STUDIES ON THE DIGENETIC TREMATODES INFECTING SOME FRESHWATER FISHES IN MALABAR, KERALA

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By

ROOPA. T.M.

PARASITOLOGY LABORATORY
DEPARTMENT OF ZOOLOGY, UNIVERSITY OF CALICUT
KERALA, 673 635, INDIA

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DEPARTMENT OF ZOOLOGY
UNIVERSITY OF CALICUT

Dr. K.P. Janardanan
Professor

Phone: Off: 401144-419
Calicut University P.O.
Kerala - 673 635
Date: 13-10-2000

CERTIFICATE

This is to certify that this thesis is an authentic record of the research work done by **Miss. Roopa, T.M.** from November 1994 to October 2000, under my supervision and guidance, that no part thereof has been presented before for any other degree and that she has passed the Ph.D. Preliminary Qualifying Examination of the University of Calicut, held in December 1996.

Dr. K.P. Janardanan

DECLARATION

I do hereby declare that the present work is original and has not been published or submitted in part or full for any degree or prize.

Calicut University,
Date: 13-10-2000

Roopa.

(Roopa, T.M.)

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ROOPA, T.M.

To My Father

CONTENTS

INTRODUCTION

Kerala, with its heavy annual rainfall and extensive system of rivers, lakes, ponds and other freshwater bodies, has a rich freshwater fish fauna. Fish forms a major source of good quality proteins in the diet of Keralites. Fish population of Kerala suffers from natural infections by digenetic trematodes, some of which are proven pathogens causing severe economic losses by rendering the flesh of food fish unpalatable. Information on the morphology and biology of digenetic trematodes is important, since it is an essential pre-requisite for formulating effective control measures against digenean infections. A review of literature showed that digenetic trematodes infecting freshwater fishes in Kerala have not been the subject of any extensive investigation. The available information is restricted to the reports on the systematics and biology of a few digeneans by Nadakal *et al.* (1969) and Mohandas (1973). I undertook the present investigation with a view to throwing more light on the morphology and biology of digeneans infecting freshwater fishes in Malabar.

The present investigation revealed that 22 out of 30 species of freshwater fishes examined were infected by 15 genera of digenetic trematodes and an unidentified echinostome metacercaria. These include 5 species of adults belonging to *Transversotrema, Caballeroia, Neopodocotyle, Haplorchoides* and *Genarchopsis,* and 13 species of metacercariae belonging to *Tetracotyle, Diplostomulum, Neascus, Diplostomum, Euclinostomum, Echinochasmus, Renicola, Encyclometra, Centrocestus, Haplorchoides* and

Acanthostomum. Life-cycles of *Diplostomum ketupanense, Transversotrema patialense, Haplorchoides mehrai* and *Acanthostomum burminis* have been established. The adults of *Echinochasmus megavitellus* and *Centrocestus formosanus* have been raised in the laboratory from their metacercariae. The echinostome metacercaria has been developed from its cercaria. Data on prevalence and intensity of digenean infections among freshwater fishes in Malabar have been collected and presented.

HISTORICAL REVIEW

Digenetic trematodes belonging to 15 genera and an unidentified echinostome metacercaria have been recovered from freshwater fishes of Malabar during the course of the present investigation. These include the metacercariae belonging to *Tetracotyle, Diplostomulum, Neastus, Diplostomum, Euclinostomum, Echinochasmus, Renicola, Encyclometra, Centrocestus* and *Acanthostomum;* the adults of *Transversotrema, Caballeroia, Neopodocotyle* and *Genarchopsis,* and both the metacercaria and adult of *Haplorchoides.* A brief review of literature of all these genera is presented here.

Larval Strigeoids

Hoffman (1961), in a synoptical review, placed the strigeoid metacercariae in four larval genera, namely, *Tetracotyle, Diplostomulum, Neascus* and *Prohemistomulum.* If the adult is not known, a strigeoid metacercaria may be placed in one of the four larval genera. As I came across the first three genera during the present investigation, a review of the 3 genera is presented here.

Tetracotyle Fillipi, 1859

The larval genus *Tetracotyle* was established by Fillipi in 1859. The characters of the genus, as given by Hoffman (1961), are: (1) fore-body oval or ovate oblong in contour and relatively thick, concave ventrally or cup-shaped; (2) hind-body present as a short rounded prominence at the

posterior end of the fore-body, sometimes inconspicuous; (3) reserve bladder consists of a large continuous space occupying the dorsal and lateral regions of the fore-body, with a sheet-like extension into the ventral lip of the anterior suctorial pocket, with small spherical calcareous concretions in the reserve excretory vessels and mostly in the anterior part of the worm; (4) a pair of lateral pseudosuckers (cotylae) on the antero-lateral edges beside the oral sucker; and (5) a true cyst of parasite origin.

The first report of a *Tetracotyle* species from India was presented by Kaw (1950) from the frog, *Rana cyanophlyctis* in Kashmir. As far as is known, 20 species of this larval genus have been described from freshwater fishes in India. These are, *T. indicus* Singh, 1957; *T. sophorensis* Singh, 1959; *T. ujjainensis* Trivedi, 1964; *T. muscularis* Chakrabarti, 1970; *T. xenentodoni* Chakrabarti, 1970; *T. glossogobi* Chakrabarti, 1970; *T. szidati* Chakrabarti and Baugh, 1970; *T. lali* Pandey, 1971; *T. lucknowensis* Pandey, 1971; *T. baughi* Pandey, 1973; *T. tandoni* Pandey, 1973; *T. aglandulata* Baugh and Chakrabarti, 1977; *T. gyanpurensis* Agrawal and Singh, 1980; *T. pandei* Agrawal and Khan, 1982; *T. srivastavai* Agrawal and Khan, 1982; *T. ramalingi* Agrawal and Khan, 1982; *T. simhai* Pandey and Tewari, 1983; *T. sanjivi* Pandey and Tewari, 1983; *T. fotedari* Tewari, 1983, and *T. satendri* Tewari and Tyagi, 1986.

Diplostomulum Brandes, 1892

Diplostomulum Brandes, 1892 is another larval genus, the adults of which are mainly parasitic in piscivorous birds. The adult belongs to the genus *Diplostomum* von Nordmann, 1832. The characters of *Diplostomulum*, as given by Hoffman (1961), are: (1) fore-body foliaceous, concave

ventrally; (2) hind-body present as a small conical prominence on the postero-dorsal part of the fore-body; (3) reserve system (bladder) consists of a system of more or less definitely arranged tubules with calcareous corpuscles, round or ellipsoidal, disposed in vesicles at the termini of small branches; (4) usually a pair of lateral suckers on the antero-lateral edges beside the oral sucker, and (5) no true cyst of parasite origin.

Kaw (1950) reported *Diplostomulum bufonis* from *Bufo viridis*, and this forms the first report of the larval genus from India. Altogether 4 species of *Diplostomulum* have been reported from freshwater fishes of India. Pandey *et al.* (1964) described *D. singhi* from *Heteropneustes fossilis;* Thapar (1967) described *D. nurius* from *Nuria daurica*, and Pandey (1970) recorded two species, *D. minutum* from *Puntius* sp. and *D. ophthalmi* from *P. sophore, P. ticto, Oxygaster bacaila, Trichogaster fasciatus* and *H. fossilis.*

Neascus Hughes, 1927

Hughes (1927) established the larval genus *Neascus* to contain the metacercariae, *N. ambloplitis* and *N. cuticola.* The characters of the genus *Neascus*, as given by Hoffman (1961), are: (1) fore-body much like *Diplostomulum*; (2) hind-body more extensively developed than in *Diplostomulum*; (3) reserve bladder more extensively developed than in *Diplostomulum* and with calcareous granules not confined to the termini of the small branches which do not end blindly but constitute anastomoses; (4) no lateral pseudosuckers or earlike processes, and (5) generally encysted with a true cyst of parasite origin.

Kaw (1950) reported *Neascus vetastai* from the freshwater fishes, *Schizothorax esocinus, S. micropogon, S. niger* and *Oreinus sinuatus,* and this forms the first report of the larval genus from India. In addition to this, 15 species have been reported from Indian fishes. These are, *N. elongatus* Singh, 1957; *N. chelai* Khera, 1958; *N. indicus,* Thapar, 1967; *N. cirrhinus* Thapar, 1967; *N. hepatica* Chakrabarti, 1970; *N. channai* Pandey, 1971; *N. xenentodoni* Pandey, 1971; *N. gussevi* Chakrabarti, 1974; *N. hoffmani* Baugh and Chakrabarti, 1977; *N. hanaksagarensis* Baugh and Chakrabarti, 1977; *N. chauhani* Agrawal and Khan, 1982; *N. hanumanthai* Agrawal and Khan, 1982; *N. moghei* Agrawal and Khan, 1982; *N. simhai* Agrawal and Khan, 1982, and *N. punctatusi* Dhanumkumari, 1994.

Genus *Diplostomum* von Nordmann, 1832

The genus *Diplostomum* von Nordmann, has more than 40 species described from all over the world on the basis of adult forms or metacercariae (Yamaguti, 1971). Of these, 4 species are known from India: *D. butei* Vidyarthi, 1937 in *Buteo rufinus; D. ketupanense* Vidyarthi, 1937 in *Ketupa zeylonensis hardwicki; D. oedicnemus* Singh, 1956 in *Burhinus oedicnemus indicum,* and *D. sterni* Gupta, 1958 in *Sterna aurantia.* Studies on life-cycles of *Diplostomum* have involved some 13 species, mostly those occurring in Europe (Niewiadomska, 1996). Complete life-cycle of an Indian species has not so far been elucidated. The available information is restricted to the reports on the metacercaria of *D. ketupanense* by Ganapati and Rao (1954) from *Catla catla* in Andhra Pradesh and Abraham and Anantaraman (1955) from the same fish in Madras and its miracidium by Ganapati and Rao (1962) from Andhra Pradesh.

Genus *Euclinostomum* Travassos, 1928

Rudolphi (1809) described *Distoma heterostomum* from *Ardea purpurea,* and Braun in 1899 transferred it to the genus *Clinostomum* Leidy, 1856 and called it *C. heterostomum.* Travassos (1928) established the genus *Euclinostomum,* shifted *C. heterostomum* to the new genus as its type, and renamed it *E. heterostomum* (Rudolphi, 1809). Since then several species have been added to the genus by various workers. Ukoli (1966), and Jhansilakshmibai and Madhavi (1997) considered only 4 species, *E. heterostomum* (Rudolphi, 1809) Travassos, 1928; *E. multicaecum* Tubangui and Masilungan, 1935; *E. minutus* Zaidi and Khan, 1975, and *E. ardeolae* El. Naffar and Khalifa, 1981, as valid. Before Ukoli's (1966) work, a total of 5 *Euclinostomum* spp. have been reported from India either as metacercariae or as adults. These are *E. heterostomum* (Rudolphi, 1809) Travassos, 1928; *E. indicum Bhalerao,* 1942; *E. heptacaecum* Jaiswal, 1957; *E. bhagavantami* Jaiswal, 1957, and *E. channai* Jaiswal, 1957. Now *E. heterostomum* is the only valid species of *Euclinostomum* known from the Indian subcontinent (Ukoli, 1966). Besides, it is a common parasite of piscivorous birds in many other parts of Asia and in Europe and Africa. The life-cycle of *E. heterostomum* has been elucidated by Donges (1974) and Jhansilakshmibai and Madhavi (1997) from Nigeria and India respectively. Metacercariae of this species have been reported from Indian freshwater fishes by Bhalerao (1942), Srivastava (1950), Jaiswal (1957), Agarwal (1959), Gupta and Agarwal (1983) and Sinha *et al.* (1988). Life-cycles have also been established for *E. minutus* in Pakistan (Zaidi and Khan, 1975) and *E. ardeolae* in Egypt (El. Naffar and Khalifa, 1981).

Genus *Transversotrema* Witenberg, 1944

Witenberg (1944) proposed the genus *Transversotrema* with *T. haasi* infecting an unidentified fish from Red Sea as the type species. Subsequently, 7 species were added to the genus. These are *T. koliense* (Olivier, 1947) Yamaguti, 1958; *T. laruei* Velasquez, 1958; *T. patialense* (Soparker, 1924) Crusz and Sathananthan, 1960; *T. licinum* Manter, 1970; *T. soparkari* Pande and Shukla, 1972; *T. chackai* Mohandas, 1973, and *T. chauhani* Agrawal and Singh, 1981. Of these, *T. patialense, T. soparkari, T. chackai* and *T. chauhani* are recorded from Indian fishes. *T. patialense* was first described in its cercarial form as *Cercaria patialensis* by Soparkar(1924) from *Melanoides tuberculatus* in Punjab. Subsequently, this cercaria was reported from the same snail species in Nellore by Anantaraman (1948), in Waltair by Rao and Ganapati (1967), in Ceylon by Crusz (1956) and in Malaysia by Betterton (1979), and from *M. anomala* in the Belgian Congo by Brien (1954). Crusz and Sathananthan (1960) recorded the metacercaria of *C. patialensis* from *Macropodus cupanus* in Ceylon and designated it *T. patialense,* and Crusz *et al.* (1964) worked out its life-cycle with *M. tuberculatus* as the first intermediate host and *M. cupanus, Ophiocephalus punctatus* and *Tilapia mossambica* as the fish hosts. They preferred to call the stages recovered from fishes as "mature metacercariae." Rao and Ganapati (1967) also gave a detailed account of the cercaria of this species from *M. tuberculatus,* and adults from *Esomus danricus, Panchax panchax* and *Catla catla.* Further, they suggested that there may not be a metacercarial stage in the life-cycle of *Transversotrema* spp. Later, Murty and Rao (1968) reported the adults of *T. patialense* from *P. panchax;* Madhavi (1980) from

Aplocheilus panchax,and Rekharani and Madhavi (1985) from *Mugil cephalus, Liza macrolepis* and *Valamugil cunnesius*. The miracidium of *T. patialense* was reported by Madhavi and Jhansilakshmibai (1994). Two other species of transversotrematid cercariae were reported from India, namely *C. chackai* Nadakal, Mohandas and Sunderaraman, 1969 ,and *C. soparkari* Pandey, 1971.

Genus *Echinochasmus* Dietz, 1909

Dietz (1909) erected the genus *Echinochasmus* with *E. coaxatus* from *Colymbus crystatus, C. griseigena, C. nigrocollis, C. caspicus, Ciconia ciconia* and *Anas platyrhyncha* as its type species. Altogether 39 species of *Echinochasmus* have been reported. Of these 10 species are from India: *E. perfoliatus* (V. Ratz, 1908) Dietz, 1909 from man, dog and cat; *E. bagulai* Verma, 1935 from the pond heron, *Ardeola grayii*, and the night heron , *Nycticorax nycticorax; E. ruficapensis* Verma, 1935 from the Indian little grebe, *Podiceps ruficollis; E. narayani* Mudaliar, 1938 from *Milvus migrans govinda; E. megavitellus* Lal, 1939 from *A. grayii; E. famelicus* (Odhner, 1911) Prudhoe, 1944 from the stork, *Leptoptilos javanicus; E. antigonus* Gupta, 1953 from *Antigone antigone; E. minutus* Karyakarte, 1970 from *Milvus migrans; E. vindhianae* Vasudev, 1973 from *Aquila rapax vindhiana,* and *E. megadermi* Salem, 1975 from *Megaderma lyra.*

Life-cycles of about 16 species of this genus have been established. From India information on life-cycles is restricted to *E. bagulai.* Ramalingam (1960) described the life-cycle from marine environment. But Yamaguti (1971) expressed doubts regarding the identity of adult flukes obtained by Ramalingam to *E. bagulai.* Life-cycle of the same species was

reported from freshwater environment by Dhanumkumari *et al.* (1991). Nath and Pande (1970a) and Madhavi (1980) reported metacercariae of *Echinochasmus* from a variety of fishes.

Genus *Caballeroia* Thapar, 1960

The genus *Caballeroia* was established by Thapar (1960) with *C. indica* from *Cirrhina fulungel* from Karnataka as its type species. Achan (1956) described *Nicollodiscus bhavani* from *Barbus hexagonolepis* from Tamil Nadu. Devraj (1972) redescribed this species and shifted it to *Caballeroia* and named it as *C. bhavani.* Srivastava (1982) agreed with this arrangement and considered this species identical to *C. indica* described by Thapar (1960) and suggested that according to the rule of priority, *C. bhavani* should become the type species of the genus. This genus remains monotypic.

Genus *Renicola* Cohn, 1904

The genus *Renicola* was proposed by Cohn (1904) with *R. pinguis* as the type species which was described by Mehlis (cited in Creplin, 1846) as *Monostomum pingue* from the crested grebe, *Podiceps cristatus.* Since then more than 47 species have been added to the genus *Renicola* from several parts of the world (Yamaguti, 1971). The adults of this genus have not so far been reported from India. The available information is limited to the description of a renicolid metacercaria by Madhavi (1980) from *Aplocheilus melastigma.*

Genus *Encyclometra* Baylis and Cannon, 1924

Rudolphi (1819) described *Distoma colubrimurorum* from *Coluber murorum*. In 1924, Baylis and Cannon proposed the genus *Encyclometra* with *E. natricis* as its type. Subsequently Dollfus (1929) shifted *D. colubrimurorum* to *Encyclometra*, and renamed it *E. colubrimurorum*. Further, Dollfus synonymized *D. allostomum* Diesing, 1850; *D. caudatum* Polonio, 1859; *D. subflavum* Sonsino, 1892; *Distoma* sp. No.I. Timotheev, 1900; *Odhneria bolognensis* Baer, 1924; *Paraplagiorchis timotheevi* Dollfus, 1924; *Orthorchis natricis* Mödlinger, 1924; *Encyclometra natricis* Baylis and Cannon, 1924, and *E. bolognensis* (Baer, 1924) Baylis and Cannon,1924 with *E. colubrimurorum*. Thus *E. colubrimurorum* (Rudolphi, 1819) Dollfus, 1929 becomes the type species of the genus. Several species have been added to the genus *Encyclometra*, and synonymy of species appears to be great, but complete accord as to which are valid is lacking. While reviewing the genus, *Encyclometra*, Yeh (1958) recognized only three species, *E. colubrimurorum*, *E. asymmetrica* Wallace, 1936, and *E. japonica* Yoshida and Ozaki, 1929, as valid. In 1958 Yamaguti listed 5 species under the genus *Encyclometra*. These are *E. colubrimurorum* (Rudolphi, 1819) Dollfus, 1929; *E. caudata* (Polonio, 1859) Dollfus, 1929; *E. japonica* Yoshida and Ozaki, 1929; *E. koreana* Park, 1940, and *E. vitellata* Gupta, 1954. Of these, Dollfus (1963) recognized *E. colubrimurorum* and *E. asymmetrica*, and synonymized *E. japonica* with *E. colubrimurorum*. Later, Srivastava and Ghosh (1968) added another species, *E. bungara* to the genus. According to Yamaguti (1971) the genus *Encyclometra* is represented by three valid species: *E. colubrimurorum*, *E. asymmetrica* and *E. bungara*. All the 3 species have been

recorded from different hosts in India (Bhalerao, 1926, 1936; Mehra, 1931; Gupta, 1954; Srivastava and Ghosh, 1968; Dwivedi and Chauhan, 1970).

Complete life-cycle of the genus has not so far been worked out. The available information is restricted to reports on the metacercariae of *E. colubrimurorum* and *E. asymmetrica* by Yamaguti (1936) and Chiang (1951) respectively. Till date there is no report on the metacercaria of *Encyclometra* from India.

Genus *Neopodocotyle* Dayal, 1950

The genus *Neopodocotyle* was established by Dayal (1950) with *N. indica* from *Callichrous bimaculatus* in Lucknow as its type species. Yamaguti (1958) reduced *Neopodocotyle* to a subgenus under *Podocotyle* (Dujardin, 1845) Odhner, 1905. However, Sircar and Sinha (1969) disagreed with this proposal and considered *Neopodocotyle* as a valid genus. Pritchard (1966) shifted *N. indica* to the genus *Allocreadium* Looss, 1900, and called it *A. indica*, but Pandey (1973a) disagreed with this view and retained it under *Neopodocotyle*. Later, Soota and Ghosh (1977) described *N. indica* Dayal, 1950 from *Tor tor* in Shillong. Gupta and Chakrabarti (1966) described *N. lucknowensis* from *Puntius sarana*. But Baugh and Chakrabarti (1970) established another genus *Puntiotrema* and transferred *N. lucknowensis* to the new genus and named it *P. lucknowensis*. In addition to the type species, the genus at present includes four more species. These are, *N. spinipora* Sircar and Sinha, 1969 from *Rita rita*; *N. mehrai* Rai, 1971 from *Puntius sarana* and *P. sophore*; *N. ballianensis* Pandey, 1973 from *Labeo calbasu*, and *N. dayali* Pandey, 1973 from *P. sarana*. The genus is restricted to India.

Genus *Centrocestus* Looss, 1899

Looss (1899) proposed the genus *Centrocestus* and designated *C. cuspidatus* as the type species which was described by him in 1896 as *Distomum cuspidatum*. Leiper (1913) described *C. cuspidatus caninus* which was considered a synonym of *C. cuspidatus* by Ransom (1921). Tanabe (1922) established another genus *Stamnosoma* to receive *S. armatum* which was closely similar to *Centrocestus*. Nishigori (1924a) added another species, *S. formosanum*, to the genus *Stamnosoma*. Faust and Nishigori (1926) considered *C. cuspidatus caninus* as a synonym of *Stamnosoma formosanum*. But Chapin (1926), Fuhrmann (1928), Witenberg (1929), Price (1932), and Stiles and Baker (1934) treated *Stamnosoma* as a synonym of *Centrocestus*. Later, Faust (1937) suppressed the genus *Stamnosoma* in favour of *Centrocestus*.

Among the species of *Centrocestus* described by various authors, Chen (1942) recognised only 3 species as valid: *C. cuspidatus* (Looss, 1896) Looss, 1899; *C. armatus* (Tanabe, 1922) Price, 1932, and *C. formosanus* (Nishigori, 1924) Price, 1932. Of these, only one species *C. formosanus*, has been reported from India. A great deal of information is available on the life-cycle of *C. formosanus*. Nishigori (1924b) first traced its life-cycle from Formosa and reported *Semisulcospira libertina* as the first intermediate host, 13 species of freshwater fishes as second intermediate hosts, and the night heron, *Nycticorax nycticorax* as the definitive host. Subsequently Chen (1942, 1948), and Zhang *et al.* (1985) worked out the life-cycle of this species from China, and Martin (1958) from Hawaii. From India Dhanumkumari *et al.* (1993) illustrated its life-cycle with *Thiara tuberculata* as the snail host,

Channa punctatus, Gambusia affinis and *Aplocheilus panchax* as second intermediate hosts, and *Tatera indica* and *Ardeola grayii* as definitive hosts. They obtained experimental infections in leghorn chicks.

Ganapati and Rao (1983) described the cercaria of this species from *Melanoides tuberculatus* in India. There have also been several reports of the occurrence of metacercariae of this species from freshwater fishes in India (Nath and Pande, 1970b; Pande and Shukla, 1972 ; Nath, 1974; Premvati and Pande, 1974; Madhavi, 1980). Rekharani and Madhavi (1985) recorded the metacercaria from brackishwater fishes of Visakhapatnam. Several workers have experimentally raised the adult flukes in various birds and mammals (Nath and Pande, 1970b; Nath, 1972a, 1974; Pande and Shukla, 1972 ; Premvati and Pande, 1974).

Genus *Haplorchoides* Chen, 1949

The genus *Haplorchoides* was proposed by Chen (1949) to accommodate some of the species of *Haplorchis* Looss, 1899. The type species was first desribed as *Distomum cahirinum* by Looss (1896) from *Bagrus bayad* in Egypt. Because of several errors in the original description, Looss (1899) redescribed it from *B. bayad* and *B. docmac,* and shifted it to *Haplorchis.* Later, Chen (1949) transferred it to the new genus *Haplorchoides,* making it the type species, and also moved Srivastava's (1935) 4 species, *Haplorchis attenuatus, H. piscicola, H. gangeticus* and *H. silundii,* and one species of Dayal (1935), *Monorchotrema takree,* to *Haplorchoides.* Subsequently additional species were described by Gupta (1955), Chatterji (1956) and Agrawal (1964), but Rai and Pande (1967) in a review of the genus considered only two Indian species, *H. attenuatus* and *H. piscicola,* as valid.

