea K.M., Nkwengulila G., Scholz T., Kostadinova A. 2013. A first insight into the barcodes for African diplostomids (Digenea: Diplostomidae): Brain parasites in *Clarias gariepinus* (Siluriformes: Clariidae). Infect. Genet. Evol. 17:62-70.
- Chibwana F.D., Nkwengulila G., Locke S.A., McLaughlin J.D., Marcogliese D.J. 2015. Completion of the life cycle of *Tylodelphys mashonense* (Sudarikov, 1971) (Digenea: Diplostomidae) with DNA barcodes and rDNA sequences. Parasitol. Res. 114:3675-3682.
- Chopra A.K., Kumar A., Singh N.R. 1983. *Diplostomulum tetrai*, a new metacercaria from a cold water fish, *Schizothorax richardsonii* (Gray, 1832) from Garhwal Himalayas. Indian J. Parasitol. 7:89-91.
- Dubois G. 1964. Du statut de quelques Strigeata La Rue, 1926 (Trematoda). I. Bulletin de la Société Neuchateloise des Sciences Naturelles 87:27-71.
- Faltýnková A. 2005. Larval trematodes (Digenea) in molluscs from small water bodies near Šeské Budšjovice, Czech Republic. Acta Parasit. 50:49-55.
- Faltýnková A., Georgieva S., Kostadinova A., Blasco-Costa I., Scholz T., Skirnisson K., 2014. *Diplostomum* von Nordmann, 1832 (Digenea: diplostomidae) in the sub-Arctic: descriptions of the larval stages of six species discovered recently in Iceland. Syst. Parasitol. 89:195-213.
- Ganapati P.N., Rao K.H. 1955. On the occurrence of metacercarial cysts of a diplostome (Trematoda) in fresh-water fishes. Proc. Indian Sci. Cong. 3:285-286.
- García-Varela M., Sereno-Uribe A.L., Pinacho-Pinacho C.D., Hernández-Cruz E., Pérez-Ponce de León G. 2016. An integrative taxonomic study reveals a new species of *Tylodelphys* Diesing, 1950 (Digenea: Diplostomidae) in central and northern Mexico. J. Helminthol. 90:668-679.
- Gupta R. 1962. Two new species of the genus *Tylodelphys* Diesing, 1850 (Trematoda: Diplostomatidae) from Indian birds. Proc. Natl. Acad. Sci. India Sect. B Biol. Sci. 32:417-420.
- Jain S.P., Gupta A.N. 1970. On a new species of the genus *Tylodelphys* Diesing, 1850 (Trematoda: Diplostomidae Poirier, 1886) from an Indian bird. Indian J. Helminthol. 22:79-83.
- Locke S.A., Al-Nasiri F.S., Caffara M., Drago F., Kalbe M., Lapiere A.R., McLaughlin J.D., Nie P., Overstreet R.M., Souza G.T.R., Takemoto R.M., Marcogliese D.J. 2015. Diversity, specificity and speciation in larval Diplostomidae (Platyhelminthes: Digenea) in the eyes of freshwater fish, as revealed by DNA barcodes. Int. J. Parasitol. 45:841-855.
- Locke S.A., McLaughlin J.D., Marcogliese D.J. 2010. DNA barcodes show cryptic diversity and a potential physiological basis for host specificity among Diplostomoidea (Platyhelminthes: Digenea) parasitizing freshwater fishes in the St. Lawrence River, Canada. Mol. Ecol. 19:2813-2827.
- Mehra H.R. 1962a. Two new species of the genus *Tylodelphys* Diesing, 1850 (Diplostomatidae) from Indian birds. Proc. Natl. Acad. Sci. India Sect. B Biol. Sci. 32:417-420.
- Mehra H.R. 1962b. A new species of the genus *Glossodiplostomum* Dubois, 1932, of the family Diplostomidae Poirier, 1886. Proc. Natl. Acad. Sci. India Sect. B Biol. Sci. 32:125-128.
- Moszczyńska A., Locke S.A., McLaughlin J.D., Marcogliese D.J., Crease T.J. 2009. Development of primers for the mitochondrial cytochrome c oxidase I gene in digenetic trematodes (Platyhelminthes) illustrates the challenge of barcoding parasitic helminthes. Mol. Ecol. Resour. 9:75-82.
- Mukherjee M., Srivastava C.B. 1989. Studies on larval strigeoid genus *Diplostomulum* Brandes, 1898, with description of a new species. Dr. B. S. Chauhan Commemoration Volume, India 13-17.
- Musiba M.J., Nkwengulila G. 2006. Occurrence of metacercariae of *Diplostomum* and *Tylodelphys* species (Diplostomidae) in *Clarias* species (Clariidae) from Lake Victoria. Tanz. J. Sci. 32:89-98.
