

STUDIES ON SOME STRIGEID METACERCARIAE OF THE  
LARVAL GENUS *DIPLOSTOMULUM* BRANDIS, 1892 FROM  
INDIAN FRESH-WATER FISHES

By

K. K. CHAKRABARTI \* and S. C. BAUGH

SUMMARY

During the course of an investigation on the metacercarial infections of the common fishes found in and around Lucknow and in Tulsipur, a number of specimens of *Puntius ticto*, *P. stigma*, *Oxygaster bacaila*, *Mystus vittatus* and *Clarias batrachus* were examined, and three species of *Diplostomulum* viz., *D. ellipticus* n. sp., *D. lucknowensis* n. sp. and *D. tulsipurensis* n. sp. have been found infesting these fishes.

INTRODUCTION

An investigation was carried out for about six years (1966-1972) on the metacercarial infections of some of the fresh-water fishes found in and around the city of Lucknow and in the environs of Tulsipur, the home town of one of the writers (KKC) in the district of Gonda, U.P. The present paper deals with the accounts of three strigeid metacercariae of the larval genus *Diplostomulum* Brandis, 1892 found in *Puntius ticto* (Hamilton), *P. stigma* (Hamilton), *Oxygaster bacaila* (Hamilton), *Mystus vittatus* (Bloch) and *Clarias batrachus* (Linnaeus).

MATERIAL AND METHODS

Fishes examined during the present investigation were regularly obtained from fishermen who used to bring their catches from the en-

---

\* Present address: B. S. N. V. Degree College, Lucknow (India).

---

Recibido el 3-1-1973

virons of Lucknow to the local market for sale. Fishes examined in Tulsipur were collected by fishermen especially engaged. Almost always live fishes were examined as the fishermen concerned were instructed to bring them alive and they were provided proper equipment. Fishes which died soon after they were caught were also examined. Live fishes, when obtained in great abundance, were maintained in the properly aerated aquaria of the Zoology Department of the University of Lucknow for a day or two, or even for a longer period when needed. Live fishes were killed either by the addition of chloroform or urethane to the container.

For examining the fishes for metacercarial infections, standard procedures like those suggested by Hoffman (1960) were followed. Cranial cavity of the fishes was opened with the help of a sharp scalpel under a dissecting binocular microscope in normal saline. Likewise eyes were examined. Metacercariae when encountered were transferred to normal saline or to Tyrode's and Locke-Lewi's solutions prepared by standard methods. A camel-hair brush or an specially prepared pipette with a long capillary end was used for handling the metacercariae.

Live metacercariae as well as fixed, stained and mounted ones were studied.

Morphological details were mostly studied in live specimens, only supplemented by observations made from stained and mounted materials. Live metacercariae were stained with freshly prepared vital stains viz., neutral red, methyl blue, methyl green and Nile blue sulphate. They were maintained in vitro (Silverman, 1965), whenever necessity arose, by using Tyrode's or Locke-Lewi's solution.

Details of the excretory system were worked out solely in live specimens often using India ink.

For fixing metacercariae, 70% alcohol, Bouin's alcoholic fluid and Zenker's fluid were used. Fixed materials, after proper washing, were stained with acetic acid alum carmine, Granacher's borax carmine and also Ehrlich's haematoxyline.

Phase-contrast microscope was used for ascertaining the presence of body spine.

Table I

Incidence of infection of the species of *Diplostomulum* obtained from different fishes

Fishes examined	Number of fishes examined		Number of fishes found infected		Incidence of infection	Metacercaria found
	At Lucknow	At Tulsipur	At Lucknow	At Tulsipur		
<i>Puntius ticto</i>	175	X	10	X	5.71% (L)	<i>D. ellipticus</i> n.sp.
<i>Puntius stigma</i>	85	X	3	X	3.52% (L)	"
<i>Oxygaster bacaila</i>	200	25	10	3	5 % (L) 12 % (T)	"
<i>Mystus vittatus</i>	72	18	5	Nil	6.94% (L)	<i>D. lucknowensis</i> n.sp.
<i>Clarias batracus</i>	48	21	Nil	2	9.52% (T)	<i>D. tulsipurensis</i> n.sp.

(L) = indicates at Lucknow  
(T) = indicates at Tulsipur

#### *Diplostomulum ellipticus* n.sp.

One hundred and seventy-five specimens of *Puntius ticto* (Hamilton) and eighty-five of *Puntius stigma* (Hamilton) obtained from the local fish market were examined during the course of the present investigation and, out of these, ten specimens of *P. ticto* and three of *P. stigma* were found infected with this metacercaria. Specimens of *Oxygaster bacaila* (Hamilton) obtained from fishermen at Lucknow fish-market and at Tulsipur were also found harbouring this strigeid metacercaria. The appended table (Table I) shows the incidence of infection of this metacercaria in *P. ticto*, *P. stigma* and *O. bacaila*.