Yamaguti (1971) listed 12 Indian species under the genus. The twelve species are, *H. attenuatus* (Srivastava, 1935) Chen, 1949 from *Macrones seenghala; H. gangeticus* (Srivastava, 1935) Chen, 1949 from *Pseudotropius athenoides; H. piscicola* (Srivastava, 1935) Chen, 1949 from *Eutropiichthys vacha; H. silundii* (Srivastava, 1935) Chen, 1949 from *Silundia gangetica; H. takree* (Dayal, 1935) Chen, 1949 from *Pseudotropius takree; H. brahmaputraensis* Gupta, 1955 from *Rita rita; H. gomtioensis* Gupta, 1955 from *Silundia gangetica; H. ritai* Gupta, 1955 from *R. rita; H. seenghali* Gupta, 1955 from *Macrones seenghala; H. parini* (Chatterji, 1957) Yamaguti, 1971 from *Wallago attu; H. macrones* (Dayal, 1949) Yamaguti, 1958 from *M. seenghala,* and *H. macronis* Agrawal, 1964 from *M. seenghala.* Pande and Shukla (1976) described 2 new species, *H. pearsoni* and *H. mehrai* from *Channa punctatus* and *Mystus vittatus* respectively, the metacercariae of which were recorded from fingerlings of over a dozen species of freshwater fishes. Gupta and Gupta (1977, 1979) added 2 new species, *H. vinodei* and *H. guptai* to the genus, but Mehra (1980) considered only two Indian species, *H. attenuatus* and *H. piscicola,* as valid. Subsequently, *H. vacha* Agrawal and Agrawal, 1981; *H. mystusi* Gupta and Singh, 1982; *H. kherai* Gupta and Govind, 1985, and *H. srivastavai* Gupta and Govind, 1985 have been reported from India. Shameem and Madhavi (1988) considered only 3 Indian species, *H. attenuatus, H. pearsoni* and *H. mehrai,* as valid.

Studies on life-cycles of the genus have been restricted to three species. El-Naffar (1980) elucidated the life-cycle of *H. cahirinus.* Agrawal and Agrawal (1981) established the life-cycle of *H. vacha,* and Shameem and Madhavi (1988) that of *H. mehrai.* In addition, metacercariae of a few

species of *Haplorchoides* have been recovered from several freshwater fishes of India. Nath (1972b) reported the metacercaria of *H. attenuatus* from *Puntius sophore* and it forms the first report of metacercaria of *Haplorchoides* from India. Pande and Shukla (1976) reported the metacercariae of *H. pearsoni* and *H. mehrai* from fingerlings of over a dozen species of freshwater fishes. Pande (1979) studied the distribution of metacercariae of *H. attenuatus* infecting *Mystus seenghala* in fingerlings of 15 species of edible fishes.

Genus *Acanthostomum* Looss, 1899

Looss (1899) created the genus *Acanthostomum* with *A. spiniceps* (Looss, 1896) from *Bagrus bayad* as its type. Altogether 27 species of *Acanthostomum* have been reported: 9 from fishes and 18 from reptiles (Yamaguti, 1971). Bhalerao (1926) described *Acanthochasmus burminis* from the water snake, *Tropidonotus piscator* in Burma, and Thapar and Ali (1929) recovered it from the same host in Lucknow. In 1936 Bhalerao shifted *A. burminis to Acanthostomum*, and named it *Acanthostomum burminis*. The same author (1940) proposed a new genus *Atrophecaecum* to accommodate this species; but Mehra (1980) and Srivastava (1982) suppressed this genus and considered *Acanthostomum burminis* as valid. Further, they treated *Acanthostomum simhai* Khalil, 1963; *Atrophecaecum hindustanensis* Baugh, 1956; *A. indicum* Simha, 1958, and *Haplocaecum proctoporum* Dwivedi, 1966 as synonyms of *Acanthostomum burminis*. Only two species of *Acanthostomum* are known from India: *A burminis* (Bhalerao, 1926) Bhalerao, 1936 from *Tropidonotus piscator*, and *A. indicum* Sinha, 1942 from a crocodile.

Of the 27 species of *Acanthostomum*, life-cycles of only 4 species have been elucidated: *A. imbutiforme* by Maillard (1973), and *A. gnerii* by Ostrowski de Nunez (1991), both parasitizing fishes, *A. brauni* by Ostrowski de Nunez (1987), and *A. burminis* by Roopa and Janardanan (1998), both infecting reptiles. Madhavi (1980) reported the metacercaria of *A. burminis* from *Aplocheilus panchax*, and Rekharani and Madhavi (1985) from *Mugil cephalus, Liza macrolepis* and *Valamugil cunnesius* in Andhra Pradesh, and Roopa and Janardanan (1998) from *Rasbora daniconius, Puntius parrah, P. melanampyx melanampyx, Mystus oculatus, M. malabaricus, Heteropneustes fossilis, Channa orientalis, Etroplus maculatus* and *Garra mullya* in Kerala.

Genus Genarchopsis Ozaki, 1925

Ozaki (1925) erected the genus *Genarchopsis* with *G. goppo* from *Mogurnda obscura* as its type species. Srivastava (1933) proposed the genus *Ophiocorchis* with its type *O. lobatum* from the stomach of *Ophiocephalus striatus*, and added another species, *O. singularis* from the intestinal caeca of the same host. He recorded two species belonging to *Progonus, P. piscicola* and *P. ovocaudatum*, from *O. punctatus*. Gupta (1951) added 3 more species to *Ophiocorchis*: *O. dasus* and *O. indicus* from the stomach of *Ophiocephalus punctatus*, and *O. faruquis* from the intestine of *Mastacembelus armatus*. Manter (1938) shifted *P. piscicola* and *P. ovocaudatum* to *Genarchopsis* , and renamed them *G. piscicola* and *G. ovocaudata* respectively. Yamaguti (1954) considered *Ophiocorchis* as a synonym of *Genarchopsis* and transferred Srivastava's two species, *O. lobatum* and *O. singularis*, to *Genarchopsis* and in 1958 shifted Gupta's three species, *O. dasus, O. indicus* and *O. faruquis*, to *Genarchopsis*. Till date thirteen species of *Genarchopsis* have been reported

from India. Of these, 11 species, *G. piscicola* (Srivastava, 1933) Manter, 1938; *G. ovocaudata* (Srivastava, 1933) Manter, 1938; *G. lobata* (Srivastava, 1933) Yamaguti, 1954; *G. singularis* (Srivastava, 1933) Yamaguti, 1954; *G. dasus* (Gupta, 1951) Yamaguti, 1958; *G. indica* (Gupta, 1951) Yamaguti, 1958; *G. faruquis* (Gupta, 1951) Yamaguti, 1958; *G. punctati* Agrawal, 1966; *G. cuchiai* Kakaji, 1969; *G. cameroni* Kakaji, 1969, and *G. wallagoni* Chandra and Banerjee, 1993, are from freshwater fishes, one species *G. melanostictus* Dwivedi, 1965, from a toad, and the other species, *G. thapari* Dwivedi, 1965, from a water snake. Rai (1971a) synonymized 8 Indian species of *Genarchopsis* with *G. goppo*. Pandey (1973b) agreed with this view and further synonymized 4 Indian species as well as *G. ozakii* Basheerullah and Elahi, 1972 and *G. bangladesensis* Basheerullah and Elahi, 1972 from Bangladesh with *G. goppo*. At present the genus *Genarchopsis* is represented by 7 species: *G. goppo* Ozaki, 1925; *G. anguillae* Yamaguti, 1938; *G. gigi* Yamaguti, 1939; *G. muelleri* (Levinsen, 1881) Yamaguti, 1954; *G. macrocotyle* Coil and Kuntz, 1960; *G. bashiri* Hafizuddin and Khan, 1973, and *G. wallagoni* Chandra and Banerjee, 1993. Of these, only 2 species, *G. goppo* and *G. wallagoni*, are recorded from India.

The life-cycle of *G. goppo* infecting the stomach of the freshwater fish, *Channa punctata* was worked out by Madhavi (1978). She recovered the cercaria of this fluke from the snail, *Amnicola travancorica*, and the metacercaria from the ostracods, *Stenocypris malcolmsoni* and *Eucypris capensis*. Recovery of only immature stages of the adult fluke from *Aplocheilus panchax* led her to believe that this fish serves as a paratenic host.

MATERIALS AND METHODS

Collection and maintenance of fish hosts:

Freshwater fishes, collected from rivers, streams, irrigation canals, ponds and paddyfields in Kasargod, Kannur, Kozhikode, Wayanad, Malappuram, Palakkad and Thrissur districts of Kerala, were used during the course of this investigation. Collections were made from January 1995 through July 1998. A list of the fishes examined, with their localities, period of collection and number examined, is presented here.

List of fish hosts examined for digenetic trematodes

Classification and name of hosts	No. of host specimens examined	Locality of collection	Period of collection
Pisces **Cypriniformes** **Cyprinidae** *Esomus barbatus* (Jerdon)	360	Kanhangad and Theerthankara in Kasargod district; Karetta, Kanhilari, Nirmalagiri, Pinarayi, Kuttimakkul and Eranholy in Kannur district; Nallur, Ramanattukara, Feroke and Puthukode in Kozhikode district; Nedungottumedu, Thalapara, Calicut University Campus and Kadakkattupara in Malappuram district	February to December 1995; January 1996; March to November 1996; June to December 1997; April 1998

Classification and name of hosts	No. of host specimens examined	Locality of collection	Period of collection
Danio aequipinnatus McClelland	295	Kanhangad in Kasargod district; Karetta, Kanhilari and Nirmalagiri in Kannur district; Vythiri, Meenangadi, Sultan Bathery and Noolpuzha in Wayanad district; Nedungottumedu in Malappuram district	September to December 1995; May and September 1996; September-November, 1997; February and July 1998
D. spinosus Day	15	Karetta in Kannur district; Vythiri and Sultan Bathery in Wayanad district; Calicut Univeristy Campus in Malappuram district	November 1995; July to October 1996; September and December 1997
Horadandia atukorali Deraniyagala	162	Kuttimakkul in Kannur district; Puthukode, Nallur and Ramanattukara in Kozhikode district; Sultan Bathery in Wayanad district; Valapad in Thrissur district	June to December 1995; January to October 1996; December 1996 to September 1997; November 1997 to May 1998
Rasbora daniconius (Hamilton)	622	Kanhangad and Theerthankara in Kasargod district; Pallikkunnu, Chirakkal, Karetta, Kanhilari, Nirmalagiri, Kuttimakkul, Eranholy and Pinarayi in Kannur district; Devagiri, Feroke, Ramanattukara, Nallur, Puthukode and Kundayithode in Kozhikode district; Vythiri, Meenangadi,	January to June and September to December 1995; February to December 1996; January to November 1997; January to March 1998

Classification and name of hosts	No. of host specimens examined	Locality of collection	Period of collection
		Sultan Bathery and Pakkam in Wayanad district; Nedungottumedu, Thalapara, Calicut University Campus and Kadakkattupara in Malappuram district	
Puntius filamentosus (Valenciennes)	21	Devagiri in Kozhikode district; Thalapara and Nedungottumedu in Malappuram district; Malampuzha in Palakkad district	October 1995; November 1996; February and November 1997; January 1998
P. melanampyx melanampyx (Day)	122	Kanhangad in Kasargod district; Nirmalagiri Kuttimakkul, Kanhilari and Karetta in Kannur district; Thamarassery, Kundayithode and Puthukode in Kozhikode district; Vythiri and Sultan Bathery in Wayanad district; Nedungottumedu, Kadakkattupara and Calicut University Campus in Malappuram district.	March to May and November-December 1995; February and May 1996; May 1997, August to November 1997.
P. parrah Day	420	Kuttimakkul, Chirakkal and Karetta in Kannur district; Feroke, Puthukode and Ramanattukara in Kozhikode district; Pakkam, Vythiri, Meenangadi and Sultan	January to September 1995; November 1995 to May 1996; August to December 1996; February to May and October to

Classification and name of hosts	No. of host specimens examined	Locality of collection	Period of collection
		Bathery in Wayanad district; Nedungottumedu and Thalapara in Malappuram district	December 1997; March to May 1998
P. sarana sarana (Hamilton)	2	Noolpuzha in Wayanad district	February 1998
P. sophore (Hamilton)	60	Kuttimakkul in Kannur district; Thalapara in Malappuram district	July to September 1995; March to September 1996; July to November 1997; March 1998
Labeo rohita (Hamilton)	62	Malampuzha in Palakkad district	November 1996; November 1997
Catla catla (Hamilton)	12	Malampuzha in Palakkad district	November 1997
Cirrhina mrigala (Hamilton)	2	Malampuzha in Palakkad district	November 1997
Garra mullya (Sykes)	9	Nedungottumedu in Malappuram district	August 1996
Cobitidae			
Somileptes gongota (Hamilton)	25	Karetta, Nirmalagiri and Kuttimakkul in Kannur district; Puthukode in Kozhikode district; Sultan Bathery in Wayanad district; Nedungottumedu in Malappuram district	January to May and October to December 1995; February to May 1996; December 1997
Siluriformes			
Bagridae			
Mystus malabaricus (Jerdon)	10	Kuttimakkul, Karetta and Nirmalagiri in Kannur district; Puthukode in Kozhikode district; Nedungottumedu in Malappuram district	March and December 1995; February and April 1996; July to September and December 1997

Classification and name of hosts	No. of host specimens examined	Locality of collection	Period of collection
M. oculatus (Valenciennes)	31	Thenhipalam in Malappuram district	October 1996
Clariidae			
Clarias batrachus (Linnaeus)	1	Malampuzha in Palakkad district	November 1996
C. dussumieri dussumieri Valenciennes	6	Thamarassery in Kozhikode district	May 1997
Heteropneustidae			
Heteropneustes fossilis (Bloch)	38	Kanhangad in Kasargod district, Pallikkunnu, Kuttimakkul, Karetta and Nirmalagiri in Kannur district; Thamarassery in Kozhikode district; Thenhipalam and Nedungottumedu in Malappuram district	August 1995; February 1996; July to November 1996; March to July and October 1997; April 1998
Atheriniformes			
Belonidae			
Xenentodon cancila Hamilton	69	Kanhilari in Kannur district; Puthukode in Kozhikode district; Valapad in Thrissur district	March 1996; September 1996; May 1997
Cyprinodontidae			
Aplocheilus lineatus (Valenciennes)	14	Kanhilari in Kannur district	November 1995 to May 1996; July to October 1996; May and September 1997; April 1998
A. panchax (Hamilton)	120	Kuttimakkul and Karetta in Kannur district; Puthukode, Nallur, Feroke, Ramanattukara, Devagiri and Kundayithode in	March to June, August, October to December 1995; March to May 1996; March to July and

Classification and name of hosts	No. of host specimens examined	Locality of collection	Period of collection
		Kozhikode district; Vythiri and Sultan Bathery in Wayanad district; Nedungottumedu, Thenhipalam and Thalapara in Malappuram district	September to November 1997
Channiformes			
Channidae			
Channa orientalis (Schneider)	42	Feroke, Ramanattukara and Puthukode in Kozhikode district; Sultan Bathery in Wayanad district; Nedungottumedu in Malappuram district;	March to December 1995; May and November 1996; April to July 1997; February 1998
C. punctatus (Bloch)	22	Kanhilari and Kuttimakkul in Kannur district; Feroke, Puthukode and Nallur in Kozhikode district; Sultan Bathery in Wayanad district; Nedugottumedu and Calicut University Campus in Malappuram district	April-May and August to October 1995; February to May 1996; November 1997; February 1998
Cichlidae			
Etroplus maculatus (Bloch)	25	Kuttimakkul in Kannur district; Nallur, Feroke, Ramanattukara and Kundayithode in Kozhikode district	November 1995; February to May and July to October 1996; May 1997
E. suratensis (Bloch)	22	Kuttimakkul in Kannur district; Nallur, Feroke and Devagiri in Kozhikode district	February and July 1996; April to August and November 1997

Classification and name of hosts	No. of host specimens examined	Locality of collection	Period of collection
Tilapia mossambica Peters	16	Ramanattukara and Nallur in Kozhikode district; Malampuzha in Palakkad district	April and October 1995; December 1996; May 1997
Belontidae			
Macropodus cupanus Valenciennes	301	Kuttimakkul, Kanhilari, Karetta and Nirmalagiri in Kannur district; Kundayithode, Feroke, Ramanattukara, Nallur, Puthukode and Devagiri in Kozhikode district; Nedungottumedu, Thenhipalam, Thalapara and Kadakkattupara in Malappuram district	January to April and July to December 1995; March to November 1996; January to August and November 1997; March to May 1998
Mastacembeliformes			
Mastacembelidae			
Mastacembelus guentheri Day	2	Nedungottumedu in Malappuram district	March 1996

The fishes collected were brought alive to the laboratory in suitable containers, and examined immediately for digenetic trematodes or maintained alive in the laboratory until used for study. Cement tanks (60 x 60 x 35 cm) or glass tanks (90 x 30 x 30 cm) containing 2 to 3 cm bottom layer of sand and pond water upto a height of 25 cm, were utilized for maintaining the fishes. The water was aerated on alternate days with an electric aerator and changed once in 3 or 4 weeks. Fishes were fed occasionally with cooked rice, beef liver, fish meal or biscuit crumbs.

Recovery and study of digenetic trematodes from fish hosts

The fishes were killed by cervical rupture and their scales, skin, gills, gill chambers and eyes were examined under a stereozoom dissecting microscope for larval digeneans. Then the skin was removed, and the muscle tissues macerated to detect the presence of metacercariae and adults. Internal organs like heart, liver, gall bladder, pancreas, intestine, kidney, urinary bladder, swim bladder, gonads and brain were dissected out from each fish, placed in separate petri-dishes containing 0.75% saline, macerated and examined under the dissecting microscope. Metacercariae and adults, when present, were carefully removed and collected in 0.75% saline taken in a petri-dish. Metacercarial cysts were mounted on a slide with saline and examined under a phase-contrast microscope for noting details of cysts and the enclosed larvae. Excysted larva was obtained either by rupturing the cyst wall with the help of fine needles or by mounting the metacercaria under cover glass and applying gentle pressure over it by fine needles. The excysted larvae and adults were studied under a phase-contrast microscope with or without supravital staining with neutral red or methylene blue (used as 0.1% aqueous solution diluted to 0.01% with 0.75% saline). Acetic orcein staining, following the procedure outlined by Webb (1991), was also used to study the metacercarial morphology. Permanent whole mounts of excysted larvae and adult flukes were prepared after fixing them in 10% formalin under slight cover glass pressure, and then staining with alum carmine, following the procedure outlined by Cantwell (1981). Data on prevalence and intensity of infection by trematodes were

collected. The terms "prevalence" and "intensity" are used here in accordance with the definition of Margolis *et al.* (1982).

Collection of snail hosts and their maintenance

Natural habitats of fishes were explored to get the first intermediate hosts of digenetic trematodes infecting the fishes. Different species of freshwater snails were collected from these habitats; they were mostly collected by hand picking. A list of the snails examined, with their localities and period of collection, is presented here.

List of snails examined for cercariae

Classification and name of hosts	Locality of collection	Period of collection
Viviparidae		
Bellamya dissimilis (Mueller)	Kuttimakkul, Kanhilari and Pinarayi in Kannur district; Sultan Bathery in Wayanad district	November, 1995; May, 1996; September 1997
Pilidae		
Pila virens (Lamarck)	Kanhangad and Theerthankara in Kasargod district; Karetta, Kanhilari, Nirmalagiri, Kuttimakkul, Pinarayi and Chirakkal in Kannur district; Kundayithode, Feroke, Nallur, Puthukode and Ramanattukara in Kozhikode district; Sultan Bathery, Vythiri and Meenangadi in Wayanad district; Nedungottumedu, Calicut University Campus, Kadakkattupara and Thalapara in Malappuram district; Malampuzha in Palakkad district; Valapad in Thrissur district	March to December 1995; January to November 1996; February to December 1997; January to March and May to July 1998

Classification and name of hosts	Locality of collection	Period of collection
Thiaridae		
Thiara tuberculata (Müeller)	Kanhangad and Theerthankara in Kasargod district; Karetta, Kanhilari, Nirmalagiri, Kuttimakkul, Pinarayi and Chirakkal in Kannur district; Kundayithode, Feroke, Nallur, Puthukode and Ramanattukara in Kozhikode district; Sultan Bathery, Vythiri and Meenangadi in Wayanad district; Nedungottumedu, Calicut University Campus, Kadakkattupara and Thalapara in Malappuram district; Malampuzha in Palakkad district; Valapad in Thrissur district	March to December 1995; January to November 1996; February to December 1997; January to March and May to July 1998
Thiara scabra (Müller)	Kanhangad and Theerthankara in Kasargod district	March and May 1996; February and November 1997
Paludomus tanschauricus (Gmelin)	Kanhilari and Karetta in Kannur district; Sultan Bathery in Wayanad district	May to December 1995; January to December 1996; February to December 1997; May to July 1998
Lymnaidae		
Lymnaea luteola (Deshayes)	Kanhangad in Kasargod district; Kanhilari, Karetta, Nirmalagiri, Kuttimakkul and Pinarayi in Kannur district; Kundayithode, Nallur, Puthukode, Feroke and Ramanattukara in Kozhikode district; Sultan Bathery, Vythiri	March to December 1995; January to November 1996; February to December 1997; January to July 1998

Classification and name of hosts	Locality of collection	Period of collection
	and Meenangadi in Wayanad district; Nedungottumedu, Thalapara and Kadakkattupara in Malappuram district; Malampuzha in Palakkad district	
Planorbidae		
Indoplanorbis exustus (Deshayes)	Kanhangad and Theerthankara in Kasargod district; Karetta, Kanhilari, Nirmalagiri, Kuttimakkul, Pinarayi and Chirakkal in Kannur district; Kundayithode, Feroke, Nallur, Puthukode and Ramanattukara in Kozhikode district; Sultan Bathery, Vythiri and Meenangadi in Wayanad district; Nedungottumedu, Calicut University Campus, Kadakkattupara and Thalapara in Malappuram district; Malampuzha in Palakkad district;Valapad in Thrissur district	March to December 1995; January to November 1996; February to December 1997; January to March and May to July 1998

The snails were brought alive to the laboratory and kept in groups of 5-8 in beakers containing pond water. The mouths of the beakers were covered with net to provide aeration and also to prevent the escape of snails. The water in the container was changed daily and the snails were fed with biscuit crumbs and/or aquatic plants. The snails which shed cercariae were isolated individually and kept in separate beakers.

Study of cercariae

Cercariae emerged from naturally infected snails were used to study the swimming behaviour and morphology. To study the swimming movements, the cercariae were transferred into a watch glass containing pond water, and observed under a stereozoom dissecting microscope. Using a micropipette, the cercariae were then transferred on to a glass slide, stained supravitally with neutral red, toluidine blue or Nile blue sulphate, a cover glass was placed over, and observed under a phase-contrast microscope to study their internal organization. By gradual removal of excess water with a piece of blotting paper, uniform pressure was exerted by the cover glass, which inhibited the vigorous activity of the larvae and made their internal organization more clear. Use of stain mixture containing equal proportions of Nile blue sulphate, neutral red and indigo carmine made the internal organization of cercariae more distinct. Collar spines and cuticular spines of cercariae were studied by staining them with dilute Lugol's iodine solution (0.01%). The spines were visible in the form of shining, light diffracting formations on the yellowish background of the body. To study the excretory system, the cercariae were stained with 0.1% Nile blue sulphate in saline and refrigerated (14°C) for 30 min to one hour. Mounting the cercariae in 1% solution of urea or 0.65% saline also facilitated the study of excretory system. Acetocarmine was used to study the genital primordia.

Study of intra-molluscan stages

Infected snails were cracked open to study the intra-molluscan stages of development. The stages were observed under a phase-contrast

microscope with or without vital staining. Measurements were taken of heat-killed cercariae and intramolluscan stages.

Collection of definitive/experimental hosts and their maintenance

Natural habitats of the fish hosts which harboured the metacercariae and the surrounding areas were explored for definitive hosts of the trematodes. The suspected definitive hosts collected were the cat fish, *Mystus malabaricus;* the water snake, *Xenochrophis piscator;* the pond heron, *Ardeola grayii,* and the cattle egret, *Bubulcus ibis.* These hosts were brought alive to the laboratory and representative specimens were dissected out and examined for natural infections by adult flukes. The rest of the specimens were maintained in the laboratory for further studies. *Mystus malabaricus* were maintained in the same way described earlier.

The common water snake, *Xenochrophis piscator,* were maintained individually in glass jars (25 x 12 X 26 cm) containing a bottom layer of pebbles and sand and tap water to a height of 10 cm. The troughs were covered with wire gauze. The water was changed occasionally and the snakes were fed with small frogs. The birds were maintained in netted cages. The cages were maintained clean and the birds were fed occasionally with infection-free fishes or frogs.

One-day-old domestic chicks and white rats were used in experimental infection studies. They were also maintained in cages and provided with adequate temperature and diet.