- Nath D. 1973. The strigeoid metacercarial fauna of the Indian pond-frog *Rana cyanophlyctis*. Indian J. Anim. Sci. 43:61-65.
- Odening K. 1970. Neue funde von Diplostomidae (Trematoda) aus vögeln des berliner tierparks. Mitteilungen aus dem Zoologische Museum in Berlin 46:167-181.
- Otachi E.O., Locke S.A., Jirsa F., Fellner-Frank C., Marcogliese D.J. 2015. Morphometric and molecular analyses of *Tylodelphys* sp. metacercariae (Digenea: Diplostomidae) from the vitreous humour of four fish species from Lake Naivasha, Kenya. J. Helminthol. 89:404-414.
- Otachi E.O., Magana A.E.M., Jirsa F., Fellner-Frank C. 2014. Parasites of commercially important fish from Lake Naivasha, Rift Valley, Kenya. Parasitol. Res. 113:1057-1067.

- Padrós F., Knudsen R., Blasco-Costa I. 2018. Histopathological characterisation of retinal lesions associated to *Diplostomum* species (Platyhelminthes: Trematoda) infection in polymorphic Arctic charr *Salvelinus alpinus*. Int. J. Parasitol. Parasites Wildl. 7:68–74.
- Pande B.P., Bhatia B.B., Rai P. 1964. On a strigeid metacercaria from *Heteropneustes fossilis* (Bloch) 'Singhi'. Proc. Natl. Acad. Sci. India Sect. B Biol. Sci. 34:245-248.
- Pandey K.C. 1970. Studies on metacercaria of freshwater fishes of India III. On two new species of *Diplostomulum* Brandes, 1892. Proc. Natl. Acad. Sci. India Sect. B Biol. Sci. 72:162–170.
- Pandey K.C., Agrawal N. 2013. Metacercarial fauna of India. Records of Zoological Survey of India, Occasional Paper No. 349. Published by the Director, Zoological Survey of India, Kolkata, India, 310 pp.
- Pandey K., Tewari S. 1984. A redescription of *Diplostomum ophthalmi* Pandey, 1968; with a note on validity of certain other Indian species. U.P. J. Zool. 4:179–184.
- Poulin R. 1999. The functional importance of parasites in animal communities: many roles at many levels? Int. J. Parasitol. 29:903–914.
- Presswell B., Blasco-Costa I. 2020. Description of *Tylodelphys darbyi* n. sp. (Trematoda: Diplostomidae) from the threatened Australasian crested grebe (*Podiceps cristatus australis*, Gould 1844) and linking of its life-cycle stages. J. Helminthol. 94: e40. e40. DOI: 10.1017/S0022149X19000142
- Rai P., Pande B.P. 1969. On the morphology and pathogenic significance of the strigeoid metacercariae in some Indian fresh water fishes. III. *Diplostomulum*. Indian J. Anim. Sci. 39:539-552.
- Sandland G.J., Goater C.P. 2000. Development and intensity dependence of *Ornithodiplostomum ptychocheilus* metacercariae in fathead minnows (*Pimephales promelas*). J. Parasitol. 86:1056–1060.
- Selbach C., Soldánová M., Feld C.K., Kostadinova A., Sures B. 2020. Hidden parasite diversity in a European freshwater system. Sci. Rep. 10:2694.
- Sereno-Uribe A.L., Andrade-Gómez L., de León G.P.P., García-Varela M. 2019. Exploring the genetic diversity of *Tylodelphys* (Diesing, 1850) metacercariae in the cranial and body cavities of Mexican freshwater fishes using nuclear and mitochondrial DNA sequences, with the description of a new species. Parasitol. Res. 118: 203–217.
- Sereno-Uribe A.L., López-Jimenez A., Andrade-Gómez L., García-Varela M. 2018. A morphological and molecular study of adults and metacercariae of *Hysteromorpha triloba* (Rudolphi, 1819), Lutz 1931 (Diplostomidae) from the Neotropical region. J. Helminthol. 93:91-99.
- Singh K.S. 1956. On some strigeids from India. J. Zool. Soc. India 8:47–56.
- Sudarikov V.E. 1974. Order Strigeidida (La Rue 1926). In: Skrjabin, K.I. (Ed.), Trematodes of animals and man. Principles of trematodology, Vol. 25. [in Russian]. Nauka, Moscow, pp. 29–244.
- Thapar G.S. 1968. Some new larval strigeids causing black spot disease in fishes from Tungabhadra dam. Indian J. Helminthol. 19: 173-182.
- Yamaguti S. 1971. Synopsis of digenetic trematodes of vertebrates. Tokyo, Japan, Keigaku Publishing.