Most of the metacercariae were found in the cranial cavity of the infected hosts, but twice they were obtained from within the eyes of *P. ticto* and *P. stigma*, once in either. In all cases, the metacercariae were found devoid of cysts. The intensity of infection was low as only one to four specimens were obtained from individual hosts. The metacercariae survived in Tyrode's and Locke-Lewis' solutions for hours and thereby enabled the writers to study them long in live condition. They performed active movements in these media.

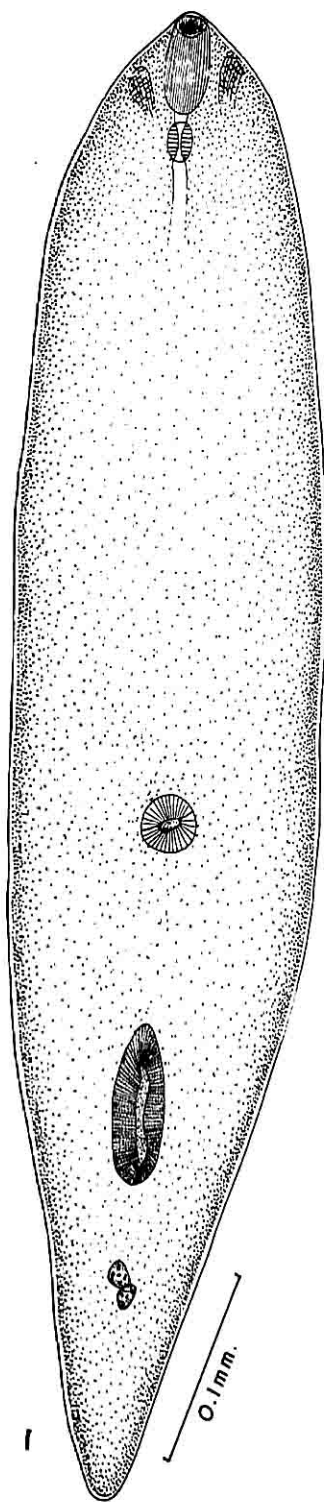


Fig. 1.—*Diplostomulum ellipticus* n.sp.  
Ventral view. Drawn from a  
mounted specimen

A careful study of these metacercariae obtained from three different fishes proved them all to be specifically identical and to represent a species hitherto undescribed.

Body (Fig. 1) aspinose, elongated and flattened with a round anterior and a bluntly pointed posterior end in live specimens, and measures 0.65-0.95 mm in length and 0.18-0.26 mm in maximum width in the middle region. It is not demarcated into a fore-body and a hind-body. Suckers fairly muscular. Oral sucker subterminal and measures  $0.05-0.07 \times 0.02-0.04$  mm. Ventral sucker circular, post-equatorial and measures 0.03-0.05 mm in diameter. Pseudosuckers weakly developed. Hold-fast organ highly developed, elliptical in outline with a slit-like opening and is located considerably behind the ventral sucker. It measures  $0.09-0.16 \times 0.06-0.09$  mm. Hold-fast gland is lacking.

Alimentary canal includes a short prepharynx, a small subglobular and feebly muscular pharynx measuring 0.02-0.04 mm in diameter; a short oesophagus leading into intestinal caeca which could be traced, in live specimens, up to the posterior end of the body. In fixed and mounted specimens, their course could hardly be traced.

Fundaments of gonads as yet poorly differentiated and are represented by two small closely juxtaposed masses of dark staining cells mesially located behind the hold-fast organ.

Excretory bladder large and V-shaped (Fig. 2), located at the posterior end of the body and opens outside by a terminal excretory pore. The cornua of the bladder extend anteriorly up to the posterior border of the hold-fast organ.

The excretory canals of *Diplostomulum* group of strigeid metacercariae fall under two heads viz., (a) a primary system and (b) a reserve system.

Komiya (1938), Rees (1955), Pearson (1961), Odening (1965) paid attention to the study of both primary and reserve systems of excretory canals in *Diplostomulum* group, but others have only studied the reserve system.

It has been possible to study only the reserve system of excretory canals in the present *Diplostomulum*. The primary system of excretory canals is greatly obscured by the extensively developed reserve system of excretory canals. The details of the reserve system are as follows:

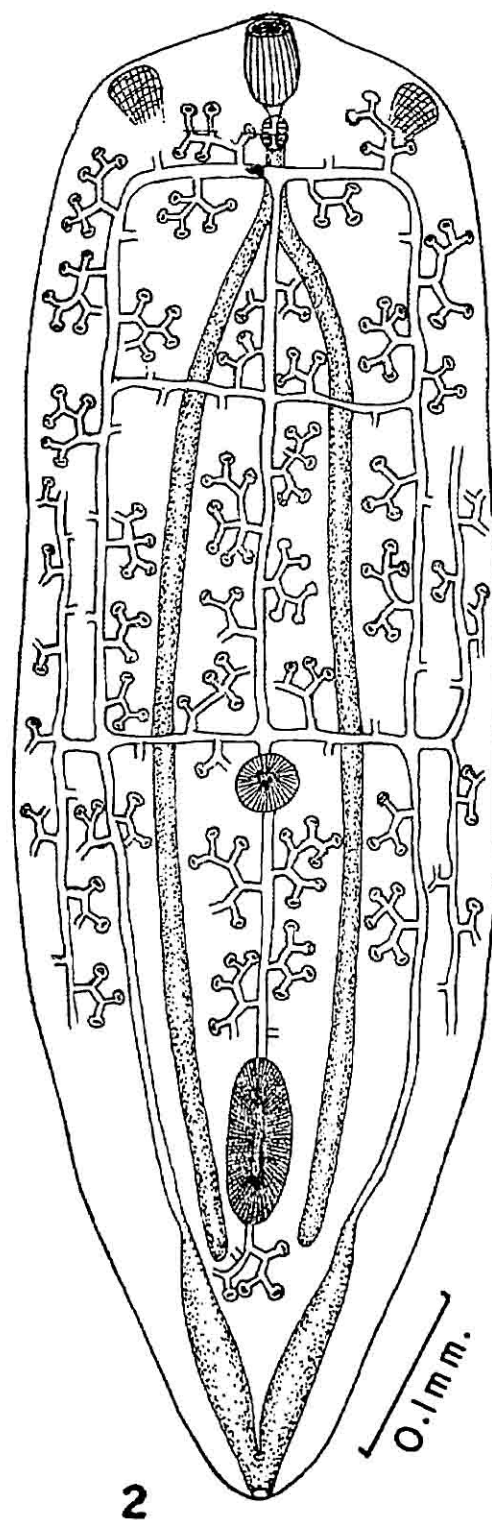


Fig. 2.—*Diplostomulum ellipticus* n.sp.  
Ventral view. Drawn from a live  
specimen showing the reserve  
excretory system

Two main excretory canals of the reserve system arise, one on each side, from the anterior end of the cornua of the excretory bladder. These canals run anteriorly through the lateral regions of the body up to the level of the esophagus where they are connected by a transverse canal—the anterior transverse canal. A median longitudinal canal arises from the anterior transverse canal and runs posteriorly along the median line of the body and, on its way, it is connected, roughly midway between the two suckers, with the main reserve excretory canals of the opposite sides by a transverse canal—the median transverse canal. Further behind but immediately in front of the ventral sucker, the median longitudinal canal is again connected with the main reserve excretory canals by another transverse canal—the posterior transverse canal. This transverse canal extends beyond the main reserve excretory canal of either side and bifurcates into an anterior and a posterior collecting canal.

The median longitudinal canal continues its course posteriad and ultimately ramifies close behind the hold-fast organ into a number of short fine branches. Small branches arise from the main reserve excretory canals, median longitudinal canal and transverse canals, and these branches repeatedly divide and redivide and these ramifications transverse the entire body. The ultimate twigs of these branches end in subglobular enlargements, each containing a calcareous corpuscle which are invariably elliptical in shape and constitute a characteristic feature of this species. The calcareous corpuscles measure  $0.008-0.012 \times 0.004-0.006$  mm. These corpuscles are dense, and they extend, forming a layer, from the level of the oral sucker up to the end of the hold-fast organ.

#### Discussion:

The larval genus *Diplostomulum* Brandis, 1892 includes to date only eight Indian species viz., *D. bufonis* Kaw, 1950; *D. pigmentata* Singh, 1956; *D. ketupanensis* Ganputi and Rao, 1962; *D. singhi* Pandey, Bhatia and Rai, 1966; *D. nurius* Thapar, 1967; *D. cerebralis* Chakrabarti, 1968; *D. ophthalmi* Pandey, 1970 and *D. minutum* Pandey, 1970. Of these, the present form closely resembles *D. singhi*, *D. cerebralis* and *D. ophthalmi*, but chiefly differs from them in the number of genital rudiments and in the pattern of the reserve excretory system. The genital rudiments are represented in *D. singhi*

by four masse, in *D. cerebralis* and *D. ophtalmi* by a single mass, whereas in the present form by two masses. The present form further differs from *D. singhi* in the presence of a prepharynx and from *D. cerebralis* and *D. ophtalmi* in having spinose cuticle.

Among the species described from foreign countries, the present form resembles *D. destructor* Szidat and Nani, 1951 and *Diplostomulum* of *Hysteromorpha triloba* Huggins, 1954. It differs from *D. destructor* in having a cup-like oral sucker and two masses of genital rudiments instead of one. The present species differs from *Diplostomulum* of *H. triloba* in the absence of the division of the body, and in the number of genital rudiments instead of one. The present species differs from *Diplostomulum* of *H. triloba* in the absence of the division of the body, and in the number of genital rudiments which are two in the former but three in the latter. Further, the present form is not associated with a cyst, but *Diplostomulum* of *H. triloba* is associated with one.