Experimental infection studies

Life-cycles of *Diplostomum ketupanense, Transversotrema patialense, Haplorchoides mehrai* and *Acanthostomum burminis* were established in the laboratory using natural and experimental hosts. Fully embryonated eggs of *H. mehrai,* collected from gravid flukes, were fed to young laboratory bred snails, *Thiara tuberculata.* The exposed snails were then maintained in the laboratory and examined at periodic intervals for developmental stages and emerging cercariae. In the case of *D. ketupanense* and *A. burminis* miracidial development could not be obtained in the laboratory.

Cercariae from natural infections or those developed in the laboratory were used in experimental infection studies. Fingerlings of fishes collected from localities, where infections with these cercariae were not found, were exposed to cercariae. The fishes were dissected at various intervals post-exposure and development of metacercariae was followed. The metacercariae were studied using the procedure outlined above.

In order to obtain adults, metacercariae from natural infections or those developed in the laboratory were fed to infection-free natural definitive hosts or experimental hosts. Metacercariae of *Diplostomum ketupanense* or fishes infected by these metacercariae were fed to *Ardeola grayii.* The transversotrematid cercariae shed by *Thiara tuberculata* were used to develop their adults in *Rasbora daniconius.* Metacercariae of *Haplorchoides mehrai* collected from *Puntius parrah* and *Rasbora daniconius* or pieces of host tissues infected by the metacercariae were fed to *Mystus malabaricus.* In the case of *Acanthostomum burminis,* infected fishes were given to *Xenochrophis piscator.*

In addition to these 4 species of flukes, the adults of *Echinochasmus megavitellus* and *Centrocestus formosanus* were raised in the laboratory using their metacercariae collected from naturally infected fishes. The metacercariae or infected fishes were fed to *A. grayii* or one-day-old chicks. The fed hosts were then maintained in the laboratory, dissected at various intervals, and the flukes at different stages of development were recovered. Flukes were also recovered from naturally infected *A. grayii*. The adults were studied using the procedure outlined above.

The 27-spined echinostome cercariae released by *Indoplanorbis exustus* were used to develop their metacercariae in *Puntius parrah* and *Horadandia atukorali*. The exposed fishes were dissected at various intervals and the echinostome metacercariae at different stages of development were recovered. The adults and metacercariae were studied using the procedure outlined above.

Measurements and sketches

Measurements were made with the aid of a calibrated ocular micrometer. Measurements are in micrometre (μm); the range is followed by the mean value in parantheses. Sketches were made with the aid of a prism type camera lucida; details were added free hand from observations made on live specimens.

RESULTS

A total of 2708 fishes belonging to 20 genera and 30 species, collected from different localities in Malabar, Kerala were examined for digenetic trematodes. Eighteen species of digeneans were recovered from 22 species of fishes during the survey. They comprised 4 species of adults, metacercariae and adults of a species and 13 species of metacercariae. Life-cycles of 4 species have been elucidated and a few stages in the life-cycles of 3 species have been recovered and reported. The various species of digeneans recovered are arranged according to the classification given by La Rue (1957).

ADULT DIGENEA

Family: Transversotrematidae Yamaguti, 1954

Genus: *Transversotrema* Witenberg, 1944

Transversotrema patialense (Soparker, 1924) Crusz and Sathananthan, 1960

Natural infections by the adult of *Transversotrema patialense* were found beneath scales of *Rasbora daniconius* collected from Theerthankara in Kasargod district of Kerala during February 1996. The characters of the present fluke agreed fully with the descriptions of *Transversotrema patialense*

given by Crusz *et al.* (1964), and Murty and Rao (1968). A brief description of the species is given here.

Description (Fig. 1a; Table 1):

Body flattened, leaf-like, transversely elongated, spinose, measured 445-453 x 649-664 (450 x 660) in size. Eyespots spherical, situated on either side of pharynx. Oral sucker absent. Acetabulum equatorial, discoid, pedunculate, behind pharynx, 95-103 x 97-115 (101 x 104) in size. Mouth subterminal, directly opens into pharynx. Pharynx spherical, muscular, measured 70-78 x 78-85 (75 x 82). Oesophagus short, 49-55(53) in length. Intestine cyclocoelid with slightly sacculated walls. Testes lobed, symmetrical, intercaecal. Left testis measured 95-103 x 111-128 (100 x 121); right testis 88-92 x 98-102 (90 x 100). Cirrus sac absent. Seminal vesicle bipartite, one half situated above and the other below the intestinal caeca, just anterior to right testis. The anterior end of seminal vesicle becomes tubular, opens into genital atrium, at anterior end of the body. Ovary small, oval, intercaecal, anterior to left testis; measured 114-123 x 71-79 (120 x 75). A short oviduct leads from ovary to ootype. Vitellaria follicular, extracaecal. Seminal receptacle present. Ootype leads into uterus which passes obliquely above the anterior border of the left testis, descends to a position between the two testes and then passes obliquely above the anterior border of the right testis and ventral to the intestine and then proceeds anteriorly to the common genital opening. Eggs large, oval, measured 138-144 x 68-80 (141 x 73) in size. Excretory bladder pear-shaped, with terminal excretory pore.

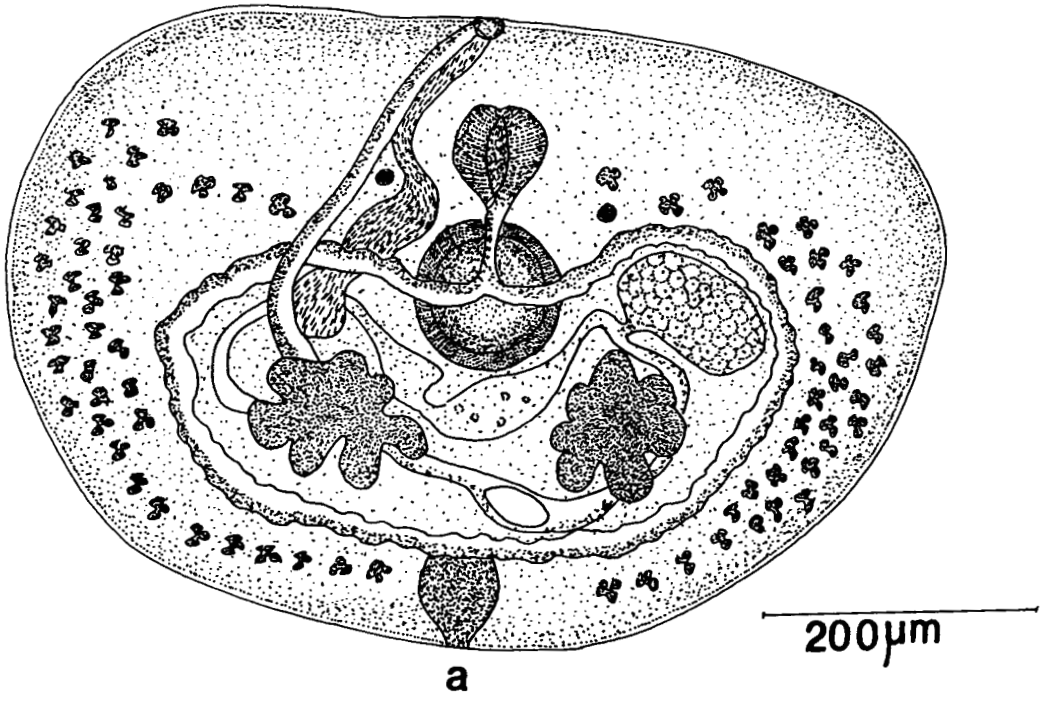


Fig. 1. *Transversotrema patialense* (Soparker, 1924) Crusz and Sathananthan,1960

a. Adult

Table 1. Measurements (μm) of adults of *Transversotrema patialense* (Soparker, 1924) Crusz and Sathananthan, 1960

Character	Range	Mean
Body size	445 - 453 x 649 - 664	450 x 660
Acetabulum	95 - 103 x 97 - 115	101 x 104
Pharynx	70 - 78 x 78 - 85	75 x 82
Oesophagus	49 - 55	53
Left testis	95 - 103 x 111 - 128	100 x 121
Right testis	88 - 92 x 98 - 102	90 x 100
Ovary	114 - 123 x 71 - 79	120 x 75
Egg	138 - 144 x 68 - 80	141 x 73

Life-cycle:

During the course of present study, the life cycle of *Transversotrema patialense* has been elucidated in the laboratory. Cercaria referable to *T. patialense* were obtained from *Thiara tuberculata* collected from Theerthankara in Kasargod district. The link between the cercaria and adult has been successfully established by experiments involving exposure of *Rasbora daniconius* to naturally emerged cercariae. Cercaria rests in water with the body hanging downward and the tail and furcae directed upward. A brief description of the cercaria is given here.

Description (Fig. 1b; Table 2):

Distomatous, pharyngeate, furcocercous, biocellate cercaria. Body flattened, leaf-like, covered with triangular spines; measured 295-388 x 491-510 (322 x 508). Tail stem measured 340-360 x 95-110 (355 x 100). Furcae measured 170-190 x 70-85 (180 x 75). Oral sucker absent. Acetabulum median, 49-53 x 45-58 (50 x 53) in size. Eyespots pigmented, spherical. Mouth ventral, much behind the anterior end, leads into pharynx. Pharynx globular, 40-52 x 42-49 (48 x 46) in size. Oesophagus 20-28 (26) long. Intestine ring-like. Testes lobed, symmetrical, placed within intestinal ring. Left testis measured 49-53 x 43-50 (50 x 46); right testis 45-49 x 47-52 (48 x 50). Seminal vesicle S-shaped, just anterior to right testis, half within and half outside intestinal loop; anterior end narrowed to form ejaculatory duct extending to genital atrium with subventral pore well anterior to mouth. Ovary oval, anterior to left testis, measured 22-32 x 36-46 (28 x 38). Excretory bladder saccular, just posterior to intestine. Cercariae develop in rediae.

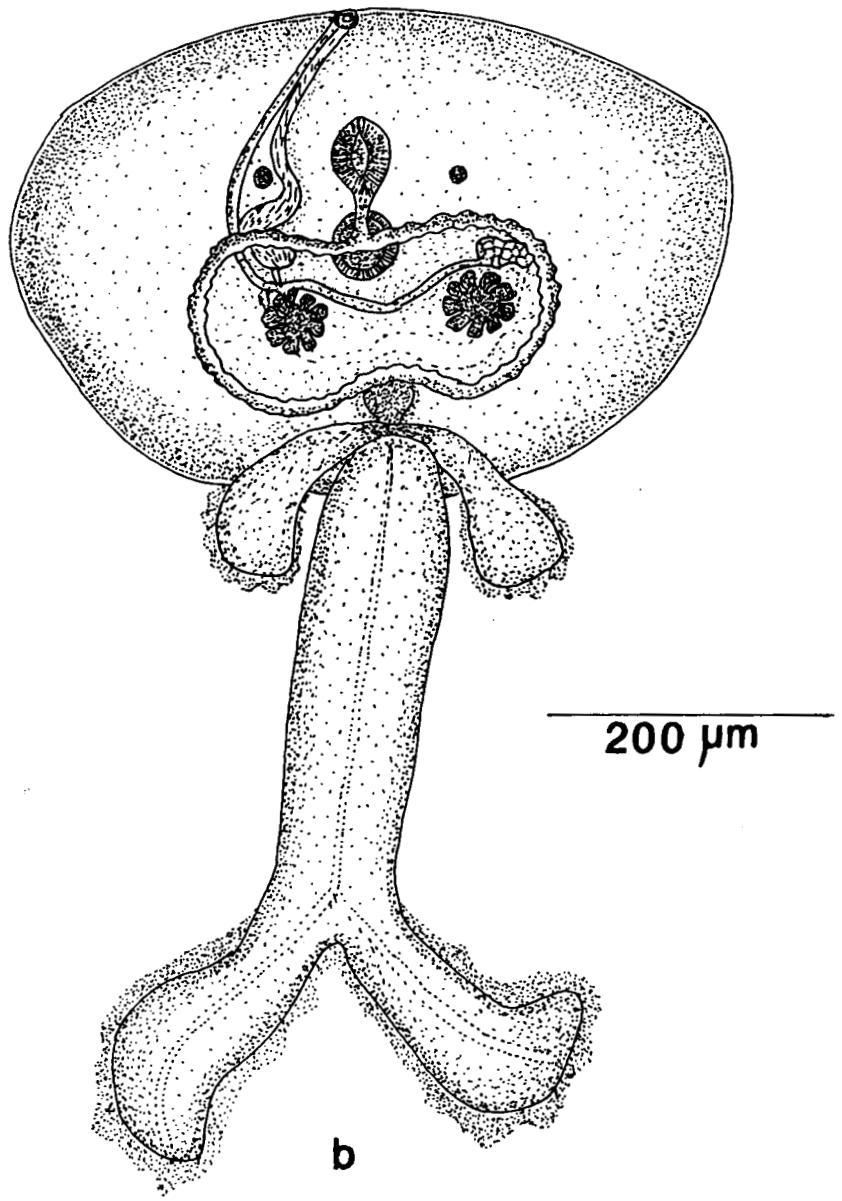


Fig. 1. *Transversotrema patialense* (Soparker,1924) Crusz and Sathananthan, 1960

b. Cercaria

Table 2. **Measurements (μm) of cercariae of *Transversotrema patialense* (Soparker, 1924) Crusz and Sathananthan, 1960**

Character	Range	Mean
Body size	295-388 x 491-510	322 x 508
Tail stem	340-360 x 95-110	355 x 100
Tail furcae	170-190 x 70-85	180 x 75
Acetabulum	49-53 x 45-58	50 x 53
Pharynx	40-52 x 42-49	48 x 46
Oesophagus	20-28	26
Left testis	49-53 x 43-50	50 x 46
Right testis	45-49 x 47-52	48 x 50
Ovary	22-32 x 36-46	28 x 38

Redia (Fig. 1c)

Body pyriform, tapering to posterior end; measured 900-950 x 460-499 (933 x 480). Pharynx globular, measured 70-83 x 140-153 (79 x 147). Gut elongated, 440-490 (485) in length.

Definitive host	:	*Rasbora daniconius* (Hamilton)
Site of infection	:	Beneath scales
Locality	:	Theerthankara in Kasargod district of Kerala.
Period of collection	:	February 1996
Prevalence of infection	:	8 of 622 (1.29%) fishes examined.
Intensity of infection	:	3 to 6
Snail host	:	*Thiara tuberculata* (Müeller)
Site of infection	:	Hepatopancreas
Locality	:	Theerthankara in Kasargod district of Kerala.
Period of collection	:	February 1996
Prevalence of infection	:	23 of 685 (3.36%) snails examined.

Remarks:

Comparative study of characters of the adult flukes recovered from *Rasbora daniconius*,and cercaria from *Thiara tuberculata* with known species of *Transversotrema*, revealed that the present stages belonged to *T. patialense* (Soparker, 1924) Crusz and Sathananthan, 1960. Soparker (1924) reported the cercaria of *T. patialense* from *Melanoides tuberculatus* from Punjab, India. Subsequently, the same cercariae were reported from the same host by Anantaraman (1948) from Nellore, and Rao and Ganapati (1967) from

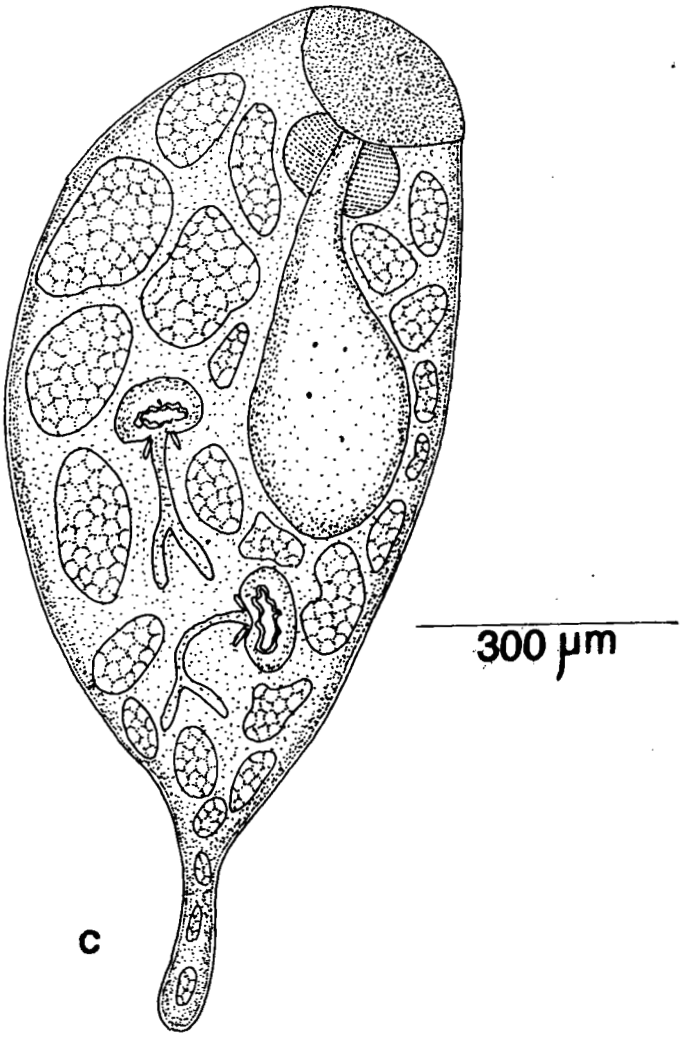


Fig. 1. *Transversotrema patialense* (Soparker,1924) Crusz and Sathananthan, 1960

c. Redia

Waltair, India. Crusz (1956) recorded it from Ceylon and Betterton (1979) from the Belgian Congo.

Observations on the development of *Cercaria patialensis* were made in the fishes, *Macropodus cupanus, Ophiocephalus punctatus* and *Tilapia mossambica* and in the snail,*M. tuberculatus* by Crusz *et al.* (1964). This forms the first experimental demonstration of the life-cycle of *T. patialense.* Further studies on the life-cycle of this species were made by Rao and Ganapati (1967) who recovered the adults from *Esomus danricus, Panchax panchax* and *Catla catla* and suggested that there may not be a metacercarial stage in the life-cycle of *Transversotrema* spp. My observations are in conformity with those made by Rao and Ganapati. Later, Murty and Rao (1968), Madhavi (1980) and Rekharani and Madhavi (1985) recorded the adults from different fishes. Its miracidium was reported by Madhavi and Jhansilakshmibai (1994). Apart from *T. patialense,* three other species of *Transversotrema* have been reported from India, namely, *T. soparkari* Pande and Shukla, 1972; *T. chackai* Mohandas, 1973, and *T. chauhani* Agrawal and Singh, 1981. Nadakkal *et al.* (1969) described *C. chackai* from *Melania tuberculata* and *M. scabra* and differentiated it from the cercaria of *C. patialensis* by differences in body size and features of the excretory system. Pandey (1971a) reported *C. soparkari* from *Melanoides tuberculatus* and differentiated it from *C. patialensis* by the arrangement of excretory

canals. All the three species differ only slightly from *T. patialense* in morphological features and are probably synonymous to it.

Family: Paramphistomidae Fischoeder, 1901

Genus: *Caballeroia* Thapar, 1960

***Caballeroia bhavani* (Achan, 1956) Devraj, 1972**

Caballeroia bhavani was found in the intestine of *Puntius sarana sarana* collected from Noolpuzha in Wayanad district during February 1998. The two fishes collected were positive for this fluke. Fifty-nine flukes were recovered from one fish and 80 from the other. The fishes exhibited mixed infection with the present fluke and the adult of *Neopodocotyle dayali.*

Description (Fig. 2a; Table 3):

Body elongated with rounded extremities, measured 3200-5376 x 1285-1489 (3840 x 1394); anterior end bears small conical transverse rows of papillae. Oral sucker terminal, 416-563 x 290-438 (512 x 384) in size. Acetabulum large, ventro-terminal, 358-600 x 412-619 (486 x 500). Oral diverticula present. Pharynx absent; oesophagus, 512-640 (563) in length. Caeca 1790-2432 (2133) long, terminate in front of acetabulum. Testes oval, pre-equatorial, oblique, intercaecal, just posterior to caecal bifurcation. Left testis measured 307-512 x 282-488 (390 x 346); right testis 387-589 x 258-519 (403 x 467) in size. Cirrus sac elongated, pear shaped, post-bifurcal, 332-458 (426) long, contains coiled seminal vesicle and elongated cirrus. Genital pore dextral, just pre-bifurcal. Ovary small, submedian, in front of acetabulum, measured 204-263 x 179-205 (220 x 188) in size. Vitellaria

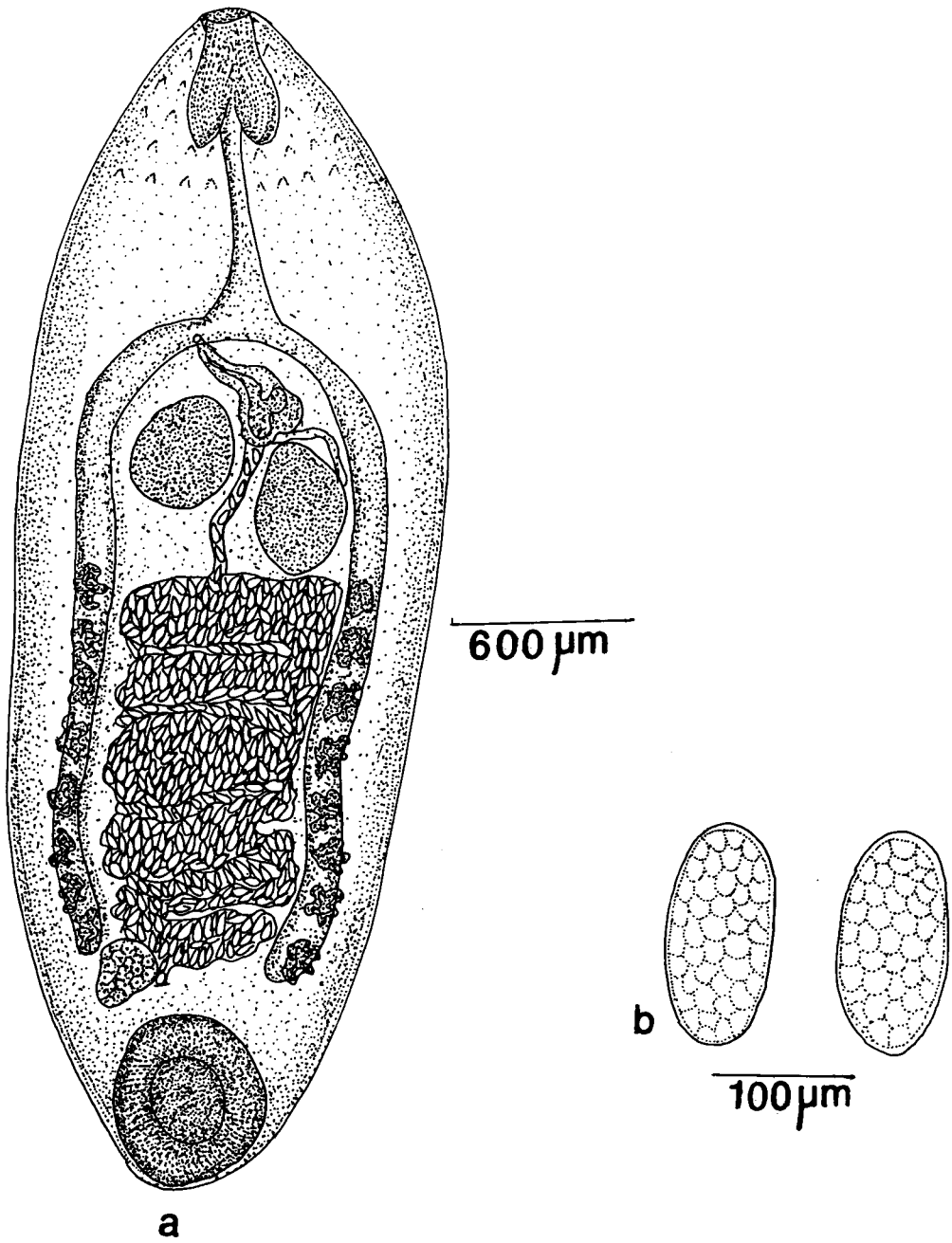


Fig. 2. *Caballeroia bhavani* (Achan, 1956) Devraj, 1972

a. Adult; b. Eggs

Table 3. Measurements (μm) of adults of *Caballeroia bhavani* (Achan, 1956) Devraj, 1972

Character	Range	Mean
Body size	3200-5376 x 1285-1489	3840 x 1394
Oral sucker	416-563 x 290-438	512 x 384
Acetabulum	358-600 x 412-619	486 x 500
Oesophagus	512-640	563
Caeca	1790-2432	2133
Left testis	307-512 x 282-488	390 x 346
Right testis	387-589 x 258-519	403 x 467
Cirrus sac	332-458	426
Ovary	204-263 x 179-205	220 x 188
Egg	171-187 x 76-80	179 x 78

follicular, extend along dorso-lateral margin of caeca from posterior region of testes to caecal end. Uterus intercaecal, extending from testes to anterior end of acetabulum and opens at genital pore. Eggs numerous, oval, measured 171-187 x 76-80 (179 x 78) in size (Fig. 2b).

Host	:	*Puntius sarana sarana* (Hamilton)
Site of infection	:	Intestine
Locality	:	Noolpuzha in Wayanad district of Kerala.
Period of collection	:	February 1998
Prevalence of infection	:	2 of 2 (100%) fishes examined
Intensity of infection	:	59-80.

Remarks:

The present species recovered from the intestine of *Puntius sarana sarana* is very similar to *Caballeroia bhavani* (Achan, 1956) Devraj, 1972 in general body form, shape and position of ovary, testes and cirrus sac, and in the distribution of vitelline follicles. As *C. bhavani* is the only valid species known under the genus *Caballeroia*, the present fluke is identified and reported here as *Caballeroia bhavani* (Achan, 1956) Devraj, 1972. This report forms the first record of *C. bhavani* from *P. sarana sarana* and from Kerala.

Family: Opecoelidae Ozaki, 1925

Genus: *Neopodocotyle* Dayal, 1950

***Neopodocotyle dayali* Pandey, 1973**

Natural infections by this fluke were found in the intestine of *Puntius sarana sarana* collected from Noolpuzha in Wayanad district during February 1998. The two fishes collected were positive for this infection. Three flukes were recovered from one fish and 2 from the other. Both the fishes were found infected by the adults of *Caballeroia bhavani* also.

Description (Fig. 3a; Table 4):

Body elongated, aspinose, with rounded anterior and posterior ends; measured 3500-3654 x 1002-1035 (3584 x 1024) in size. Oral sucker terminal, 280-315 x 278-362 (308 x 354) in size. Acetabulum pre-equatorial, round, measured 280-336 (323). Pre-pharynx absent; pharynx globular; muscular, measured 120-132 x 229-235(128 x 230) in size. Oesophagus 200-275 (245) long, bifurcates into caeca just in front of acetabulum. Caeca 2900-3165 (2995) long, reaching to posterior end of body. Testes round, tandem, post-equatorial. Anterior testis measured 409-440 (433) while the posterior one 428-485 (439). Cirrus sac extracaecal, extends from anterior level of acetabulum to anterior end of pharynx; measured 485-531 x 250-346 (530 x 265); contained tubular seminal vesicle and short ejaculatory duct. Ovary oval or pear-shaped, pre-equatorial, measured 245-295 x 232-288 (270 x

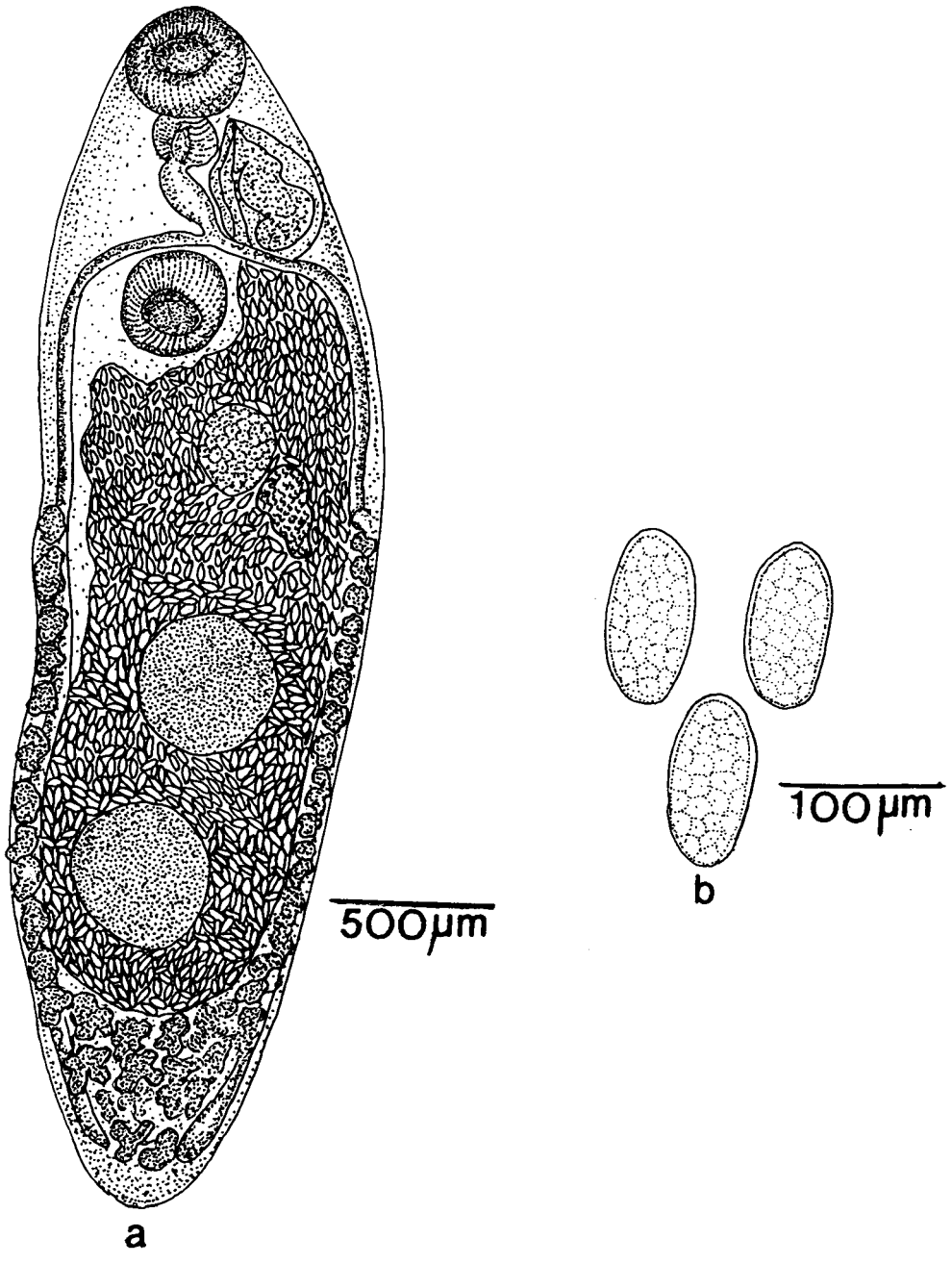


Fig. 3. *Neopodocotyle dayali* Pandey, 1973

a. Adult; b. Eggs

Table 4. **Measurements (μm) of adults of *Neopodocotyle dayali* Pandey, 1973**

Character	Range	Mean
Body size	3500-3654 x 1002-1035	3584 x 1024
Oral sucker	280-315 x 278-362	308 x 354
Acetabulum	280-336	323
Pharynx	120-132 x 229-235	128 x 230
Oesophagus	200-275	245
Caeca	2900-3165	2995
Anterior testis	409-440	433
Posterior testis	428-485	439
Cirrus sac	485-531 x 250-346	530 x 265
Ovary	245-295 x 232-288	270 x 255
Egg	96-111 x 49-72	101 x 60

255). Oviduct arises from the anterior end of ovary and opens to the ootype. Seminal receptacle elongated, lying postero-lateral to ovary. Vitelline follicles mostly lateral, extend from ovary to the end of caeca, but merge together, in the post-testicular region. Uterus coiled, extending from anterior testis to the level of acetabulum and opens at the genital pore by a short metraterm located on the left side of oesophagus. Eggs numerous, oval, operculated, 96-111 x 49-72 (101 x 60) in size (Fig. 3b).

Host	:	*Puntius sarana sarana* (Hamilton)
Site of infection	:	Intestine
Locality	:	Noolpuzha in Wayanad district of Kerala.
Period of collection	:	February 1998
Prevalence of infection	:	2 of 2 (100%) fishes examined
Intensity of infection	:	2 and 3

Remarks:

The present fluke recovered from the intestine of *Puntius sarana sarana* has pre-equatorial ovary, post-equatorial testes which are arranged in tandem and extracaecal cirrus sac. Based on these characters, it is included under the genus *Neopodocotyle* Dayal, 1950. As far as is known, only 5 species have been reported under this genus: *Neopodocotyle indica* Dayal, 1950 from *Callichrous bimaculatus; N. spinipora* Sircar and Sinha, 1969 from *Rita rita; N. mehrai* Rai, 1971 from *Puntius sarana* and *P. sophore;*

N. balliaensis Pandey, 1973 from *Labeo calbasu*, and *N. dayali* Pandey, 1973 from *P. sarana*. A comparison of characters of all the known species with those of the present form revealed that the present fluke is identical with *Neopodocotyle dayali* Pandey, 1973, and is accordingly reported here. Recovery of this species from *P. sarana sarana* at Noolpuzha in Wayanad district forms a new geographical record.

Family : Heterophyidae Odhner, 1914

Genus : *Haplorchoides* Chen, 1949

***Haplorchoides mehrai* Pande and Shukla, 1976**

Adult

Ten *Mystus malabaricus* were collected from Karetta and Kuttimakkul in Kannur, Puthukode in Kozhikode and Nedungottumedu in Malappuram districts of Kerala during March and December 1995; April 1996 and December 1997. Six specimens were positive for *Haplorchoides mehrai*. The intensity of infection varied from 3 to 8. The characters of the present fluke agreed fully with the descriptions of *Haplorchoides mehrai* given by Pande and Shukla (1976) and Shameem and Madhavi (1988). Mixed infections by this fluke and metacercariae of 27-spined echinostome and *Centrocestus formosanus* were occasionally found in *M. malabaricus*. A brief description of the adult is presented here.

Description (Fig. 4a; Table 5):

Body elliptical, spinose, measured 785-1401 x 251-480 (1092 x 325) in size. Oral sucker subterminal, 49-90 x 51-89 (62 x 68) in size. Acetabulum small, enclosed in ventrogenital sac; divisible into a nucleated part and a non-nucleated part. Ventrogenital sac oval, submedian, just behind caecal bifurcation, measured 23-70 x 23-82 (44 x 50). Pre-pharynx 92-246 (162) long; pharynx 39-62 x 37-62 (48 x 46) in size. Oesophagus 20-63 (42) long. Caeca narrow, extend beyond posterior border of testis; measured 193-501 (371). Testis single, large, round to oval, in the middle of hind-body; measured 108-203 x 116-234 (168 x 169). Cirrus sac absent. Seminal vesicle bipartite; ejaculatory duct short, opening together with metraterm into ventrogenital sac. Ovary oval, median, pre-testicular, measured 62-112 x 77-128 (86 x 97). Seminal receptacle postero-lateral to ovary; measured 54-112 x 39-100 (82 x 70). Vitellaria follicular, consisting of tear-shaped follicles, distributed lateral and posterior to testis. Uterus with ascending and descending limbs, occupying all available space behind ventrogenital sac. Eggs oval, operculated, 26-36 x 13-26 (32 x 19) in size. Excretory bladder saccular, excretory pore terminal.

Metacercaria

Natural infections by the metacercaria were found in 11 species of fish hosts collected from various localities in Kerala during January 1995 -

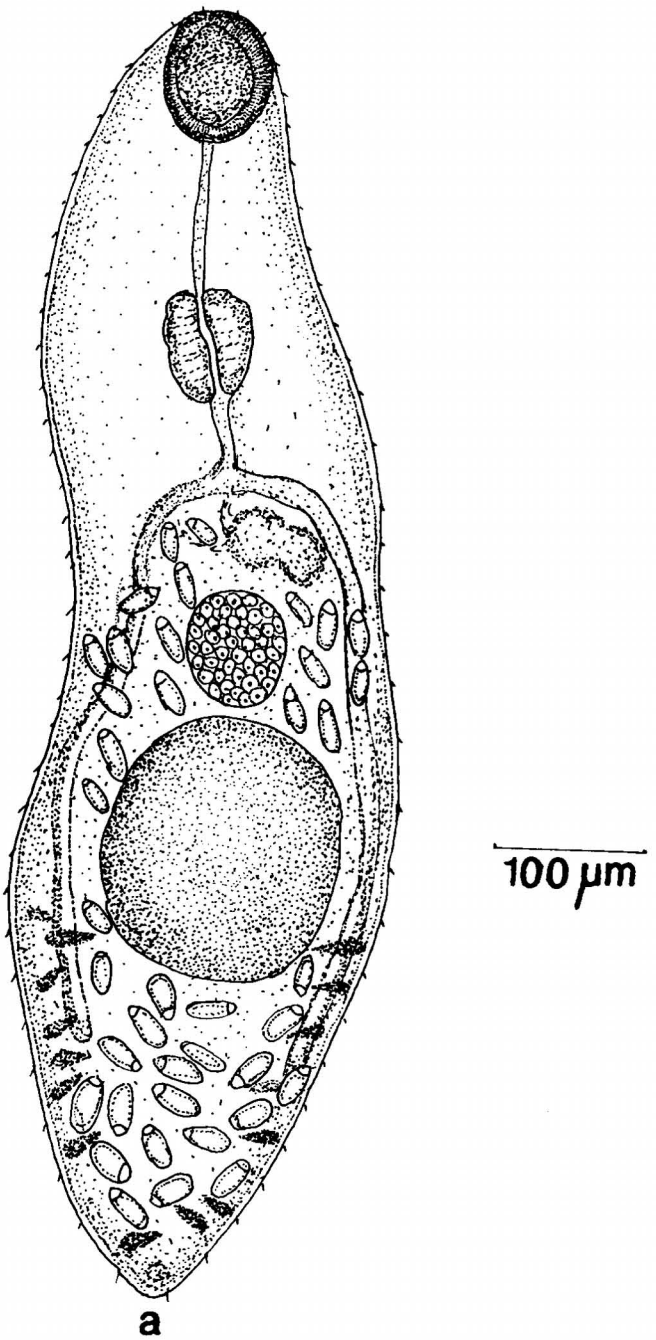


Fig. 4. *Haplorchoides mehrai* Pande and Shukla, 1976

a. Adult

Table 5. **Measurements (μm) of adults of *Haplorchoides mehrai* Pande and Shukla, 1976**

Character	Range	Mean
Body size	785-1401 x 251-480	1092 x 325
Oral sucker	49-90 x 51-89	62 x 68
Ventrogenital sac	23-70 x 23-82	44 x 50
Prepharynx	92-246	162
Pharynx	39-62 x 37-62	48 x 46
Oesophagus	20-63	42
Caeca	193-501	371
Testis	108-203 x 116-234	168 x 169
Ovary	62-112 x 77-128	86 x 97
Seminal receptacle	54-112 x 39-100	82 x 70
Egg	26-36 x 13-26	32 x 19

May 1998. The number of host species examined, their locality of collection and site, prevalence and intensity of metacercarial infection are summarised in the Table 6. All the specimens agreed fully with the descriptions of *Haplorchoides mehrai* metacercaria given by Pande and Shukla (1976) and Shameem and Madhavi (1988). Mixed infections by this metacercaria and a 27-spined echinostome metacercaria were found in *Horadandia atukorali, Puntius parrah* and *Mystus malabaricus.* Metacercariae of *Centrocestus formosanus* and the present metacercariae were frequently found in *P. melanampyx melanampyx, P. parrah, P. sophore* and *M. malabaricus. Rasbora daniconius* was found infected by metacercariae of *Diplostomum ketupanense, H. mehrai* and *Acanthostomum burminis.*

Description:

Cysts small, white, round to oval with double layered wall; measured 146-225 x 123-201 (179 x 157) in size. The larva remains folded inside cyst cavity (Fig. 4b, Table 7).

Excysted metacercaria (Fig. 4c; Table 7):

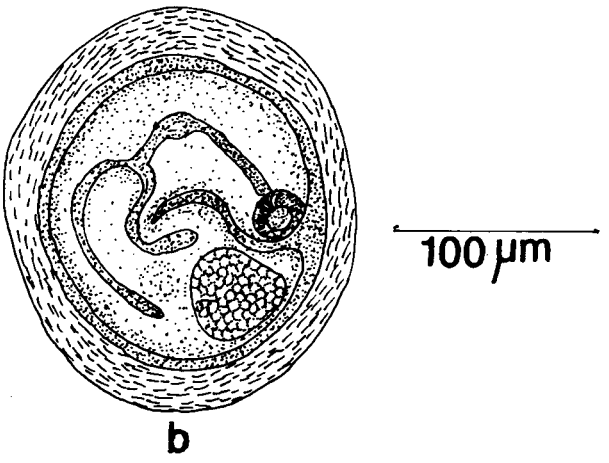
Body elongated, spinose, 290-525 x 90-215 (430 x 170) in size. Oral sucker subterminal, 35-60 x 40-63 (45 x 50) in size. Acetabulum small, enclosed in ventrogenital sac; divisible into a nucleated part and a non-nucleated part. Ventrogenital sac submedian, located behind the caecal

Table 6. List of host species examined, their locality of collection, and site prevalence and intensity of infection by metacercariae of *Haplorchoides mehrai* Pande and Shukla, 1976

Name of fish	Site of infection	No. of fishes		Prevalence of Infection	Intensity of infection	Locality
		Exa-mined	Infected			
(1)	(2)	(3)	(4)	(5)	(6)	(7)
Esomus barbatus (Jerdon)	Fins and gills	360	138	38.33	20 to 39	Kanhangad and Theerthankara in Kasargod; Karetta, Kanhilari Pinarayi, Kuttimakkul and Eranholy in Kannur; Ramanattukara, Feroke and Puthukode in Kozhikode, and Nedungottumedu, Thalapara and Kadakkattupara in Malappuram districts of Kerala
Horadandia atukorali Deraniyagala	Fins, muscles and gills	162	120	74.07	12 to 17	Kuttimakkul in Kannur; Puthukode, Nallur and Ramanattukara in Kozhikode, and Sultan Bathery in Wayanad districts of Kerala
Rasbora daniconius (Hamilton)	Fins, muscles and gills	622	451	72.51	9 to 135	Kanhangad in Kasargod; Karetta, Kanhilari, Nirmalagiri, Kuttimakkul and Pinarayi in Kannur; Feroke, Ramanattukara, Nallur, Puthukode and

(1)	(2)	(3)	(4)	(5)	(6)	(7)
						Kundayithode in Kozhikode; Vythiri, Pakkam and Sultan Bathery in Wayanad; Nedungottumedu, Thalapara, Calicut University Campus and Kadakkattupara in Malappuram districts of Kerala.
P. filamentosus (Valenciennes)	Fins, scales, muscles and gills	21	16	76.19	6 to 18	Devagiri in Kozhikode; Thalapara and Nedungottumedu in Malappuram districts of Kerala
P. melanampyx melanampyx (Day)	Scales, fins and muscles	122	83	68.03	8 to 15	Kuttimakkul, Kanhilari and Karetta in Kannur, Kundayithode and Puthukode in Kozhikode; Sultan Bathery and Vythiri in Wayanad, and Nedungottumedu and Kadakkattupara in Malappuram districts of Kerala
P. parrah Day	Scales, fins, muscles, gills, kidney and liver	420	392	93.33	31 to 123	Kuttimakkul, Chirakkal and Karetta in Kannur; Feroke, Puthukode and Ramanattukara in Kozhikode; Pakkam, Vythiri, Meenangadi and Sultan Bathery

(1)	(2)	(3)	(4)	(5)	(6)	(7)
						in Wayanad,and Nedungottumedu and Thalapara in Malappuram districts of Kerala
P. sophore (Hamilton)	Scales,fins, muscles and gills	60	42	70	6 to 19	Kuttimakkul in Kannur, and Thalapara in Malappuram districts of Kerala
Garra mullya (Sykes)	Fins	9	2	22.22	1 to 3	Nedungottumedu in Malappuram district of Kerala
Mystus malabaricus (Jerdon)	Fins	10	6	60	1 to 8	Kuttimakkul and Karetta in Kannur; Puthukode in Kozhikode; Nedungottumedu in Malappuram districts of Kerala
M. oculatus (Valenciennes)	Fins	31	2	6.45	3 to 6	Thenhipalam in Malappuram district of Kerala
Heteropneustes fossilis (Bloch)	Fins and gills	38	12	31.57	2 to 8	Kanhangad in Kasargod; Kuttimakkul and Karetta in Kannur, and Nedungottumedu in Malappuram districts of Kerala



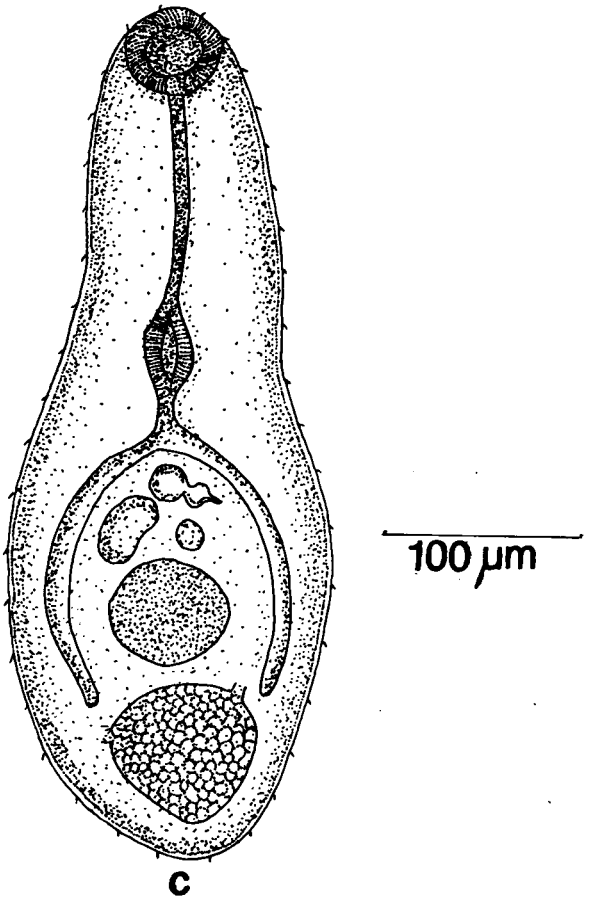


Fig. 4. *Haplorchoides mehrai* Pande and Shukla, 1976

b. Encysted metacercaria; c. Excysted metacercaria

Table 7. **Measurements (μm) of metacercariae of *Haplorchoides mehrai* Pande and Shukla, 1976**

Character	Range	Mean
Encysted metacercaria	146-225 x 123-201	179 x 157
Excysted metacercaria	290-525 x 90-215	430 x 170
Oral sucker	35-60 x 40-63	45 x 50
Ventrogenital sac	23-59 x 25-59	32 x 44
Pre-pharynx	63-162	111
Pharynx	31-46 x 20-40	39 x 30
Oesophagus	17-46	33
Caeca	150-201	180
Excretory bladder	62-131 x 77-131	98 x 106

bifurcation and measured 23-59 x 25-59 (32 x 44). Pre-pharynx 63-162 (111) long; pharynx muscular, 31-46 x 20-40 (39 x 30) in size. Oesophagus 17-46 (33) long; caeca narrow, reaching behind the posterior border of testis; measured 150-201 (180). Testis intercaecal, in the posterior half of body. Ovary, seminal vesicle and seminal receptacle rudimentary. Excretory bladder globular, filled with dark, small concretions; measured 62-131 x 77-131 (98 x 106). Excretory pore terminal.

Life-cycle:

The life-cycle of *H. mehrai* was established by Shameem and Madhavi (1988) with *Melanoides tuberculatus* (= *Thiara tuberculata*) as the snail host, *Puntius sophore* as the second intermediate host and four species of cat fishes as the definitive hosts. During the course of the present study, the life-cycle of *H. mehrai* has been established in the laboratory. The snails, *Thiara tuberculata* were exposed to fully embryonated eggs shed by the adult worms, and the development of intramolluscan stages and cercariae were followed.

Sporocyst (Fig. 4d):

Snails fed with fully embryonated eggs developed sporocysts within a week. Sporocysts appeared as a lobulated mass filled with germ balls and young rediae; measured 116-424 x 54-116 (238 x 88).

Redia (Fig. 4e):

Redia developed in the hepatopancreas of snail host and started releasing cercariae 70 days post-exposure to embryonated eggs. Body elongated, measured 205-693 x 99-162 (440 x 129). Pharynx globular, measured 23-45 x 23-39 (33 x 30) in size. Gut long, narrow, 36-139 x 19-45 (79 x 29) in size. Redial cavity filled with a number of germ balls and cercariae at various stages of development.