*Diplostomulum lucknowensis* n.sp.

Out of ninety specimens of *Mystus vittatus* (Bloch) examined during the period of the present investigation, only five were found infected with this metacercaria. Seventy-two host specimens were procured from Lucknow fish market and eighteen from fisherman at Tulsipur, but infection was found only in the former. The table (Table I) shows the incidence of infection of this metacercaria in *M. vittatus*. Metacercariae were found free in the cranial cavity, but in the eyes of two. Regarding the intensity of infection, two to five metacercariae were found in the cranial cavity, but only one or two in the eyes. They performed active movements when kept in saline or in Tyrode's solution.

Body (Fig. 3) spineless, elongated and flattened with a round anterior and a bluntly conical posterior end, but not demarcated into a fore-body and hind-body. It measures 0.44-0.72 mm in length and 0.20-0.34 mm in maximum width. Suckers well-developed. Oral sucker cup-like, subterminal and measures 0.04-0.05 × 0.05-0.04 mm. Ventral sucker circular, located far behind the equatorial line of the body and measures 0.03-0.05 mm in diameter. Pseudosuckers weakly developed. Hold-fast organ strongly muscular, elliptical in outline, and located close behind the ventral sucker. It measures 0.06-0.10 × 0.04-0.06 mm. Hold-fast gland absent.

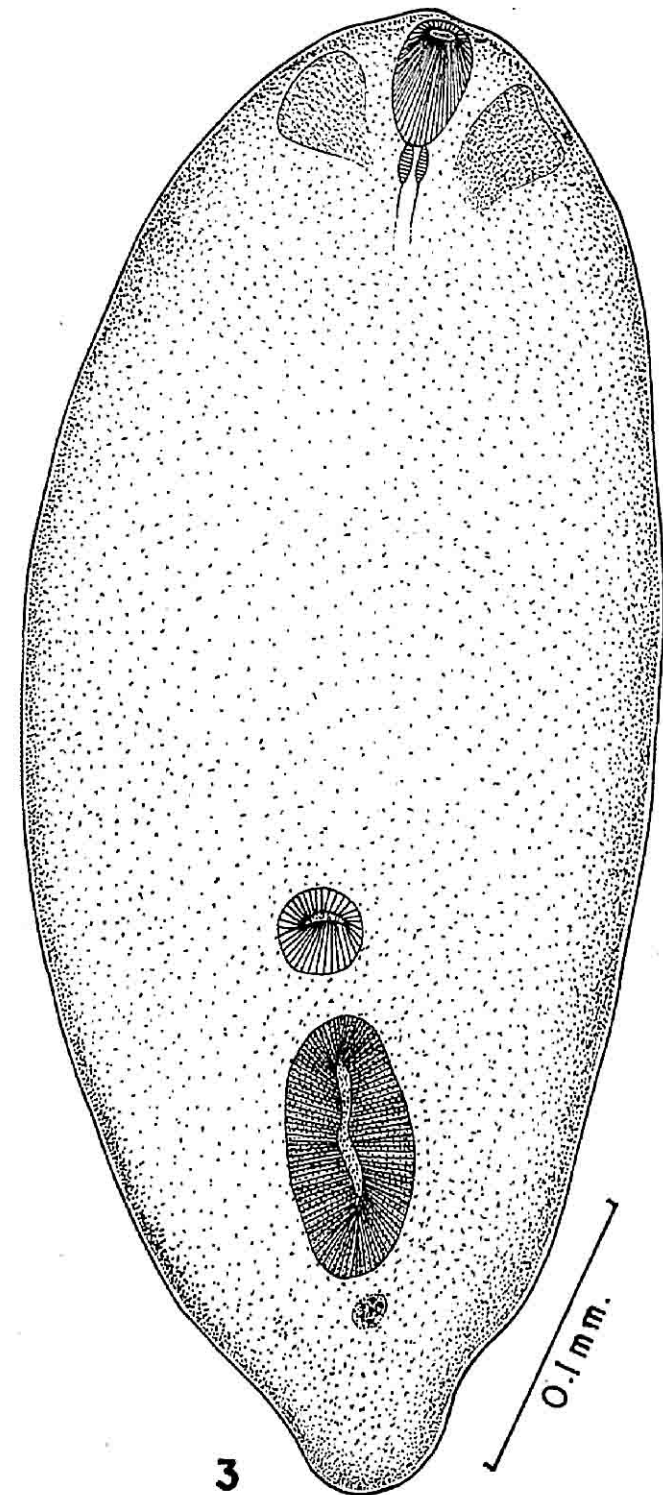


Fig. 3.—*Diplostomulum lucknowensis* n.sp.  
Ventral view. Drawn from a  
mounted specimen



former has only one mass of genital rudiment, while the latter has two. Further, the elliptical calcareous corpuscles of *D. ellipticus* serve well to distinguish it from the present species in which they are round.

Among the species described from outside India, the present from resembles *D. mordax* Szidat and Nani, 1951 and *D. ictaluri* Haderlie, 1953. It differs from *D. mordax* in the absence of the division of the body and in the form of three well-differentiated masses. From *D. ictaluri*, the present species also differs in the number of genital rudiments; in the former, three masses of cells constitute the rudiment of the genitalia, whereas in the latter only one mass of cells represents the same. In two other points, the present species differs from *D. ictaluri* viz., the former lacks the hold-fast gland, while the latter possesses it; and, further, the present from is not associated with a cyst but *D. ictaluri* is associated with a fibrous cyst.

*Diplostomulum tulsipurensis* n.sp

Sixty-nine specimens of *Clarias batrachus* (Linnaeus) were examined during the course of the present investigation, of which forty-eight were procured from the local fish market and twenty-one from fishermen at Tulsipur. None of the fishes obtained from the local fish market was found infected, but two of them examined at Tulsipur (Table I) were found infected with this metacercaria. Metacercariae were found in the body cavity of the host and were not associated with any cyst. In all eighteen metacercariae were obtained, ten from one host specimen and eight from the other. When taken out in saline and Tyrode's solution, they performed active movements.

Body (Fig. 5) spinose, elongated and flattened with a round anterior and a bluntly conical posterior end, measuring  $0.74-1.30 \times 0.24-0.40$  mm. Like the preceding ones, in this metacercaria, too, body is not marked into a fore-body and a hind-body. Suckers well-developed. Oral sucker cup-like, subterminal and measures  $0.04-0.07 \times 0.02-0.04$  mm. Ventral sucker circular, postequatorial and measures  $0.03-0.06$  mm in diameter. Pseudosuckers as yet weakly developed. Hold-fast organ fairly muscular, elliptical in outline with a slit-like opening and situated slightly behind the ventral sucker. It measures  $0.06-0.12 \times 0.04-0.07$  mm. No gland is found associated with the hold-fast organ.

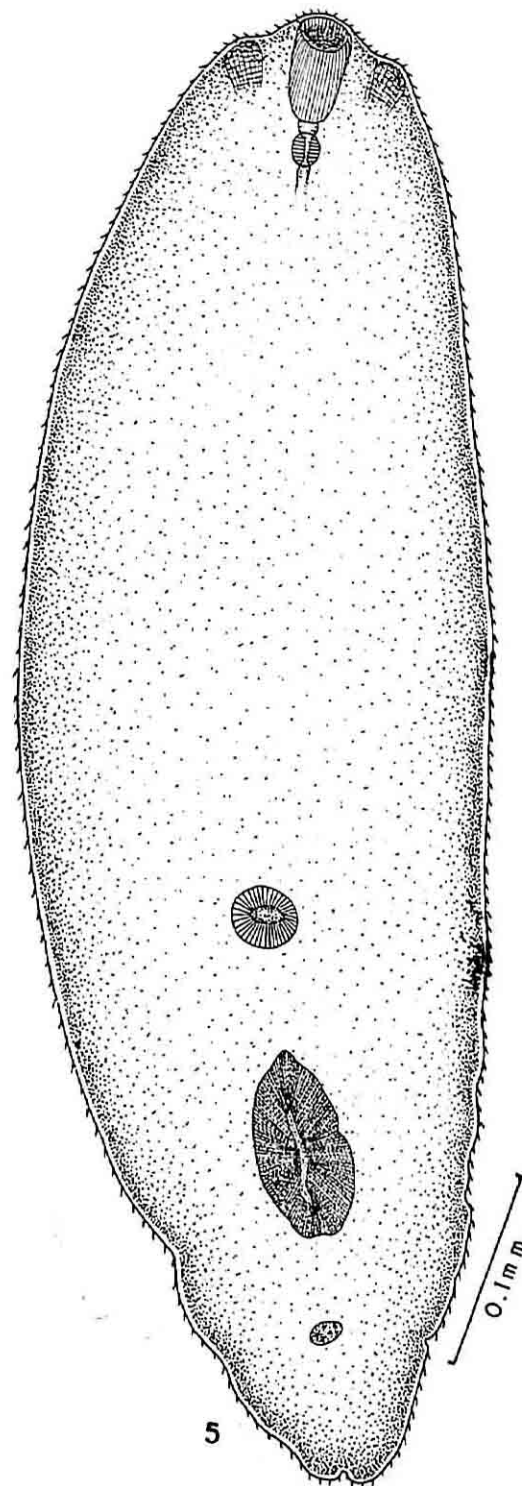


Fig. 5.—*Diplostomulum tulsipurensis*  
Fig. 5.—*Diplostomulum tulsipurensis* n.sp.  
Ventral view. Drawn from a