Cercaria:

Infected snails released the cercariae 70 days post-exposure to embryonated eggs. Cercariae identical to that described by Shameem and Madhavi (1988), were found in *Thiara tuberculata* collected from Kuttimakkul and Kanhilari in Kannur; Sultan Bathery and Meenangadi in Wayanad, and Nedungottumedu in Malappuram districts of Kerala. Cercariae emerged from snails throughout day time and they swim continually with short intervals of rest. The cercariae, exposed to the fingerlings of *Rasbora daniconius* and *Puntius parrah,* developed into metacercariae of *H. mehrai.* A brief description of the cercaria is furnished below.

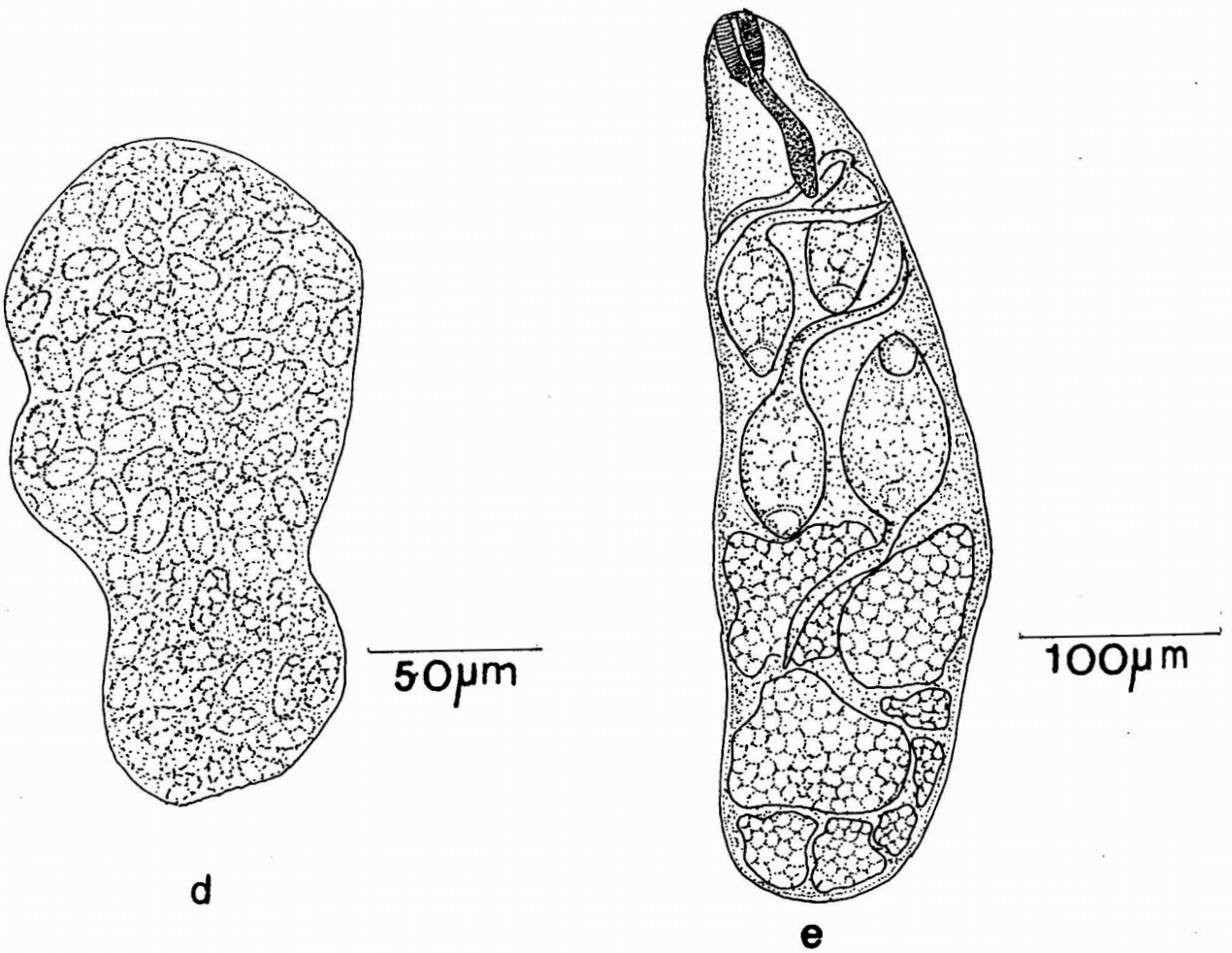


Fig. 4. *Haplorchoides mehrai* Pande and Shukla, 1976

d. Sporocyst; e. Redia

Description (Fig. 4f, Table 8):

Cercaria monostome, opisthorchioid and pleurolophocercous. Body elongate, spinose, measured 123-437 x 85-156 (271 x 121) in size. Tail slender, longer than body, inserted in a socket at posterior end of body and measured 274-482 x 30-47 (379 x 38). Caudal fin folds well developed. Two lateral finfolds covering the anterior one third of tail and dorsoventral finfold originating dorsally one third of distance from anterior end, continuing over the tip of tail to ventral side and terminating at the midventral region of tail. Oral sucker pyriform, subterminal, 33-54 x 36-62 (48 x 45) in size, modified into a piercing organ. Pre-pharynx short; pharynx globular, 23-50 (39) wide. Eyespots prominent, on either side of prepharynx at a distance of 69-125 (96) from anterior end of body. Penetration glands 7 pairs, arranged in 4 rows between excretory bladder and eyespots. Gland ducts run in 2 narrow bundles, divide into 4 groups of enlarged ducts near oral sucker and open individually through pores on pre-oral lobe in a 3, 4, 4, 3 pattern. Genital rudiment triangular, situated in front of excretory bladder. Excretory bladder globular, lined by flat cells containing granular material; measured 43-140 x 77-137 (99 x 104). Flame cell formula, 2[(2+2+2) + (2+2+2)] = 24.

Definitive host : *Mystus malabaricus* (Jerdon)

Site of infection : Intestine

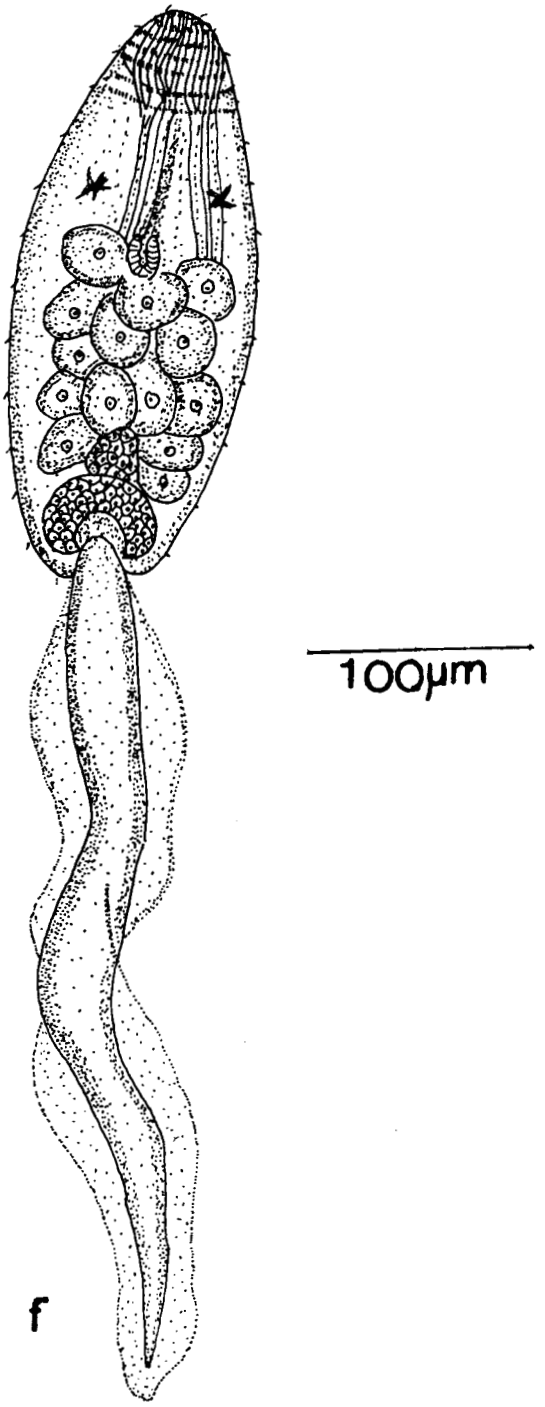


Fig. 4. *Haplorchoides mehrai* Pande and Shukla, 1976

f. Cercaria

Table 8. Measurements (μm) of cercariae of *Haplorchoides mehrai* Pande and Shukla, 1976

Character	Range	Mean
Body size	123-437 x 85-156	271 x 121
Tail size	274 - 482 x 30-47	379 x 38
Oral sucker	33-54 x 36-62	48 x 45
Pharynx	23-50	39
Excretory bladder	43-140 x 77-137	99 x 104

Locality	:	Karetta and Kuttimakkul in Kannur, Puthukode in Kozhikode, and Nedungottumedu in Malappuram districts of Kerala.
Period of collection	:	March and December 1995, April 1996 and December 1997
Prevalence of infection	:	6 of 10 (60%) fishes examined
Intensity of infection	:	3 to 8.
Fish hosts, locality of collection, site, and prevalence and intensity of infection	:	Table 6
Snail host	:	*Thiara tuberculata* (Müeller)
Locality	:	Kuttimakkul and Kanhilari in Kannur, Sultan Bathery and Meenangadi in Wayanad, and Nedungottumedu in Malappuram districts of Kerala.
Prevalence of infection	:	280 of 685 (40.87%) snails examined.

Remarks:

The life-cycle of *Haplorchoides mehrai* Pande and Shukla, 1976 was worked out by Shameem and Madhavi (1988). All the stages recovered during the present investigation were identical to those recorded by Shameem and Madhavi (1988). Pande and Shukla (1976) reported the adult

of *H. mehrai* from the cat fish, *Mystus vittatus* from Uttar Pradesh, and its metacercaria in fingerlings of 15 species of freshwater fishes. These are, *Puntius sophore, P. chola, P. ticto, Chela laubuca, Nandus nandus, Cirrhinus reba, Oxygaster phulo, Ambassis ranga, Amblypharyngodon mola, Esomus danricus, Ompok bimaculatus, M. vittatus, Xenentodon cancilla,Osteobrame cotio* and *Colisa lalius.* Shameem and Madhavi (1988) recorded the adult from *M. gulio, M. keletius, M. vittatus* and *Nangra robusta* from Chilka lake (Orissa), and observed the metacercaria in *P. sophore.* During the present investigation the adults of *H. mehrai* were found infecting *M. malabaricus* and the metacercariae were recovered from 11 species of freshwater fishes (Table 6). Recovery of adult of *H. mehrai* from *M. malabaricus* and its metacercaria from *Esomus barbatus, Horadandia atukorali, Rasbora daniconius, Puntius filamentosus, P. melanampyx melanampyx, P. parrah, Garra mullya, M. malabaricus, M. oculatus* and *Heteropneustes fossilis* in Kerala constitutes new host and geographical records for this fluke.

Family: Hemiuridae Lühe, 1901

Genus: *Genarchopsis* Ozaki, 1925

***Genarchopsis goppo* Ozaki, 1925**

Natural infections by this fluke were found in the stomach of *Channa orientalis* collected from Sultan Bathery in Wayanad district of Kerala during October-November 1995, and February1998. The prevalence of infection was 35.71% (15 of 42 fishes), and the intensity of infection varied

from 3 to 8. Mixed infections with the metacercariae of *Encyclometra colubrimurorum* and of *Centrocestus formosanus* and the adult of *G. goppo* were frequently observed in *C. orientalis*.

Description (Fig. 5a; Table 9):

Body large, muscular, aspinose, cylindrical, with round anterior and posterior ends; measured 1550-2496 x 733-780 (2207 x 760) in size. Oral sucker subterminal, oval, highly muscular, measuring 218-250 x 280-328 (234 x 308) in size. Acetabulum large, post-equatorial, measuring 452-484 x 438-516 (467 x 499), situated at a distance of 890-1421 (1121) from anterior end. Distance between two suckers 722-1410 (982). Pre-pharynx absent; pharynx globular, muscular, 93-109 x 109-125 (99 x 114) in size. Oesophagus short, provided with a pouch and measured 70-72 x 44-46 (71 x 45) in size. Caeca reaching to posterior end of the body and unite in front of vitellaria. Testes irregular in outline, diagonal, situated on either side behind acetabulum. Right testis measured 134-295 x 218-250 (206 x 233); left testis 218-297 x 187-250 (246 x 217) in size. Cirrus sac long, pear-shaped, measured 219-300 x 210-298 (230 x 246); just behind caecal bifurcation; encloses a curved, winding seminal vesicle and pars prostatica. Genital pore post-bifurcal, in front of right caecum. Ovary spherical, intercaecal, submedian, post-testicular, in front of caecal union; measured 130-284 x 192-281 (182 x 205) in size. Vitellaria divided into two compact, diagonal masses lying at the posterior end of body; right one measured 154-242 x 47-156 (193 x 89); left one 109-218 x 47-94 (163 x 70) in size. Uterine coils occupy pre- and post-acetabular intercaecal areas. Metraterm muscular. Eggs yellowish brown, ovoid, with one polar filament. Eggs

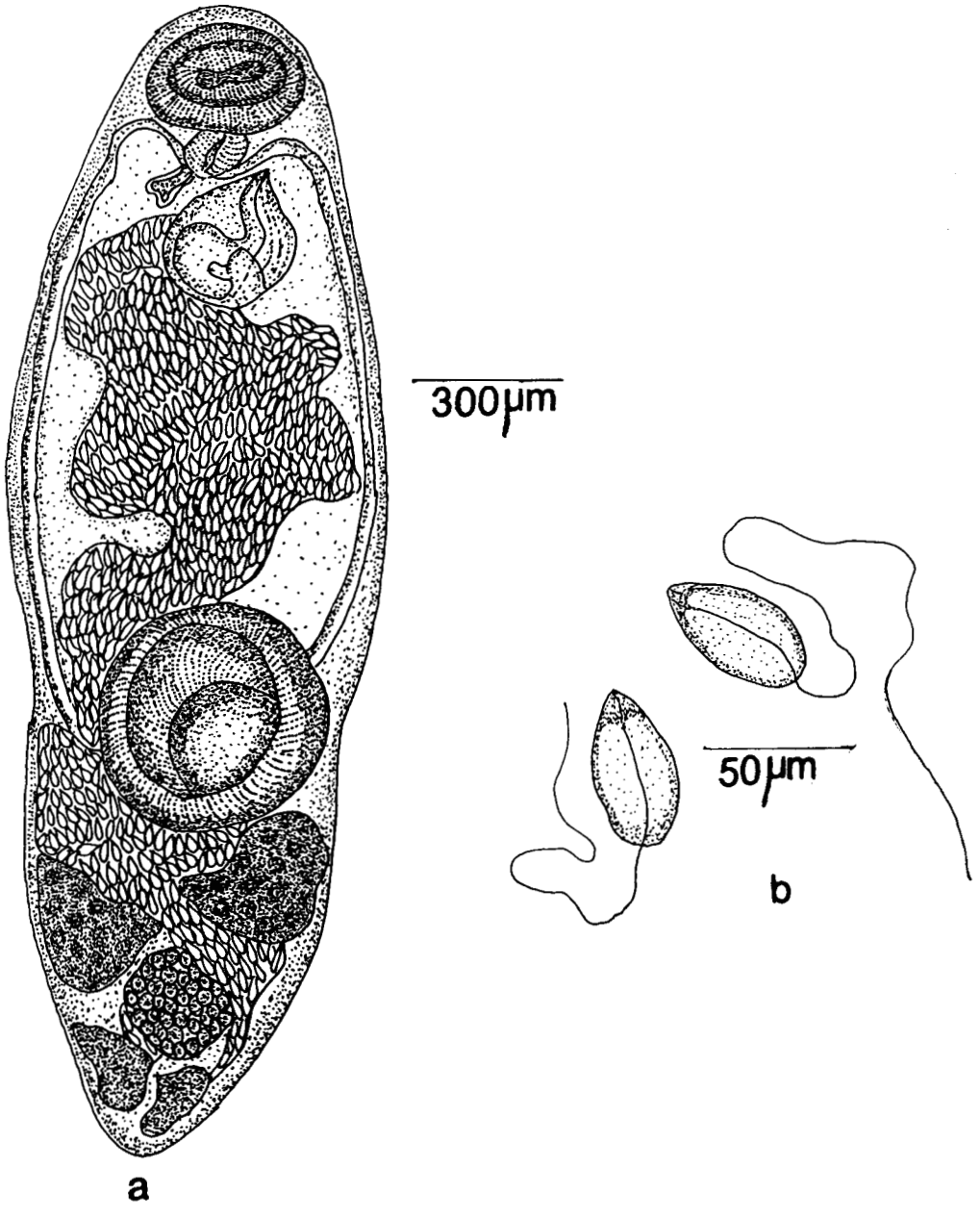


Fig. 5. *Genarchopsis goppo* Ozaki, 1925

a. Adult; b. Eggs

Table 9. Measurements (μm) of adults of *Genarchopsis goppo* Ozaki, 1925

Character	Range	Mean
Body size	1550-2496 x 733-780	2207 x 760
Oral sucker	218-250 x 280-328	234 x 308
Acetabulum	452-484 x 438-516	467 x 499
Distance from anterior end to acetabulum	890-1421	1121
Distance between suckers	722-1410	982
Pharynx	93-109 x 109-125	99 x 114
Oesophagus	70-72 x 44-46	71 x 45
Right testis	134-295 x 218-250	206 x 233
Left testis	218-297 x 187-250	246 x 217
Cirrus sac	219-300 x 210-298	230 x 246
Ovary	130-284 x 192-281	182 x 205
Right vitellaria	154-242 x 47-156	193 x 89
Left vitellaria	109-218 x 47-94	163 x 70
Egg	41-62 x 23-30	46 x 27
Egg filament	106-297	220

measured 41-62 x 23-30 (46 x 27) in size; filament 106-297 (220) long (Fig. 5b).

Host	:	*Channa orientalis* Schneider
Site of infection	:	Stomach
Locality	:	Sultan Bathery in Wayanad district of Kerala.
Period of collection	:	October -November 1995; February 1998.
Prevalence of infection	:	15 of 42 (35.71%) fishes examined.
Intensity of infection	:	3 to 8

Remarks:

The characters of the present fluke recovered from the stomach of *Channa orientalis* agreed fully with the descriptions of *Genarchopsis goppo* Ozaki, 1925, suggesting its identity to *G. goppo.*

Altogether 11 species of *Genarchopsis* have been reported from Indian freshwater fishes. Rai (1971a) and Pandey (1973b) synonymised 10 species with *G. goppo,* the type species reported by Ozaki (1925). The other species, *G. wallagoni* described by Chandra and Banerjee (1993), differs only slightly from *G. goppo,* and is probably synonymous to it. *G. goppo* has previously been reported from different freshwater fishes of India by several workers. Srivastava (1933) reported it from *Ophiocephalus striatus,* Gupta (1951) from *O. punctatus* and *Mastacembelus armatus,* Agrawal (1966) from *Channa punctatus,* Kakaji (1969) from *Mystus seenghala* and *Amphipnous cuchia,* Rai (1971b) from *O. punctatus, M. armatus* and *Mystus vittatus,* Pandey (1973b) from *C. striatus* and *Clarias batrachus,* and Madhavi (1978) from *C. punctatus.* Besides, Madhavi (1978, 1980) implicated *Aplocheilus panchax* as a paratenic host in the life-cycle of *G. goppo.* The finding of this fluke in *C. orientalis* in

Sultan Bathery represents a new host record and the first record of *G. goppo* from Kerala.

LARVAL DIGENEA

Family: Strigeidae Railliet, 1919

Tetracotyle metacercaria sp. I

Seventy out of 295 (23.73%) *Danio aequipinnatus*, collected from Karetta in Kannur and Nedungottumedu in Malappuram districts of Kerala during September and November 1995, May and September 1996 and February1998 were infected by this trematode. The intensity of infection varied from 6 to 14. Mixed infections with the metacercaria of *Centrocestus formosanus* and *Tetracotyle* metacercaria sp.I were frequently observed in *D. aequipinnatus*.

Description:

Cysts oval, milky-white, measuring 1092-1248 x 811-982 (1192 x 895) in size. Cyst wall double-layered; outer layer thick, fibrous and opaque; inner layer thin, hyaline and transparent. The larva lies curled up inside the cyst wall compactly filling the cavity (Fig. 6a; Table 10).

Excysted metacercaria (Fig. 6b; Table 10):

Body thick, oval, aspinose, divided into fore- and hind-bodies. Fore-body large, measured 780-936 x 702-905 (864 x 827), concave ventrally, with widened anterior end; hind-body small, bluntly conical, measured 234-374 x 246-437 (293 x 312). Oral sucker terminal, round to oval, 109-156 x 85-150

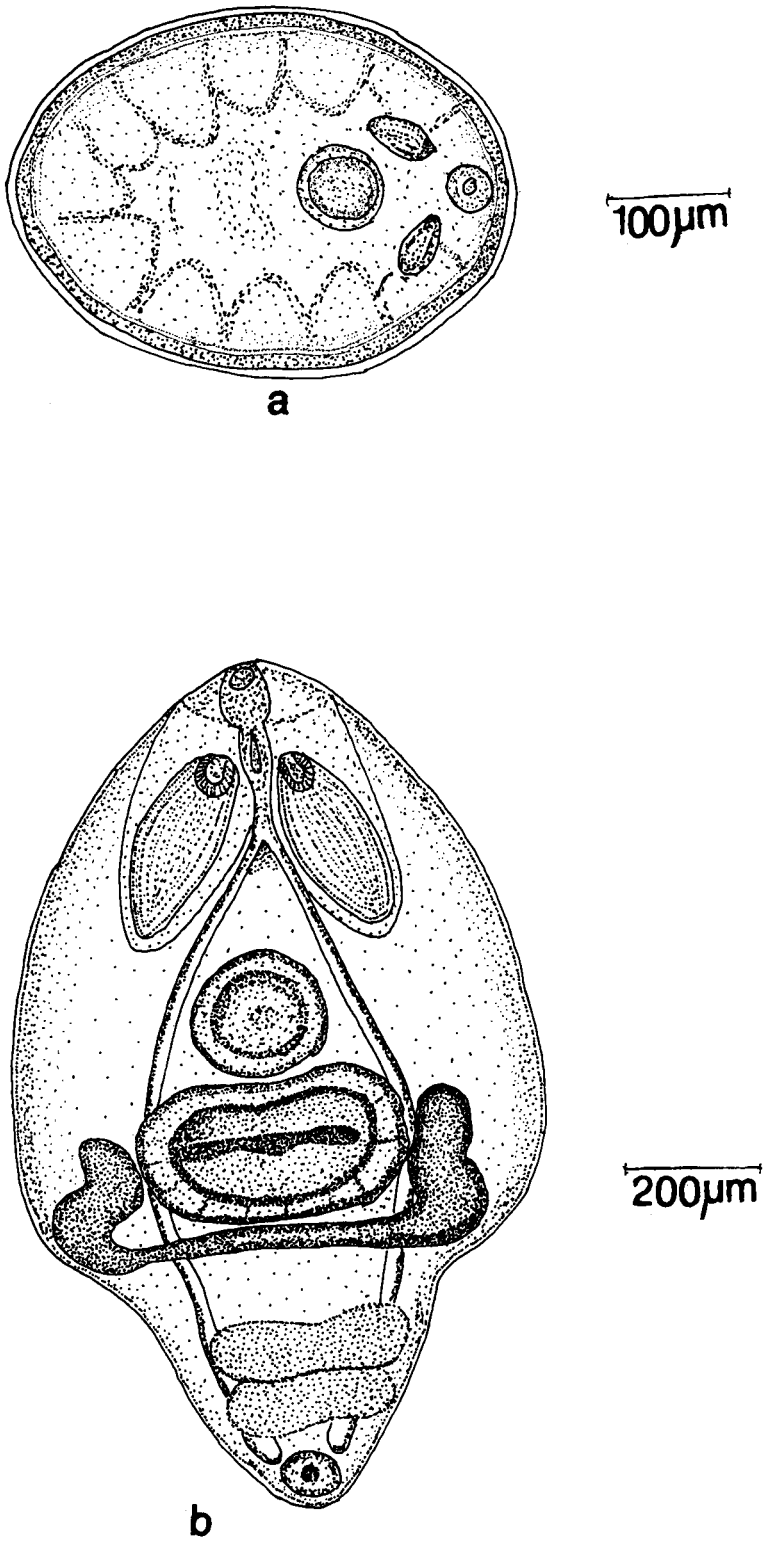


Fig. 6. *Tetracotyle* metacercaria sp. I

a. Encysted metacercaria; b. Excysted metacercaria

Table 10. Measurements (μm) of metacercariae of *Tetracotyle* sp. I

Character	Range	Mean
Encysted metacercaria	1092-1248 x 811-982	1192 x 895
Fore-body	780-936 x 702-905	864 x 827
Hind-body	234-374 x 246-437	293 x 312
Oral sucker	109-156 x 85-150	127 x 125
Acetabulum	124-195 x 156-223	173 x 191
Accessory sucker	265-359 x 156-187	304 x 172
Hold -fast organ	171-234 x 280-406	195 x 347
Hold-fast gland	811-1248 x 46-123	964 x 75
Pharynx	47-70 x 39-47	61 x 51
Oesophagus	93-156	127
Caeca	936-1045	979

(127 x 125) in size. Acetabulum oval, pre-equatorial in the fore-body; measured 124-195 x 156-223 (173 x 191). Two large accessory suckers present, one on either side of oral sucker and measured 265-359 x 156-187 (304 x 172). Hold-fast organ well-developed, highly muscular, situated posterior to acetabulum; measured 171-234 x 280-406 (195 x 347). Hold-fast gland large, semicircular, measured 811-1248 x 46-123 (964 x 75). Pre-pharynx absent. Pharynx small, subglobular, 47-70 x 39-47 (61 x 51) in size. Oesophagus 93-156 (127) long. Caeca 936-1045 (979) long, extend upto the posterior end of hind-body. The rudiments of gonads represented by 2 masses, situated in tandem in posterior region of hind-body. Excretory bladder small, oval with subterminal excretory pore. Reserve excretory system extensively developed, consisting of large number of canals forming a network and contained refractory granules.