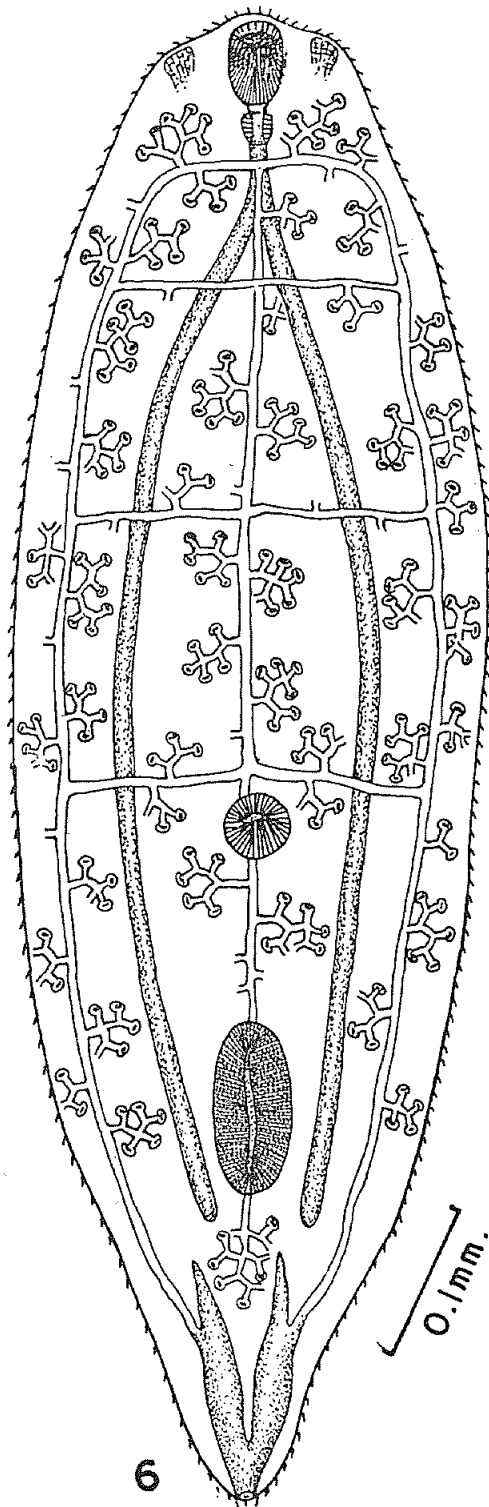


Fig. 6.—*Diplostomulum tulsipurensis* n.sp.  
Ventral view. Drawn from a live  
specimen showing the reserve  
excretory system

A prepharynx present. Pharynx weakly muscular, oval or roughly globular and measures 0.01-0.03 mm in diameter. Oesophagus short. Intestinal caeca are not discernible in stained and mounted specimens, but in live specimens they could be traced extending almost up to the cornua in live specimens they could be traced extending almost up to the cornua of the excretory bladder.

Gonads are represented by a single mass of dark staining cells, located posterior to the hold-fast organ.

Excretory bladder (Fig. 6) V-shaped, located at the hind end of the body and opens outside by a terminal excretory pore.

As in the species described in the foregoing pages, here too, the excretory canals of the reserve system have been worked out and are detailed below:

Two main excretory canals of the reserve system arise laterally from the anterior part of the cornua of the excretory bladder. Each of these canals runs anteriorly through the lateral region of the body and, on reaching the level of the oesophagus, it joins its fellow of the opposite side by a transverse canal — the anterior transverse canal. A median longitudinal canal arises from the anterior transverse canal and runs backward along the middle line of the body up to the level of the posterior border of the hold-fast organ where it terminates into a number of fine branches. It is connected, during its course, with the main reserve excretory canals by three additional transverse canals of which the hindmost canal runs just in front of the ventral sucker. Short branches arise from all these canals viz., the main reserve excretory canals, median longitudinal canal and transverse canals. These branches further ramify and the ramifications extend throughout the entire body. The fine terminal twigs of these branches end in subglobular enlargements each of which contains a calcareous corpuscle shaped like a short blunt rod with round ends. These corpuscles measure 0.010-0.012 w 0.002-0.003 mm.

#### Discussion:

Of all the species hitherto recorded from India, including those described in the foregoing pages, the present form bears a close resemblance to *D. cerebralis* Chakrabarti, 1968; *D. ophthalmi* Pandey, 1970; *D. ellipticus* n.sp. and *D. lucknowensis* n.sp. The various measurements and chief characters of these species and of the pre-

Table II  
Various measurements and chief characters of *D. tulsipurensis* n.sp. and of the allied Indian species

	<i>D. cerebralis</i> Chakrabarti	<i>D. ophthalmi</i> Pandey	<i>D. ellipticus</i> n.sp.	<i>D. lucknowensis</i> n.sp.	<i>D. tulsipurensis</i> n.sp. (Present form)
Body size	0.86 - 1.23 ×	0.65 - 1.12 ×	0.65 - 0.95 ×	0.42 - 0.72 ×	0.74 - 1.30 ×
Shape and size	0.18 - 0.33 cup-like	0.25 - 0.30 circular	0.18 - 0.26 cup-like	0.20 - 0.32 cup-like	0.24 - 0.40 cup-like
Oral sucker	0.051 - 0.073 ×	0.04 - 0.06	0.05 - 0.07 ×	0.04 - 0.05 ×	0.04 - 0.07 ×
Ventral sucker	0.035 - 0.042		0.02 - 0.04	0.02 - 0.03	0.02 - 0.04
Pseudosucker	0.042 - 0.056	0.07 - 0.08	0.03 - 0.05	0.03 - 0.05	0.03 - 0.06
	absent	absent	present	present	present
Hold-fast-organ	0.12 - 0.17 ×	?	0.09 - 0.16 ×	0.06 - 0.10 ×	0.08 - 0.12 ×
Pharynx	0.05 - 0.08		0.06 - 0.09	0.04 - 0.06	0.04 - 0.07
Gen. rud.	0.02 - 0.03	0.01 - 0.03	0.02 - 0.05	0.01 - 0.03	0.01 - 0.03
Shape of excretory corpuscles	one mass elliptical	one mass oval	two masses elliptical	one mass round	one mass rod-like
Origin of main res. ex. canal	lateral side of cornua of ex. bl.	lateral side of cornua of ex. bl.	ant. end of cornua of ex. bl.	ant. end of cornua of ex. bl.	lateral side of cornua of ex. bl.

Interrogation marks indicate data lacking.

All measurements in millimetres.

ex. bl.: Excretory bladder.

res. ex. canal: reserve excretory canal.

Gen. rud. Genital rudiment.

sent form are tabulated (Table II). The present form differs from *D. ellipticus* and *D. lucknowensis* in having a spinose cuticle, and from *D. cerebralis* and *D. ophthalmi* in having pseudosuckers. From *D. ellipticus*, it also differs in the number of genital rudiments: in the present form, the genital rudiment is represented by a single mass of cells, whereas in *D. ellipticus* it is constituted by two masses. In the shape of the calcareous corpuscles, the present species differs from *D. lucknowensis* and *D. ophthalmi*: the former has blunt rod-like corpuscles, while latter two have oval corpuscles. Another important feature distinguishing the present form from both *D. ellipticus* and *D. lucknowensis* concerns the origin of the main reserve excretory canals which in the present species arise from the sides of the anterior part of the cornua of the excretory bladder and not from the ends of the cornua as they do in the other two. Lastly, all these species remarkably differ from one another in the details of the reserve excretory system.

As far as the writer has been able to consult the relevant literature on foreign species of *Diplostomulum*, the present species bears a resemblance to *D. scheuringi* originally described by Hughes (1929) from Douglas Lake fishes and later by Van Cleave and Mueller (1934) from Oneida Lake fishes. Hughes (1929) described the body of this metacercaria as being demarcated into a fore-body and a hind-body. Van Cleave and Mueller (1934), too, appear to have made a similar statement regarding the demarcation of the body (vide Hoffman, 1960). The present form differs from *D. scheuringi* in entirely lacking the division of the body into two parts. It further differs in having cuticular spines on the body and rod-like calcareous corpuscles in the reserve excretory system. Lastly, the shaped of the excretory bladder sharply distinguished the two. But have a V-shaped excretory bladder: in *D. scheuringi*, the excretory bladder has bifid cornua as evident from the figure given by Hoffman (1960), but not so in the present species. Strangely, Etges (1961) in his work on the life history of *D. scheuringi* depicts the cornua of the excretory bladder as extending anteriorly up to the esophageal bifurcation.

The present form resembles to a certain measure *D. truttae* Lal, 1953, but differs from it in lacking the division of the body, in the shape of the oral sucker and of the calcareous corpuscles. Body is not divided in the writers' form, but it is divided in *D. truttae* into a fore-body and a hind-body. Regarding the shape of the oral sucker,



it is cup-shaped in the writers' form, but circular in *D. truttae*; lastly, the excretory corpuscles are rod-like in the former, but spherical in the latter.

#### ACKNOWLEDGEMENTS

The writers have pleasure in expressing their grateful thanks to Professor P. D. Gupta, Head of the Department of Zoology, Lucknow University, for providing laboratory facilities; to Dr. Ramesh Gupta, Head of the Department of Zoology, J. N. Degree College, Lucknow, for so kindly placing his personal microscope at our disposal which made possible the study of the metacercariae infesting the fishes found in the environs of Tulsipur, and to Mother M. Madeleine, I.B.V.M. of Loreto Convent Lucknow, for translating some Spanish literature.

One of us (K K C) further feels pleasure in expressing his gratefulness to the Management and Principal of B. S. N. V. Degree College, Lucknow, for kindly allowing him to pursue the work in the Zoology Laboratory of the College, and to the authorities of the University Grants Commission, Government of India, New Delhi, for kindly sanctioning a research grant which enabled him to continue the work after he took up the present assignment in the College.