Fish host	:	*Danio aequipinnatus* McClelland
Site of infection	:	Muscles near to vertebral column
Locality	:	Karetta in Kannur, and Nedungottumedu in Malappuram districts of Kerala.
Period of collection	:	September and November 1995; May and September 1996,and February 1998
Prevalence of infection	:	70 of 295 (23.73%) fishes examined.
Intensity of infection	:	6 to 14.

Remarks:

The present metacercaria recovered from *Danio aequipinnatus* is characterised by the presence of a double-layered cyst wall and a pair of accessory suckers on either side of oral sucker. It is, therefore, identified as

belonging to the larval genus *Tetracotyle* Fillipi, 1859 of the family Strigeidae Railliet, 1919. Several species of *Tetracotyle* have been described by different workers from various parts of the world. The first report of the genus from India was made by Kaw (1950) who described *T. ranae* from the frog, *Rana cyanophlyctis*. Since then 20 species have been added to this larval genus from India: *Tetracotyle indicus* Singh, 1957; *T. sophorensis* Singh, 1959; *T. ujjainensis* Trivedi, 1964; *T. muscularis* Chakrabarti, 1970; *T. xenenotodoni* Chakrabarti, 1970; *T. glossogobi* Chakrabarti, 1970; *T. szidati* Chakrabarti and Baugh, 1970; *T. lali* Pandey, 1971; *T. lucknowensis* Pandey, 1971; *T. baughui* Pandey, 1973; *T. tandoni* Pandey, 1973; *T. aglanulata* Baugh and Chakrabarti, 1977; *T. gyanpurensis* Agrawal and Singh, 1980; *T. pandei* Agrawal and Khan, 1982; *T. srivastavai* Agrawal and Khan, 1982; *T. ramalingui* Agrawal & Khan, 1982; *T. simhai* Pandey and Tewari, 1983; *T. sanjivi* Pandey and Tewari, 1983; *T. fotedari* Pandey and Tewari, 1983, and *T. satendri* Tewari and Tyagi, 1986.

The present metacercaria is comparable to *T. lucknowensis* reported by Pandey (1971b) from *Channa striatus* in several respects. The exact identity of the fluke can be revealed by raising adults from metacercariae. Therefore, the present form recovered from *D. aequipinnatus*, is tentatively reported here as *Tetracotyle* metacercaria sp. I.

Tetracotyle metacercaria sp. II

Twenty out of 22 *Etroplus suratensis*, collected from Nallur in Kozhikode district of Kerala during April-August 1997, were found to be infected by this metacercaria. The intensity of infection varied from 7 to 10.

Description:

Cysts oval to elongate, white, 2124-2304 x 1000-1545 (2219 x 1254) in size. Cyst wall single ; the larva remains folded inside the cyst wall (Fig. 7a; Table 11).

Excysted metacercaria (Fig. 7b; Table 11):

Body elongated, foliaceous, aspinose, measured 1622-2483 x 842-1024 (2024 x 956). Oral sucker terminal, round to oval, 128-234 x 179-250 (178 x 194) in size. Acetabulum pre-equatorial, 202-258 x 179-245 (208 x 196) in dimensions. Distance between suckers 374-666 (499). Two small accessory suckers, one on either side of oral sucker; measured 202-270 x 179-223 (217 x 191). Hold-fast organ equatorial, transversely elongated, with a median slit-like opening; measured 171-282 x 343-538 (236 x 410) in size. Hold-fast gland 'U'-shaped, 588-1024 x 109-255 (874 x 184) in size. Pharynx absent; oesophagus 179-250 (216) in length. A pair of lateral diverticulum present just before the caecal bifurcation. Caeca 1216-2048 (1668) long, extend upto the posterior end of body. Rudiments of gonads represented by a small mass of dark staining cells located at posterior end of the body. Excretory bladder 'V' shaped; excretory pore terminal.

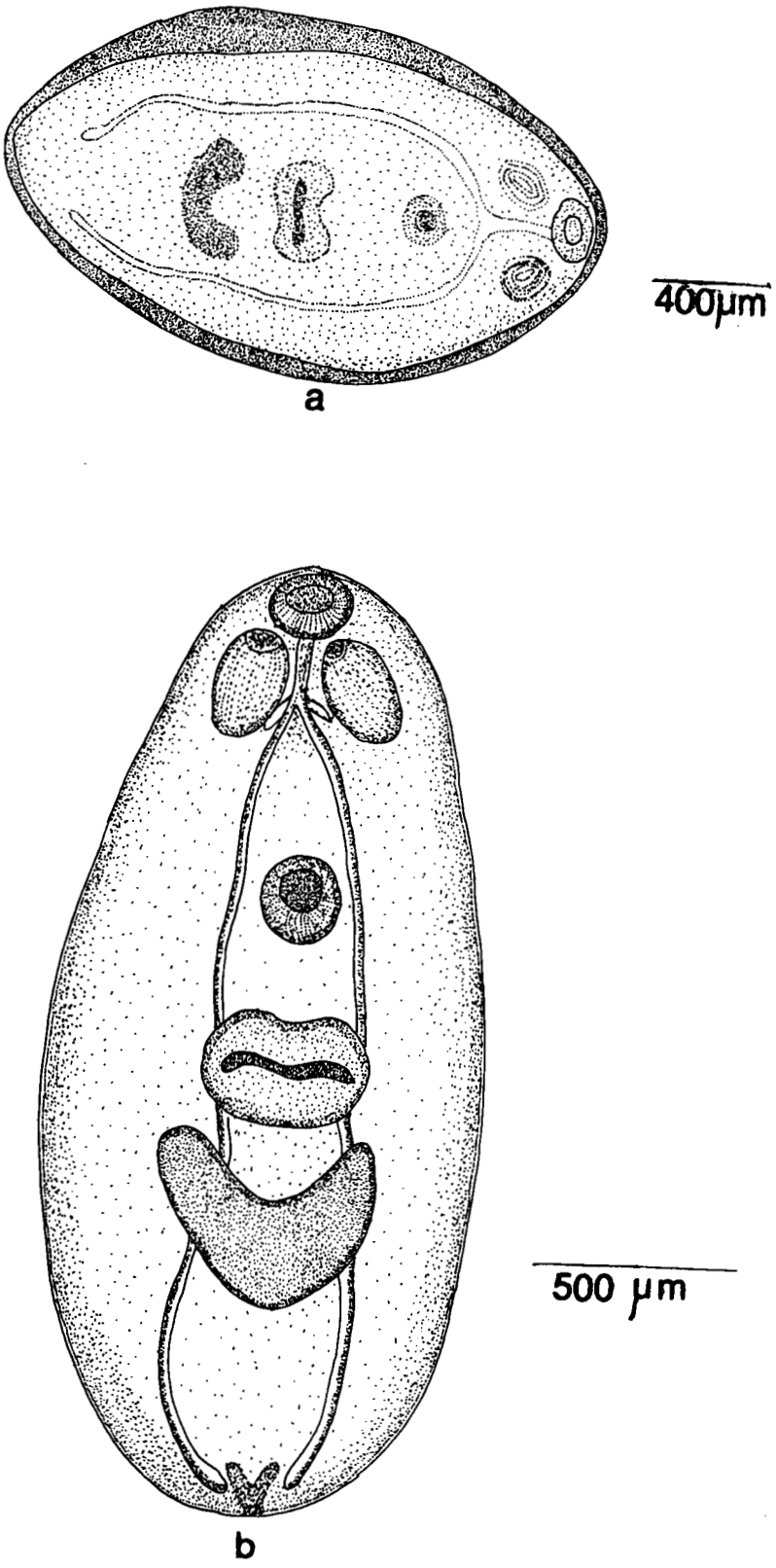


Fig. 7. *Tetracotyle* metacercaria sp. II

a. Encysted metacercaria; b. Excysted metacercaria

Table 11. Measurements (μm) of metacercariae of *Tetracotyle* sp. II

Character	Range	Mean
Encysted metacercaria	2124-2304 x 1000-1345	2219 x 1254
Excysted metacercaria	1622-2483 x 842-1024	2024 x 956
Oral sucker	128-234 x 179-250	178 x 194
Acetabulum	202-258 x 179-245	208 x 196
Distance between suckers	374-666	499
Accessory suckers	202-270 x 179-223	217 x 191
Hold-fast organ	171-282 x 343-538	236 x 410
Hold-fast gland	588-1024 x 109-255	874 x 184
Oesophagus	179-250	216
Caeca	1216-2048	1668

Fish host	:	*Etroplus suratensis* (Bloch)
Site of infection	:	Mesentery of visceral organs
Locality	:	Nallur in Kozhikode district of Kerala.
Period of collection	:	April - August 1997
Prevalence of infection	:	20 of 22 (90.91%) fishes examined.
Intensity of infection	:	7 to 10

Remarks:

The present metacercaria found attached to the mesentery of visceral organs of *Etroplus suratensis* is identified as belonging to the larval genus *Tetracotyle* Fillipi, 1859 of the family Strigeidae Railliet, 1919. It differs from *Tetracotyle* metacercaria sp I (present report) in the shape of body and hold-fast organ, and in the absence of pharynx. Besides it is recorded from a different fish host. A comparison of characters of the known species of *Tetracotyle* revealed that it resembles *Tetracotyle xenentodoni* described by Chakrabarti (1970) from *Xenentodon cancilla* in several morphological features, but there are minor differences also. The exact identity of the metacercaria could not be ascertained because experimental attempts to raise adults were futile. Therefore, the present metacercaria is tentatively reported here as *Tetracotyle* metacercaria sp. II.

Family : Strigeidae Railliet, 1919

Diplostomulum sp.

Natural infections by the metacercariae were found in the muscles of *Labeo rohita* collected from Malampuzha in Palakkad district of Kerala. The

prevalence of infection was 66.13% in a total of 62 specimens examined during November of 1996 and 1997. The intensity of infection varied from 8 to 25. Mixed infection with the metacercariae of *Diplostomulum* sp., *Echinochasmus megavitellus* and *Centrocestus formosanus,* were frequently observed in *L. rohita.*

Description:

Cysts large, oval to round, globular, measured 1561-1843 x 1408-1613 (1702 x 1510) in size. Cyst wall double-layered; outer layer thick, fibrous and black; inner layer thin, gelatinous and transparent. The larva lies curled up inside the cyst wall (Fig. 8a; Table 12).

Excysted metacercaria (Fig. 8b; Table 12).

Body pear-shaped, aspinose, divided into a foliaceous fore-body and a conical hind-body. Fore-body measured 1792-2150 x 1228-1612 (1971 x 1491) in size; hind-body 716-947 x 844-1152 (853 x 1032) in size. Oral sucker subterminal, round to oval, measuring 128-202 x 156-192 (160 x 168) in size. Acetabulum located near the middle of fore-body, measured 156-274 x 202-307 (208 x 256) in size. Distance between suckers 1331-1485 (1425). Accessory suckers well-developed, muscular, saccular with deep depressions, extending from the level of pharynx to caecal bifurcation; measured 588-717 x 192-282 (646 x 221) in size. Hold-fast organ large, located in the posterior region of fore-body, measuring 324-640 x 360-499

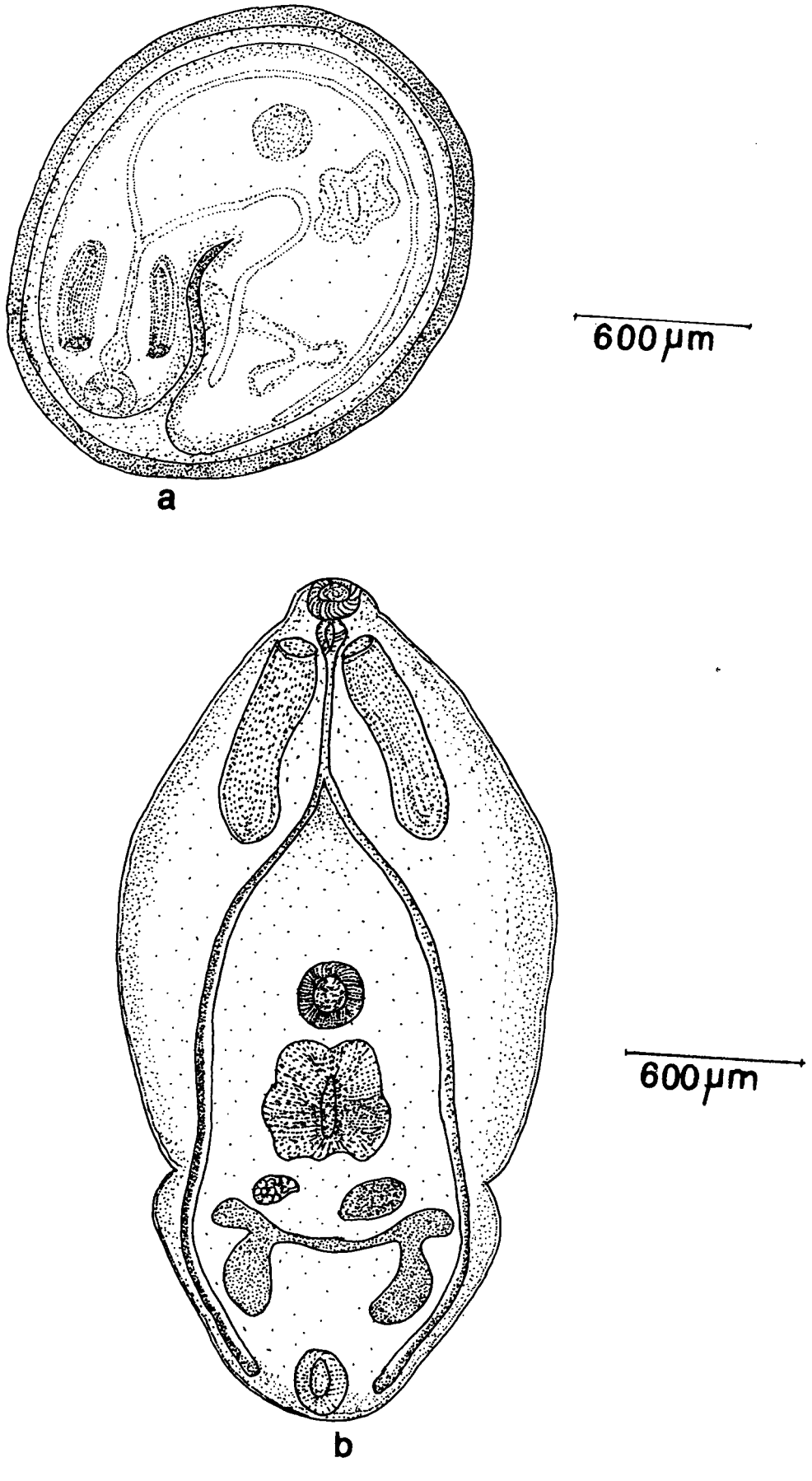


Fig. 8. *Diplostomulum* sp.

a. Encysted metacercaria; b. Excysted metacercaria

Table 12. Measurements (μm) of metacercariae of *Diplostomulum* sp.

Character	Range	Mean
Encysted metacercaria	1561-1843 x 1408-1613	1702 x 1510
Fore-body	1792-2150 x 1228-1612	1971 x 1491
Hind-body	716-947 x 844-1152	853 x 1032
Oral sucker	128-202 x 156-192	160 x 168
Acetabulum	156-274 x 202-307	208 x 256
Distance between suckers	1331-1485	1425
Accessory suckers	588-717 x 192-282	646 x 221
Hold-fast organ	324-640 x 360-499	495 x 428
Pharynx	78-109 x 78-102	95 x 92
Oesophagus	371-384	378
Caeca	2048-2380	2253
Anterior testis	121-187 x 78-171	172 x 158
Posterior testis	362-440 x 440-603	400 x 516
Ovary	62-78 x 62-129	70 x 86

(495 x 428) in size. Hold-fast gland present. Pharynx globular, muscular, 78-109 x 78-102 (95 x 92) in size. Oesophagus 371-384 (378) long. Caeca extend up to the posterior region of hind-body, 2048-2380 (2253) in length. Testes well developed, asymmetrical. Anterior testis oval to pyriform, 121-187 x 78-171 (172 x 158) in size; posterior testis 'H' shaped,362-440 x 440-603 (400 x 516) in size. Ovary oval, located in front of posterior testis, in level with anterior testis and measured 62-78 x 62-129 (70 x 86). Bursa copulatrix present at the hind end of body. Excretory bladder 'V'-shaped; excretory pore terminal.

Fish host	:	*Labeo rohita* (Hamilton)
Site of infection	:	Muscles
Locality	:	Malampuzha in Palakkad district of Kerala.
Period of collection	:	November of 1996 and 1997
Prevalence of infection	:	41 of 62 (66.13%) fishes examined.
Intensity of infection	:	8 to 25

Remarks:

The present metacercaria recovered from the muscles of *Labeo rohita* resembles the larval genus *Diplostomulum* Brandes, 1892, the adults of which are mainly parasitic in the intestine of fish eating birds. The adult belongs to the genus *Diplostomum* von Nordmann, 1832. Altogether 4 species of *Diplostomulum* have been reported from freshwater fishes of

India: *D. singhi* Pandey *et al.* 1964; *D. nurius* Thapar, 1967; *D. minutum* Pandey, 1970, and *D. ophthalmi* Pandey, 1970. The present form is distinctly different from all these species by possessing large, highly muscular, lateral suckers with deep depressions. Therefore, it is not included in any of the known species of *Diplostomulum* and is reported here as *Diplostomulum* sp. It is expected that recovery of adults of the 4 known species of metacercariae will result in the loss of specific status for some of these species.

Family : Strigeidae Railliet, 1919

***Neascus* metacercaria sp. I**

Sixty-five of 69 (94.2%) *Xenentodon cancila,* collected from Puthukode in Kozhikode and Valapad in Thrissur districts of Kerala during March and September 1996 and May 1997, were positive for this metacercaria. Forty-five of 120 (37.5%) *Aplocheilus panchax,* collected from Karetta in Kannur and Vythiri in Wayanad districts of Kerala during October-December 1995, March-April 1996, September-October 1997, were also positive. The intensity of infection varied from 25 to 33 larvae.

Description:

Cysts oval, translucent, measuring 624-1248 x 546 - 936 (936 x 721) in size; cyst wall single. The larva lies curled up in cyst fluid, occupying only part of cyst cavity (Fig. 9a, Table 13).

Excysted metacercaria (Fig. 9b; Table 13):

Body elongated, foliaceous, spinose, divided into fore- and hind-bodies. Fore-body leaf-like, measuring 600-924 x 246-477 (748 x 366). Hind-body small, oval, measuring 223-323 x 169-254 (284 x 230) in size. Oral sucker terminal, oval, 28-68 x 32-72 (48 x 53) in size. Acetabulum post-equatorial, 57-73 x 53-75 (60 x 58) in dimensions. Hold-fast organ oval, situated at posterior end of fore-body, 130-218 x 146-162 (166 x 155) in size. Hold-fast gland transversely elongated. Pharynx absent; oesophagus very short, bifurcates into caeca reaching to the level of rudimentary gonads in the hind-body. Caeca measuring 965-991 (975) in length. Gonads rudimentary, represented by three darkly staining masses, tandemly placed in the posterior region of hind-body. A bursa copulatrix present at the hind-body, opened outside through a subterminal genital pore; measures 74-77 x 75-79 (75 x 77) in size. Excretory bladder V-shaped, opening out by terminal excretory pore. Reserve excretory system extensively developed, consisting of two main reserve excretory canals arising from the cornua of excretory bladder and running anteriorly along the lateral regions of body up to the level of oral sucker where they are joined by a transverse canal.

Fish host	:	*Aplocheilus panchax* (Hamilton) *Xenentodon cancila* Hamilton
Site of infection	:	Liver
Locality	:	Karetta in Kannur, Puthukode in Kozhikode, Vythiri in Wayanad and Valapad in Thrissur districts of Kerala.
Period of collection	:	October to December 1995; March, April

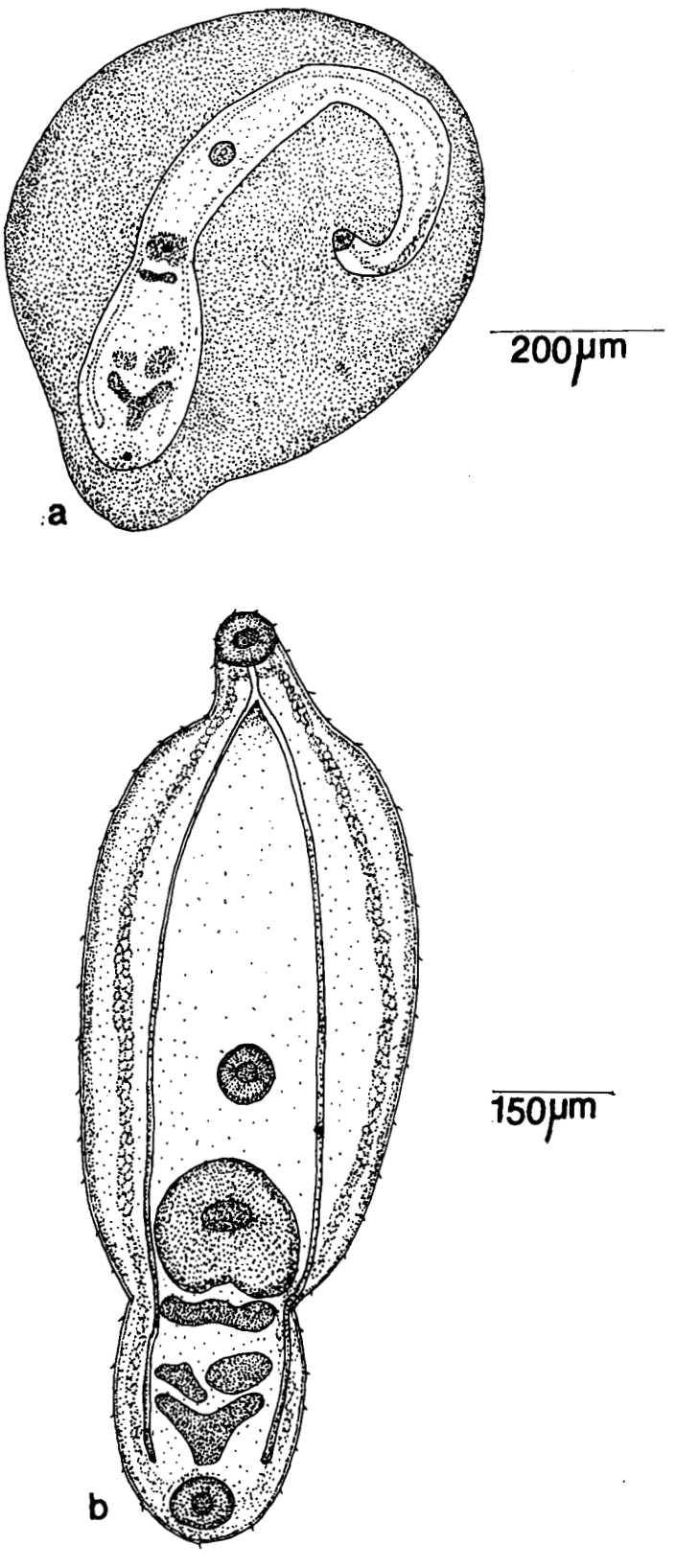


Fig. 9. *Neascus* metacercaria sp. I

a. Encysted metacercaria; b. Excysted metacercaria

Table 13. Measurements (μm) of metacercariae of *Neascus* sp. I

Character	Range	Mean
Encysted metacercaria	624-1248 x 546-936	936 x 721
Fore-body	600-924 x 246-477	748 x 366
Hind-body	223-323 x 169-254	284 x 230
Oral sucker	28-68 x 32-72	48-53
Acetabulum	57-73 x 53-75	60 x 58
Hold-fast organ	130-218 x 146-162	166 x 155
Caeca	965 - 991	975
Bursa copulatrix	74-77 x 75-79	76 x 77

and September 1996 and May, September and October 1997

Prevalence of infection : 65 of 69 (94.2%) *X. cancila* examined.
45 of 120 (37.5%) *A.panchax* examined.