#### SUMARIO

Durante el curso de una investigación sobre infecciones por metacercarias en peces comunes de Lucknow y alrededores, y en Tulsipur (India), fueron examinados ejemplares de *Puntius ticto*, *P. stigma*, *Oxygaster bacaila*, *Mystus vittatus* y *Clarias batrachus*. Tres especies de *Diplostomum*: *D. ellipticus*, n. sp., *D. Lucknowensis*, n. sp. y *D. tulsipurensis*, n. sp. se encontraron infectando estos peces.

#### REFERENCES

- CHAKRABARTI, K. K., 1968: On a new strigeid metacercaria, *Diplostomulum cerebralis* n.sp., from an Indian fresh-water fish. *Zool. Anz.*, 181, 303-306.  
 ETGES, F. J., 1961: Contributions to the life history of the brain fluke of newts and fish, *Diplostomulum scheuringi* Hughes, 1929 (Trematoda: *Diplostomatidae*). *J. Parasit.*, 47, 453-458.

- GANPATI, P. N. and K. HANUMANTHA RAO, 1962: Ecological and life-history studies on strigeid metacercaria. (Trematoda: *Diplostomatidae*) from fresh-water fishes of Andhra Pradesh. *Parasitology*, 52, 517-525.  
 HADERLIE, E. C., 1953: Parasites of the fresh-water fishes of Northern California. *Univ. California Pub. Zool.*, 57, 303-440.  
 HOFFMAN, G. L., 1960.—Synopsis of *Strigeoidea* (Trematoda) of fishes and their life cycles. *Fishery Bull. Fish and Wildlife Service*, 60, 439-469.  
 HUGHINS, E. J., 1954.—Life history of a strigeid trematode, *Hysteromorpha triloba* (Rudolphi, 1819) Lutz. II. Sporocyst through adult. *Tran. Amer. Micr. Soc.*, 73, 221-236.  
 HUGHES, R. C., 1929.—Studies on the trematode family *Strigeidae* (*Holostomidae*). XIX. *Diplostomulum scheuringi* sp. nov. and *D. vegrandis* (LaRue). *J. Parasit.*, 15, 267-271.  
 KAW, B. L. 1950.—Studies on Helminthology: Helminth parasites of Kashmir. I. 'Trematoda'. *Indian J. Helminth.*, 2, 67-126.  
 KOMIYA, Y., 1938.—Die Entwicklung des Exkretion systems einiger Trematodenlarven aus Alster und Elbe, nebst Bemerkungen über ihren Entwicklungszyklus *Z. Parasitenk.*, 10, 340-385.  
 LAL, M. B., 1953.—A new trematode metacercaria from the eyes of trout. *Nature*, 171, 130-131.  
 ODENING, K., 1965.—Der Lebenszyklus von *Neodiplostomum spathoides* Dubois (Trematoda, *Strigeida*) im Raum Berlin nebst Beiträgen zur Entwicklungsweise verwandter Arten. *Zool. Jb. Syst.*, 92, 523-624.  
 PANDE, B. P., B. B. BHATIA and P. RAI., 1964.—On a strigeid metacercaria from *Heteropneustes fossilis* Bloch ('Singhi'). *Proc. Nat. Acad. Sci. India*, B, 34, 245-248.  
 PANDEY, K. C., 1970.—Studies on metacercariae of fresh water fishes of India. III. On two new species of *Diplostomulum* Brandes, 1892. *Proc. Indian Acad. Sci.* 72, 162-170.  
 PEARSON, J. C., 1961.—Observations on the morphology and life-cycle of *Neodiplostomum intermedium* (Trematoda: *Diplostomatidae*) *Parasitology*, 51, 133-172.  
 SILVERMAN, P. H., 1965.—Recent advances in Parasitology, 3, 159-222.  
 SINGH, R. N. 1956.—Studies on a new strigeid metacercaria parasitic in cyprinoid fishes. *Proc. Nat. Acad. Sci. India*, B, 26, 274-277.  
 SZIDAT, L. and A. NANI, 1951.—Diplostomiasis cerebri del pejerrey. Una grave epizootia que afecta la economía nacional producida por larvas de trematodes que destruyen el cerebro de los pejerreyes. *Rev. Inst. Nac. Invest. Cien. Nat. Buenos Aires Cien Zool.*, 1, 323-384.  
 REES, G., 1955.—The adult and diplostomulum stage (*Diplostomulum phoxini* Faust) of *Diplostomum palmatoides* Dubois and experimental demonstration of part of the life cycle. *Parasitology*, 45, 295-312.  
 THAPAR, G. S., 1967.—Some new larval strigeids causing black spot disease in fishes from Tungabhadra dam. *Indian J. Helminth.*, 19, 173-182.  
 \*VAN CLEAVE, H. J., and J. MUELLER, 1934.—Parasites of Oneida Lake fishes. III. A biological and ecological survey of worm parasites. *Roosevelt Wildlife Ann.*, 3, 161-373.  
 \* Not consulted in original.