Intensity of infection : 25 to 33

Remarks:

The present metacercaria recovered from the liver of *Aplocheilus panchax* and *Xenentodon cancila* has foliaceous and spinose body which is divided into fore- and hind-bodies. Besides, it has oval-shaped hold-fast organ, transversely elongated hold-fast gland and reserve excretory system. Based on these characters, the present form is included under the larval genus *Neascus* Hughes, 1927 of the family Strigeidae Railliet, 1919. The first report of *Neascus* in India was made by Kaw (1950) who described *N. vetastai* from *Schizothorax esocinus, S. micropogon, S. niger* and *Oreinus sinuatus.* Fifteen species belonging to this genus have been recorded from India since then. These are *N. elongatus* Singh, 1957; *N. chelai* Khera, 1958; *N. indicus* Thapar, 1967; *N. cirrhinus* Thapar, 1967; *N. hepatica* Chakrabarti, 1970; *N. channai* Pandey, 1971; *N. xenentodoni* Pandey, 1971; *N. gussevi* Chakrabarti, 1974; *N. hoffmani* Baugh and Chakrabarti, 1977; *N.hanaksagarensis* Baugh and Chakrabarti, 1977; *N. chauhani* Agrawal and Khan, 1982; *N. hanumanthai* Agrawal and Khan, 1982; *N. moghei* Agrawal and Khan, 1982; *N. simhai* Agrawal and Khan, 1982, and *N. punctatusi* Dhanumkumari, 1994.

A comparison of characters of the present form with that of the known species of *Neascus* revealed that it resembles *N. hepatica* described by

Chakrabarti (1970) from *Xenentodon cancilla* in several respects. The exact identity of the fluke can be revealed by raising adults from metacercariae. Therefore, the present form is tentatively reported here as *Neascus* metacercaria sp. I.

Neascus metacercaria sp. II

Liver tissue of 4 out of 301 (1.33%) *Macropodus cupanus*, collected from Kuttimakkul in Kannur district during August-September 1996, was found infected by this metacercaria. The intensity of infection varied from 9 to 12.

Description:

Cysts elongate-oval, 858-961 x 420-635 (920 x 535) in size; cyst wall double-layered. Metacercaria lies curled up inside the cyst cavity (Fig. 10a; Table 14).

Excysted metacercaria (Fig. 10b; Table 14):

Body elongated, foliaceous, aspinose, divided into fore- and hind-bodies. Fore-body broad, leaf-like, 777-936 x 500-624 (830 x 532) in size; hind-body conical, 312-485 x 312-390 (410 x 338) in size. Oral sucker terminal, 47-62 x 53-63 (56 x 59) in size. Acetabulum measured 34-56 x 49-62 (44 x 55). Hold-fast organ large, situated just above the hind-body; 156-187 x 200-265 (168 x 223) in size. Hold-fast gland present. Pre-pharynx absent. Pharynx globular, muscular, measured 33-41 x 36-42 (40 x 41). Oesophagus 80-110 (105) in length, bifurcates into caeca reaching to the

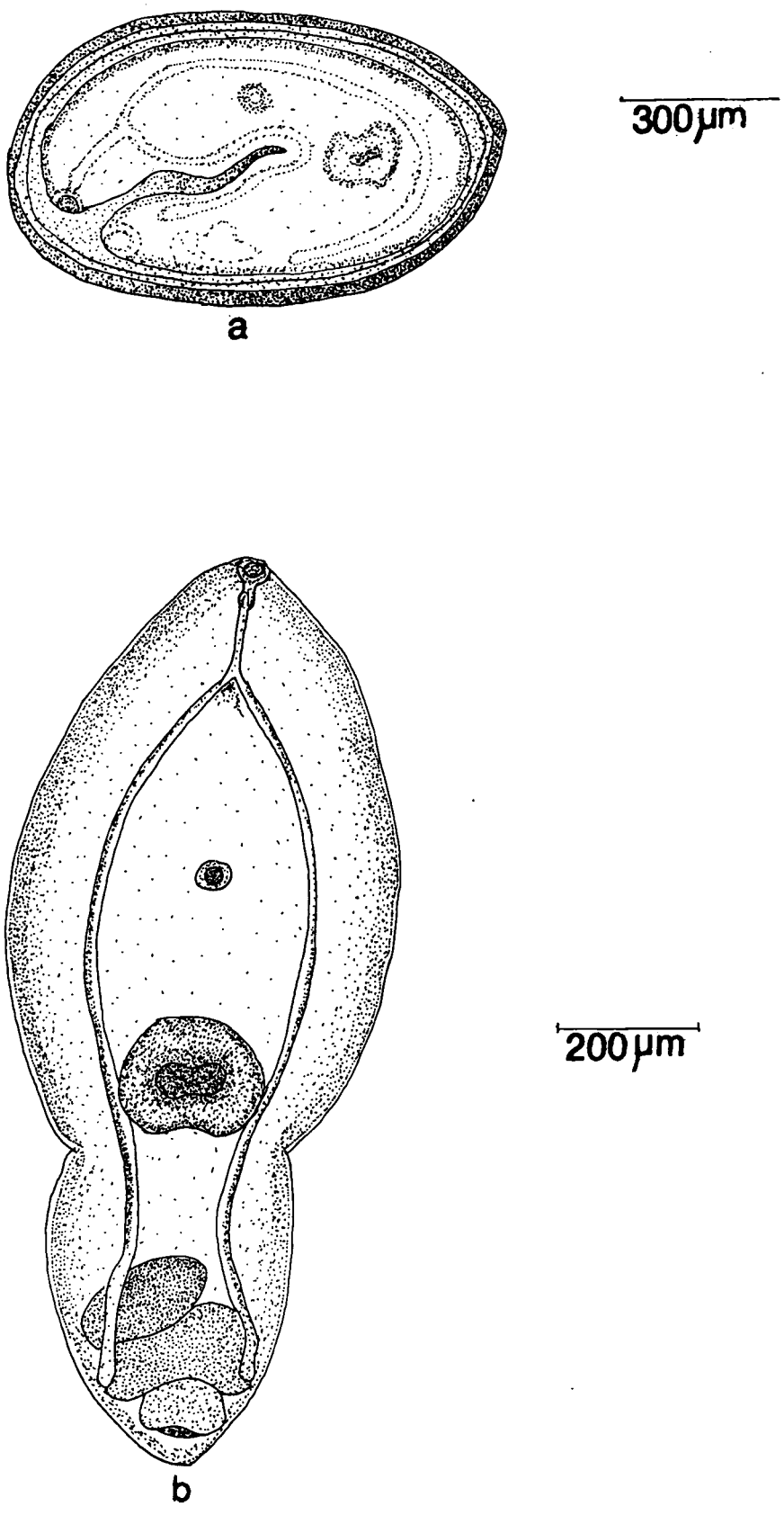


Fig. 10. *Neascus* metacercaria sp. II

a. Encysted metacercaria; b. Excysted metacercaria

Table 14. Measurements (μm) of metacercariae of *Neascus* sp. II

Character	Range	Mean
Encysted metacercaria	858-961 x 420-635	920 x 535
Fore-body	777-936 x 500-624	830 x 532
Hind-body	312-485 x 312-390	410 x 338
Oral sucker	47-62 x 53-63	56 x 59
Acetabulum	34-56 x 49-62	44 x 55
Hold-fast organ	156-187 x 200-265	168 x 223
Pharynx	33-41 x 36-42	40 x 41
Oesophagus	80-110	105
Caeca	951-1032	1003
Anterior testis	42-112 x 78-208	92 x 155
Posterior testis	78-112 x 203-216	85 x 209
Bursa copulatrix	83-95 x 104-116	89 x 111

level of testes; caeca measuring 951-1032 (1003) in length. Testes tandem, at posterior end of hind body. Anterior testis measured 42-112 x 78-208 (92 x 155), and posterior testis 78-112 x 203-216 (85 x 209) in size. Ovary represented by a mass of cells, just above anterior testis. A bursa copulatrix present at the hind-body, opened outside through a subterminal genital pore; measured 83-95 x 104-116 (89 x 111) in size. Excretory bladder 'V'-shaped; excretory pore terminal. Reserve excretory system extensively developed.

Fish host	:	*Macropodus cupanus* Valenciennes
Site of infection	:	Liver
Locality	:	Kuttimakkul in Kannur district of Kerala
Period of collection	:	August-September 1996
Prevalence of infection	:	4 of 301 (1.33%) fishes examined
Intensity of infection	:	9 to 12

Remarks:

The present metacercaria recovered from the liver of *Macropodus cupanus* is identified as belonging to the larval genus *Neascus*, Hughes 1927 of the family Strigeidae Railliet, 1919. It differs from *Neascus* metacercaria sp. I in having a double-layered cyst wall, aspinose body, muscular pharynx, long oesophagus and well developed testes. Further, it infects a different fish host. A comparison of characters of the present form with that of the known species of *Neascus* revealed that it shows some resemblance to *N. xenentodoni* described by Pandey (1971c) from *Xenentodon cancila*. Experimental attempts to raise the adults from metacercariae were

unsuccessful, and therefore, the identity of the larva cannot be revealed. The present form is tentatively reported here as *Neascus* metacercaria sp. II.

Family: Diplostomidae Poirier, 1886

Genus: *Diplostomum* von Nordmann, 1832

***Diplostomum ketupanense* Vidyarthi, 1937**

Metacercaria

Natural infections by the metacercariae were found in the muscle tissues of *Rasbora daniconius* collected from Kuttimakkul, Pinarayi, Kanhilari and Karetta in Kannur; Feroke, Kundayithode and Puthukode in Kozhikode; Sultan Bathery, Meenangadi and Vythiri in Wayanad, and Nedungottumedu and Calicut University Campus in Malappuram districts of Kerala during September-December 1995, February-June and October-December 1996, January-May and September-November 1997 and January-March 1998. The prevalence of infection was 67.52% in a total of 622 specimens examined, and intensity of infection varied from 10 to 80. Mixed infections with metacercariae of *D. ketupanense, Haplorchoides mehrai* and *Acanthostomum burminis* were frequently observed in *R. daniconius.*

Description:

Cysts large, white, oval, 936-1482 x 702-1029 (1209 x 860) in size; cyst wall double-layered, with an outer, thick fibrous layer, measuring 46-254 (148), and an inner, thin, hyaline layer of 39-103 (67) thickness (Fig. 11a; Table 15).

Excysted metacercaria (Fig. 11b; Table 15):

Metacercarial body pear-shaped, divisible into a distinct foliaceous fore-body, and a conical hind-body. Fore-body spinose, measured 468-1029 x 500-748 (748 x 624). Hind-body aspinose, measured 358-499 x 296-452 (429 x 374). Oral sucker subterminal, measured 62-93 x 62-115 (78 x 88). Accessory suckers well-developed, semicircular, strongly muscular, with shallow depressions; situated on either side of oral sucker, and measured 84-187 x 62-146 (135 x 104). Acetabulum located near the middle of fore-body, 53-100 x 78-107 (77 x 92) in size. Hold-fast organ large, circular, muscular, with a narrow median slit; situated just behind acetabulum and measured 130-185 x 94-270 (157 x 182) in size. Hold-fast gland bilobed, just behind hold-fast organ. Pharynx well-developed, 38-54 (46) long and 30-67 (49) wide; oesophagus short, bifurcates anterior to acetabulum into caeca measuring 795-817 (805); caeca extend upto posterior region of hind-body. Testes well-developed, asymmetrical; anterior testis roughly oval or pyriform, 61-78 x 77-154 (70 x 97) in size; posterior testis 'H'-shaped, situated medially in hind-body; its transverse limb measured 107-300 (189) and vertical limb 99-190 (151).

. Ovary oval, located in front of posterior testis, in level with anterior testis, and measured 33-65 x 46-100 (49 x 77). A bursa copulatrix present at the hind end of body. Excretory bladder 'V'-shaped, at posterior end of body and filled with excretory concretions. Excretory pore terminal.

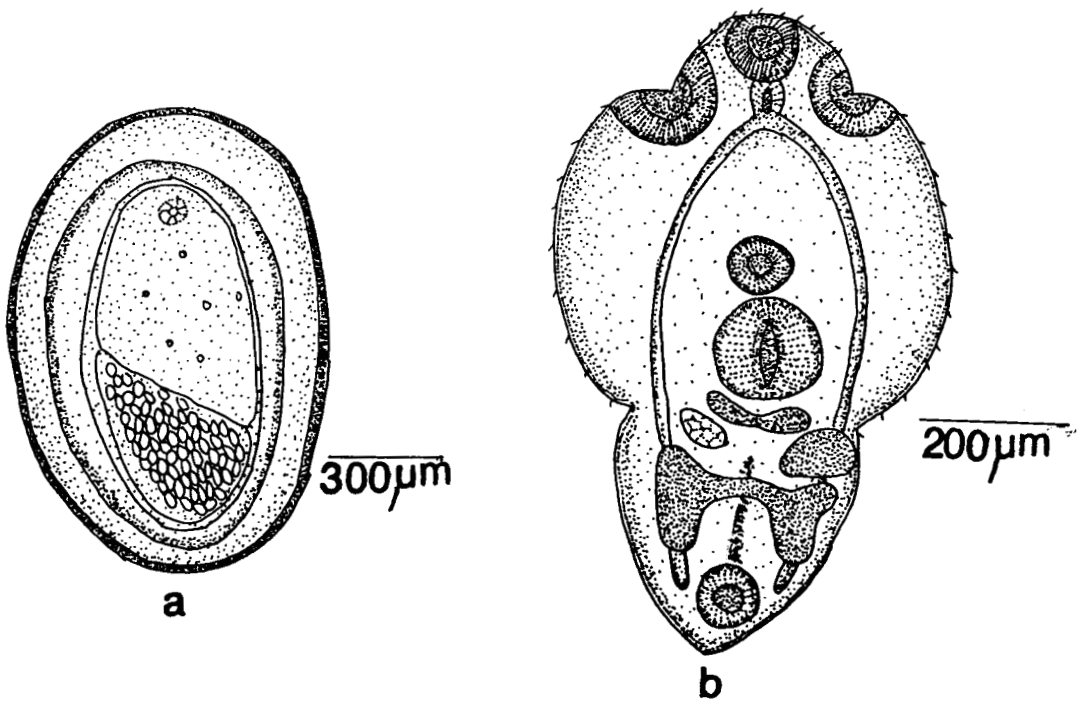


Fig. 11. *Diplostomum ketupanense* Vidyarthi, 1937

a. Encysted metacercaria; b. Excysted metacercaria

Table 15. **Measurements (μm) of metacercariae of *Diplostomum ketupanense* Vidyarthi, 1937**

Character	Range	Mean
Encysted metacercaria	936-1482 x 702-1029	1209 x 860
Fore-body	468-1029 x 500-748	748 x 624
Hind-body	358-499 x 296-452	429 x 374
Oral sucker	62-93 x 62-115	78 x 88
Accessory suckers	84-187 x 62-146	135 x 104
Acetabulum	53-100 x 78-107	77 x 92
Hold-fast organ	130-185 x 94-270	157 x 182
Pharynx	38-54 x 30-67	46 x 49
Caeca	795-817	805
Anterior testis	61-78 x 77-154	70 x 97
Posterior testis	107-300 x 99-190	189 x 151
Ovary	33-65 x 46-100	49 x 77

Adult:

Adult flukes were obtained from the small intestine of *Ardeola grayii* collected from the locality where the infected fishes were collected. The flukes were identical with *Diplostomum ketupanense* Vidyarthi, 1937. Four *A. grayii* were fed with fishes containing naturally infected metacercariae or those carrying 25-day-old experimentally developed metacercariae, and examined at various intervals post-infection. In one instance as many as 35 flukes were recovered from a single host. Juvenile flukes, obtained from birds after 2-days post-infection,measured 995-1301 x 370-410 (1125 x 400), and were very similar to excysted metacercariae in their organization. Further development was rapid, and 9-day old flukes were mature with fully developed eggs in their uteri. These flukes were similar to those recovered from natural infections of *A. grayii*. A brief description of the flukes is furnished below (Fig. 11c; Table 16).

The fore-body similar to that of metacercariae; measured 523-733 x 346-639 (628 x 493). Hind-body long, cylindrical, aspinose, 354-795 x 249-454 (574 x 359) in size. Oral sucker subterminal, 40-78 x 63-84 (62 x 74). Accessory suckers 31-62 x 77-125 (47 x 101) in size. Acetabulum anterior to middle of fore-body, measured 61-94 x 69-92 (78 x 81). Hold-fast organ massive, with a narrow median slit; just behind acetabulum and measured 124-235 x 125-215 (179 x 170). Hold-fast gland bilobed. Pharynx well-developed, 46-62 x 35-62 (54 x 48) in size. Oesophagus long; caeca narrow, long, reaching posterior extremity. Testes in hind body; anterior testis oval, measured 78-140 x 78-158 (109 x 118); posterior testis H-shaped; its transverse axis measured 246-481 (291) and vertical limbs measured 207-

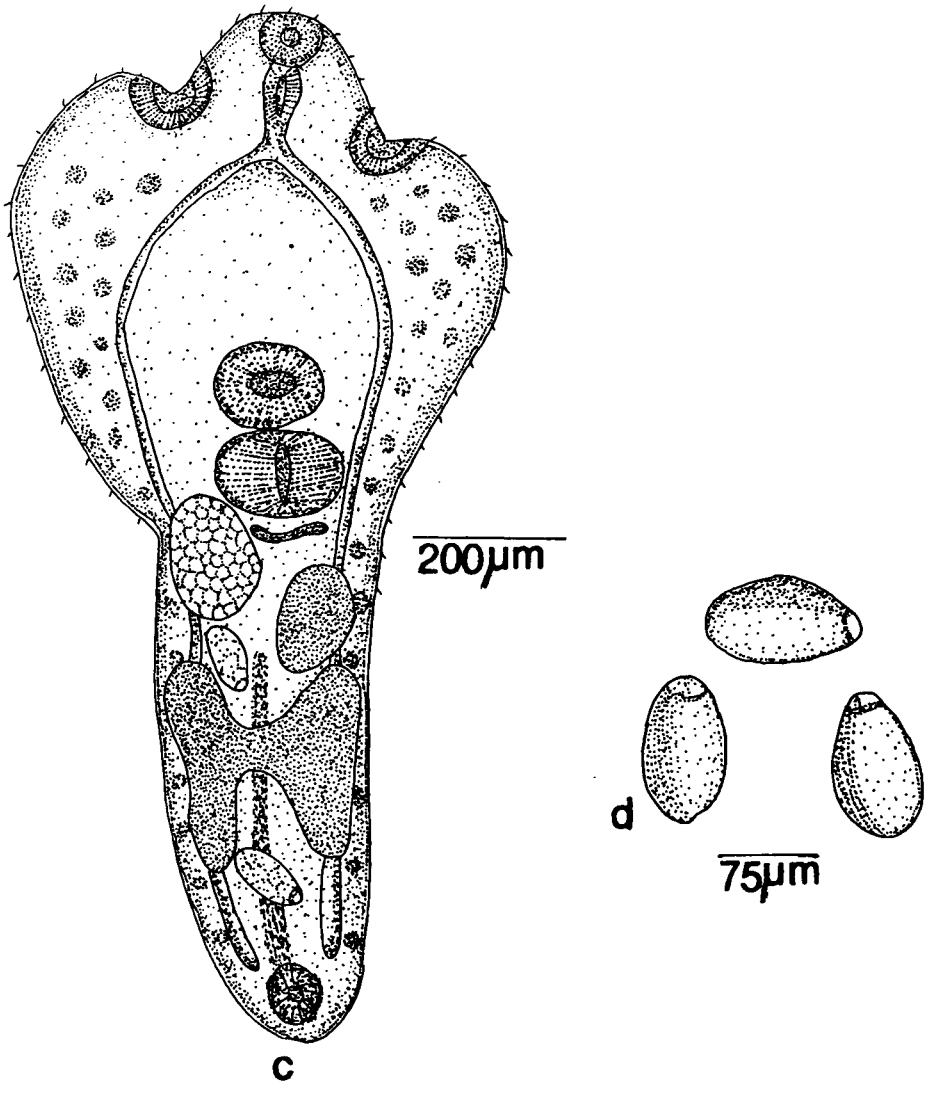


Fig. 11. *Diplostomum ketupanense* Vidyarthi, 1937

c. Adult; d. Eggs

Table 16. Measurements (μm) of adults of *Diplostomum ketupanense* Vidyarthi, 1937

Character	Range	Mean
Fore-body	523-733 x 346-639	628 x 493
Hind-body	354-795 x 249-454	574 x 359
Oral sucker	40-78 x 63-84	62 x 74
Accessory suckers	31-62 x 77-125	47 x 101
Acetabulum	61-94 x 69-92	78 x 81
Hold-fast organ	124-235 x 125-215	179 x 170
Pharynx	46-62 x 35-62	54 x 48
Anterior testis	78-140 x 78-158	109 x 118
Posterior testis	246-481 x 207-270	291 x 230
Ovary	69-171 x 93-140	122 x 116
Egg	75-101 x 62-78	90 x 70

270 (230). Ovary oval, measured 69-171 x 93-140 (122 x 116). Vitellaria follicular, extending from the level of acetabulum to copulatory bursa. Uterus well-developed, contained 2 or 4 eggs. A bursa copulatrix present at the hind end of body opens out through a subterminal genital pore. Eggs oval, yellowish brown, operculate, 75-101 x 62-78 (90 x 70) in size (Fig. 11d; Table 16).

Cercaria

Natural infections by the cercariae were found in 125 of 751 (16.64%) *Indoplanorbis exustus* examined. Infected snails were obtained only during the monsoon period (June to September). Cercarial emergence occurred throughout day time, though it was maximum in the morning. Cercariae exhibited characteristic swimming behaviour with short resting periods alternating with brief swimming activity. During resting, the cercariae remain suspended in the water column with the body hanging downward, tail stem turned upward and the furcae diverged at an angle of about 60° to the tail stem. The cercaria emerged from the snail,and exposed to *Rasbora daniconius* developed into the present metacercaria.

Description (Fig. 11e; Table 17):

Distomatous, pharyngeate, furcocercous cercaria. Body elongate-oval, measured 200-223 x 53-77 (212 x 69). Tail stem measured 292-300 x 33-53 (297 x 41), provided with sensory hairs. The furcae almost equal in length to tail stem, leaf-like and end in sharp points. Tail attachment terminal.

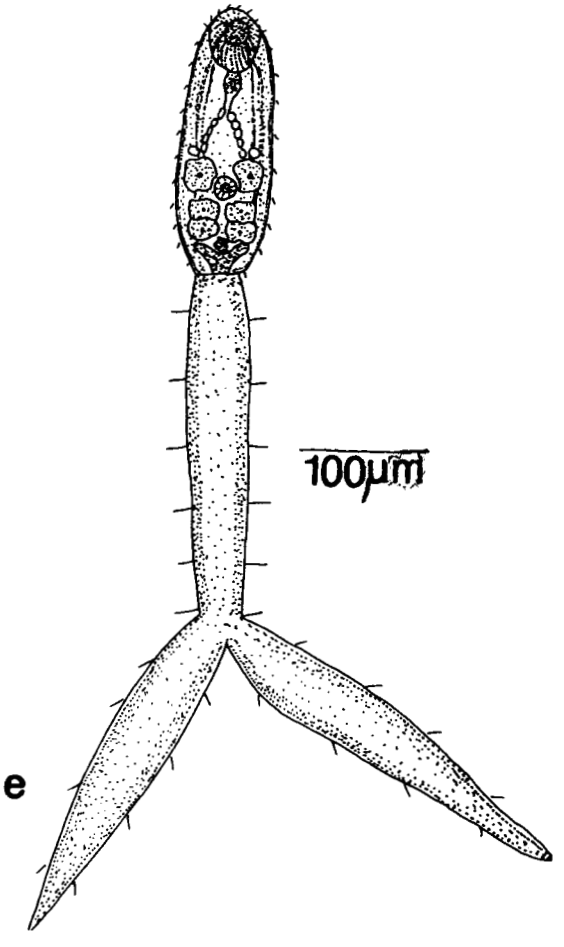


Fig. 11. *Diplostomum ketupanense* Vidyarthi, 1937

e. Cercaria

Table 17. **Measurements (μm) of cercariae of *Diplostomum ketupanense* Vidyarthi, 1937**

Character	Range	Mean
Body	200-223 x 53-77	212 x 69
Tail stem	292-300 x 33-53	297 x 41
Anterior organ	23-38 x 23-27	30 x 25
Acetabulum	28-34 x 21-25	30 x 23
Pharynx	19-27 x 17-23	24 x 21

Body tegument thick, spinose. Anterior organ ovoid, highly muscular, measured 23-38 x 23-27 (30 x 25). Two yellow eyespots, rounded or oval, just anterior to the first pair of penetration glands. Acetabulum median, 28-34 x 21-25 (30 x 23) in size. Mouth subterminal, leads to small pre-pharynx; pharynx muscular, measured 19-27 x 17-23 (24 x 21). Oesophagus short; caeca extending up to the anterior end of penetration glands. Penetration glands 3 pairs, with finely granular contents and large nuclei; one pair pre-acetabular and 2 pairs post-acetabular. The ducts of penetration glands of each side form separate bundles, run anteriorly and open out through 3 pairs of pores at the anterior margin. Cystogenous glands numerous.

Genital primordium is a mass of cells located anterior to excretory bladder. Excretory bladder tripartite, filled with granular concretions. Flame cell formula $2\left[(2+2) + (2+2+2) + (2)\right] = 24$. The cercariae developed in sporocysts.

Sporocyst:

Description:

Mother and daughter sporocysts were recovered from the hepatopancreas of *Indoplanorbis exustus*. They were yellow, thread-like structures which were so tangled in the liver mass of the snail that it was not easy to separate individual sporocyst. The mother sporocysts elongate, 1216-1420 (1318) in size; each with 3-5 daughter sporocysts and many germ balls (Fig. 11f). There is an opening in the anterior region through which daughter sporocysts escape. The daughter sporocysts similar to mother sporocysts, but with larger dimensions; measure 1350-2025 (1687) in length,

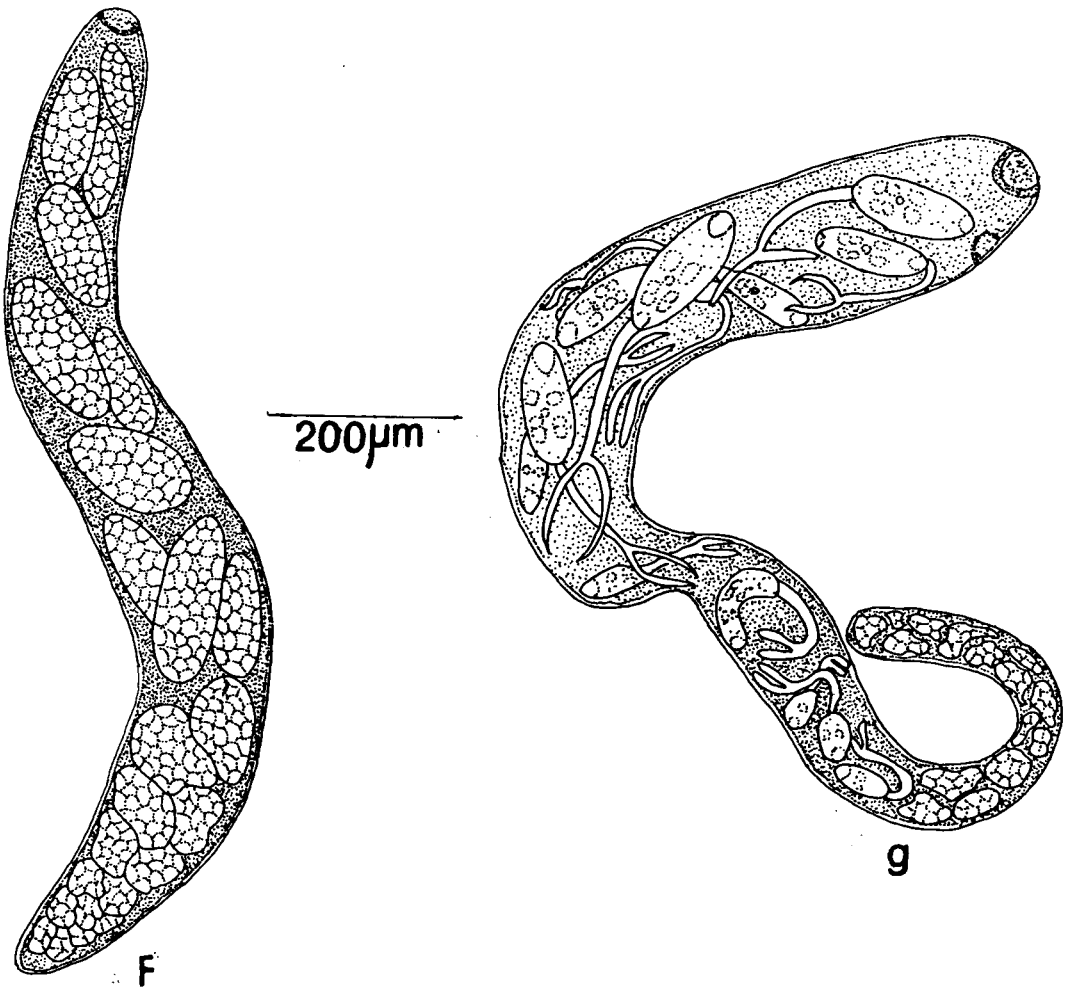


Fig. 11. *Diplostomum ketupanense* Vidyarthi, 1937

f. Mother sporocyst; g. Daughter sporocyst

and enclose 15 to 20 cercariae and a few germ balls at various stages of development (Fig. 11g).

Fish host	:	*Rasbora daniconius* (Hamilton)
Site of infection	:	Muscles
Locality of Collection	:	Kuttimakkul, Pinarayi, Kanhilari and Karetta in Kannur; Feroke, Kundayithode and Puthukode in Kozhikode; Sultan Bathery, Meenangadi and Vythiri in Wayanad, and Nedungottumedu and Calicut University Campus in Malappuram districts of Kerala.
Period of collection	:	September-December 1995, February-June and October-December 1996, January-May and September-November 1997 and January-March 1998.
Prevalence of infection	:	420 of 622 (67.52%) fishes examined
Intensity of infection	:	10 to 80
Definitive host	:	*Ardeola grayii* Sykes
Site of infection	:	Intestine
Locality of collection	:	Sultan Bathery in Wayanad and Calicut University Campus in Malappuram districts of Kerala.
Snail host	:	*Indoplanorbis exustus* (Deshayes)
Site of infection	:	Hepatopancreas

Locality : Kuttimakkul in Kannur district of Kerala

Prevalence of infection : 125 of 751 (16.64) snails examined.

Remarks:

The characters of the present metacercaria and of the adult developed from it suggest that this fluke belongs to the genus *Diplostomum* von Nordmann, 1832. Only 4 species of the genus are known from India: *D. butei* Vidyarthi, 1937 in *Buteo rufinus*; *D. ketupanense* Vidyarthi, 1937 in *Ketupa zeylonensis hardwicki*; *D. oedicnemus* Singh, 1956 in *Burhinus oedicnemus indicum*, and *D. sterni* Gupta, 1958 in *Sterna aurantia*. A comparative study of characters of the present form with that of the known *Diplostomum* spp. revealed that it undoubtedly belongs to *D. ketupanense*. Life-cycles are known for only some of the species, mostly those occurring in Europe. As far as is known, complete life-cycle of an Indian species of *Diplostomum* has not been worked out. The available information is limited to the reports on the metacercaria of *D. ketupanense* by Ganapati and Rao (1954) from Andhra Pradesh and Abraham and Anantaraman (1955) from Madras, and its miracidium by Ganapati and Rao (1962). In addition, 5 species of metacercariae, the adults of which are not known, have been reported under the larval genus *Diplostomulum* Brandes, 1892. These are *D. bufonis*, *D. singhi*, *D. nurius*, *D. minutum* and *D. ophthalmi* (Kaw, 1950; Pandey *et al.*, 1964; Thapar, 1967; Pandey, 1970). It is expected that elucidation of life-cycles of these metacercariae will result in the loss of specific status for some of these species.

Diplostomum ketupanense has a three-host life-cycle. Cercariae emerge from the planorbid snail, *Indoplanorbis exustus*, and then encyst in the

muscles of *Rasbora daniconius*. The metacercariae developed into adult flukes in 9 days in the intestine of *Ardeola grayii*.

Cercaria of *Diplostomum ketupanense* recovered from *Indoplanorbis exustus* during the present investigation agrees fully with *Cercariae indicae* II Sewell, 1922 reported from the same snail species in Calcutta. Mohandas (1974) recorded this cercaria from the same host in Kerala. Since the present cercaria and that described by Sewell (1922) are similar in organization and are infecting the same host, they are in all probability identical.

The metacercaria of *D. ketupanense* was first reported by Ganapati and Rao (1954) from *Catla catla*, and later by Abraham and Anantaraman (1955) from the same fish. The present metacercaria is identical with that reported by the above authors in morphology and morphometry. Recovery of this metacercaria from *Rasbora daniconius* constitutes a new host and geographical record for this parasite.

Family: Clinostomidae Lühe, 1901

Genus: *Euclinostomum* Travassos, 1928

***Euclinostomum heterostomum* (Rudolphi, 1809) Travassos, 1928**

Six of 25 (24%) *Etroplus maculatus*, collected from Feroke in Kozhikode district of Kerala from February to May 1996, were infected by *Euclinostomum heterostomum* metacercariae. Nine of 42 (21.43%) *Channa orientalis*, collected from Ramanattukara and Feroke in Kozhikode, Sultan Bathery in Wayanad and Nedungottumedu in Malappuram districts of

Kerala during November 1996, April and May 1997, and February 1998, were also positive for this infection. Mixed infections with the metacercariae of *Centrocestus formosanus*, *Encyclometra colubrimurorum*. and *Euclinostomum heterostomum* were frequently observed in *C. orientalis*.

Description:

Cysts oval or round, measured 2065-3987 x 1746-3249 (2926 x 2538). The larva remains curled up inside the cyst wall (Fig. 12a; Table 18).

Excysted metacercaria (Fig. 12b; Table 18):

Body elongate, linguiform, rounded at both ends, 2222-5987 x 1928 - 1543 (5323 x 1351) in size. Oral sucker subterminal, 384-461 x 768-985 (410 x 808). Acetabulum large, strongly muscular, pre-equatorial, measured 390-545 x 350-560 (400 x 442). Distance between suckers 187-245 (233). Pharynx indistinct. Oesophagus 90-120 (104) long, bifurcates in front of acetabulum. Caeca 1840-3886 (3367) long, narrow, diverticulate, extend to posterior end of body. Each caecum gives off long, narrow, unequal, lateral branches; left caecum with 10 branches and right with 12 branches. The branches measured 409-1152 (617) in length. Testes tandem, intercaecal. Anterior testis reniform, measured 102-205 x 158-219 (128 x 177), located at a distance of 2458-5120 (3365) from anterior end of body. Posterior testis 'U'-shaped, 210-366 x 188-268 (276 x 209) in size. Cirrus sac oval, situated within the concavity of anterior testis; measured 230-458 x 184-389 (318 x 243); encloses a convoluted tubular seminal vesicle which leads into a short ejaculatory duct. Cirrus sac opens into genital atrium. Ovary round to oval, intertesticular, slightly dextral, 128-157 x 183-219 (138 x 169) in size. Oviduct short, enters the ootype. Uterus coiled, emerges from the ootype,

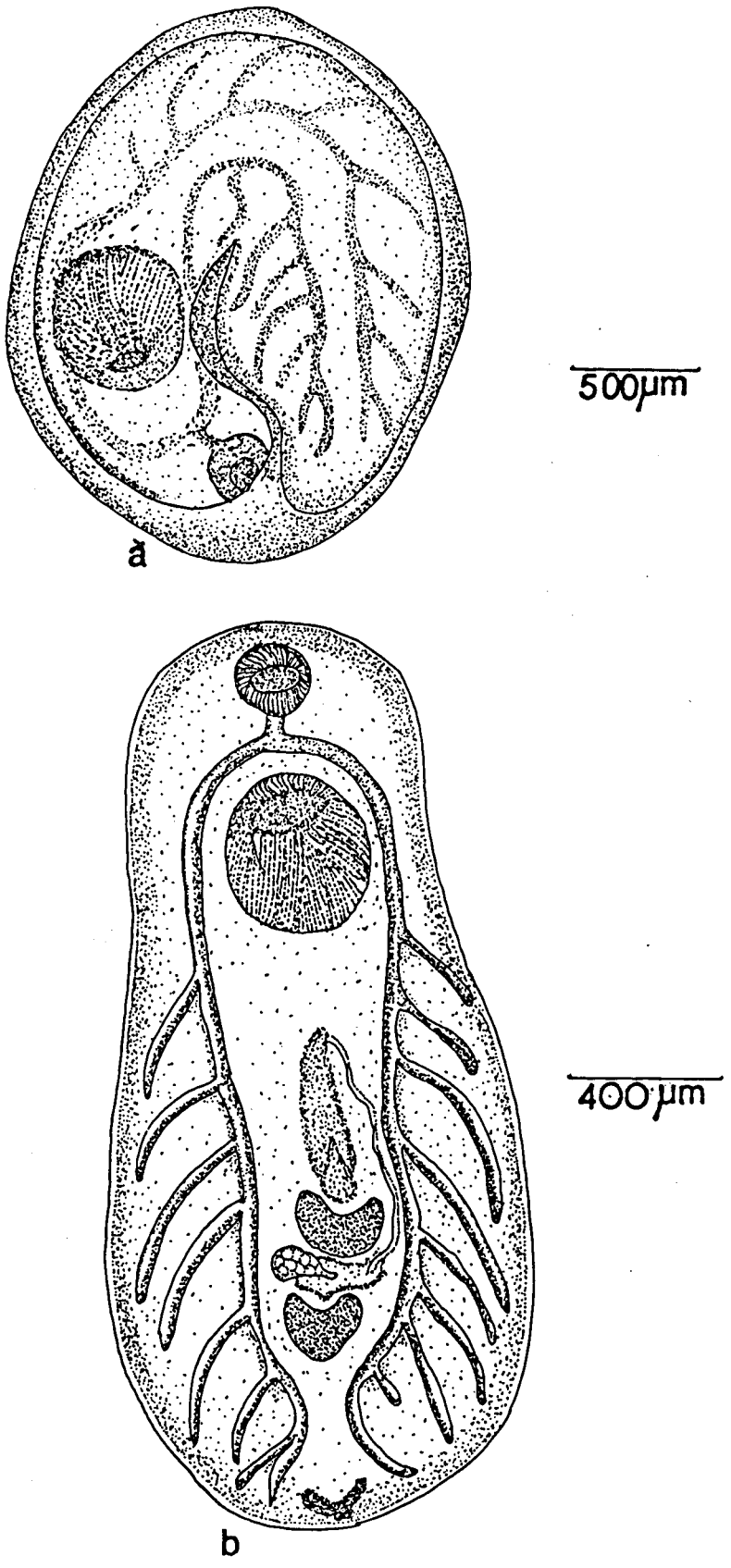


Fig. 12. *Euclinostomum heterostomum* (Rudolphi, 1809) Travassos, 1928

a. Encysted metacercaria; b. Excysted metacercaria

Table 18. Measurements (μm) of metacercariae of *Euclinostomum heterostomom* (Rudolphi, 1809) Travassos, 1928

Character	Range	Mean
Encysted metacercaria	2065-3987 x 1746-3249	2926 x 2538
Excysted metacercaria	2222-5987 x 1928-1543	5323 x 1351
Oral sucker	384-461 x 768-985	410 x 808
Acetabulum	390-545 x 350-560	400 x 442
Distance between suckers	187 x 245	233
Oesophagus	90 - 120	104
Caeca	1840-3886	3367
Caecal branches	409-1152	617
Anterior testis	102-205 x 158-219	128 x 177
Posterior testis	210-366 x 188-268	276 x 209
Cirrus sac	230-458 x 184-389	318 x 243
Ovary	128-157 x 183-219	138x169

runs forward beyond the middle of body. Metraterm opens into genital atrium. Genital pore located in the middle of body just above the anterior testis. Excretory bladder small, 'V'-shaped, located at the hind end of body, opens outside by a subterminal excretory pore.

Fish hosts	:	*Etroplus maculatus* (Bloch) *Channa orientalis* (Schneider)
Site of infection	:	Muscles
Locality	:	Feroke and Ramanattukara in Kozhikode, Sultan Bathery in Wayanad and Nedungottumedu in Malappuram districts of Kerala.
Period of collection	:	February-May and November 1996, April-May 1997 and February 1998
Prevalence of infection	:	6 of 25 (24%) *E. maculatus* examined 9 of 42 (21.43%) *C. orientalis* examined
Intensity of infection	:	10 to 22

Remarks:

The characters of the present metacercaria suggest that it belongs to the genus *Euclinostomum*. Since Ukoli (1966) synonymised all the Indian species of *Euclinostomum* with *E. heterostomum* and this was agreed to by Jhansilakshmibai and Madhavi (1997), the present form undoubtedly belongs to *E. heterostomum*. The metacercariae of *E. heterostomum* have previously been reported from several freshwater fishes in India. Bhalerao

(1942), Srivastava (1950), Gupta and Agarwal (1983) and Sinha *et al.* (1988) reported this metacercaria from *Channa punctatus;* Jaiswal(1957) from *C. punctatus* and *C. marulius;* Agarwal (1959) from *Notopterus notopterus,* and Jhansilakshmibai and Mahdavi (1997) from *C. punctatus* and *C. orientalis.* During the present investigation, the metacercariae were collected from *C. orientalis* and *Etroplus maculatus.* Recovery of the metacercaria from *E. maculatus* constitutes a new host record for this species. Besides, this forms the first report of the metacercaria from Kerala. The life-cycle of *E. heterostomum* was first traced by Donges (1974) in Western Nigeria. From India Jhansilakshmibai and Madhavi (1997) furnished a detailed account on the life-cycle of this species and implicated *Indoplanorbis exustus* as the snail host, *Channa punctatus* and *C. orientalis* as the second intermediate hosts and *Ardeola grayii* and *Bubulcus ibis* as the definitive hosts. During the present investigation, natural infections by the cercaria and adult of *E. heterostomum* could not be observed.

Family: Echinostomatidae (Looss, 1902) Poche, 1926

Echinostome metacercaria

One hundred and twenty-two of 360 (33.89%) *Esomus barbatus,* 133 of 162 (82.1%) *Horadandia atukorali,* 30 of 420 (7.14%) *Puntius parrah,* 3 of 10 (30%) *Mystus malabaricus,* 2 of 38 (5.26%) *Heteropneustes fossilis* and 3 of 22 (13.64%) *Channa punctatus* were found infected by this metacercaria. The

fishes were collected from Kuttimakkul, Kanhilari and Karetta in Kannur, Feroke and Puthukode in Kozhikode and Meenangadi and Sultan Bathery in Wayanad districts of Kerala from June to September, 1995 and January to November 1997. The intensity of infection varied from 5 to 38. Mixed infections by this metacercaria, *Centrocestus formosanus* metacercaria and the adult of *Haplorchoides mehrai* were occasionally found in *M. malabaricus*. *P. parrah* was found simultaneously infected by this metacercaria and *C. formosanus* metacercaria.

Description:

Cysts spherical to oval, found attached to the base of gill filaments and gill arches; measured 148-254 x 145-231 (195 x 170) in size. The larva remains curled up inside the double-layered cyst wall (Fig. 13a; Table 19).

Excysted metacercaria (Fig. 13c; Table 19)

Body elongate-oval, spinose, 315-462 x 112-146 (346 x 125) in size. Body spines more prominent in anterior region. Collar well developed, 89-146 (108) wide, with 27 conspicuous spines (Fig. 13b). Spine arrangement characteristic: 4 corner spines on each ventral lappet, 2 oral and 2 aboral; 6 laterals on each side in single row; 7 dorsals, 4 oral and 3 aboral. Oral sucker subterminal, 24-54 x 33-46 (37 x 39) in size. Acetabulum post-equatorial, larger than oral sucker, and measured 38-61 (49) in diameter. Distance between suckers 147-223 (195). Pre-pharynx 15-30 (19) long; pharynx muscular, 29-49 x 16-23 (34 x 20) in size. Oesophagus solid, 89-158 (121) long, consisting of 9 rectangular cells having distinct nuclei; bifurcates in front of acetabulum. Caeca with 8 cells each, terminating blindly near posterior end; measured 154-285 (180) in length. Genital primordia consist

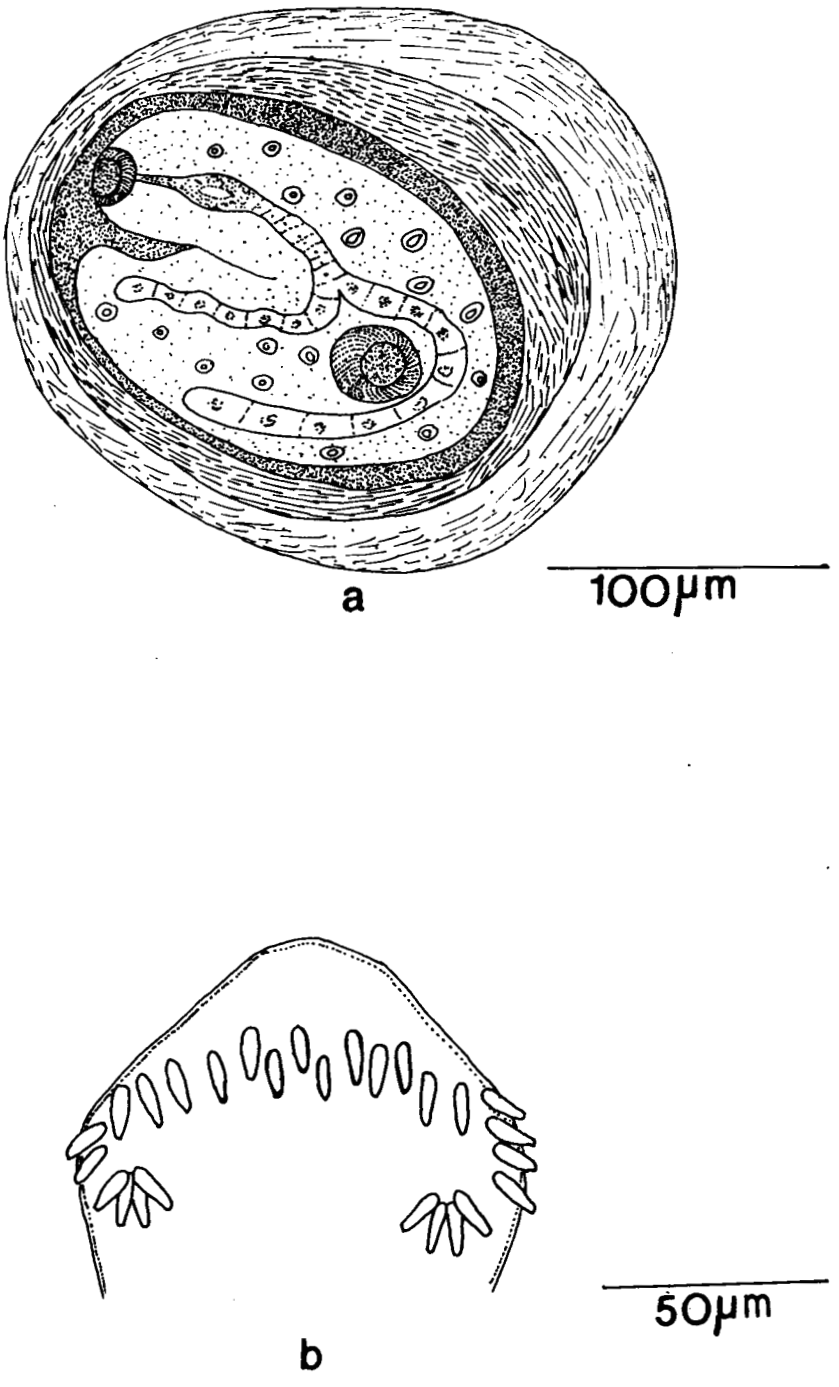


Fig. 13. Echinostome metacercaria

a. Encysted metacercaria; b. Head collar showing spines

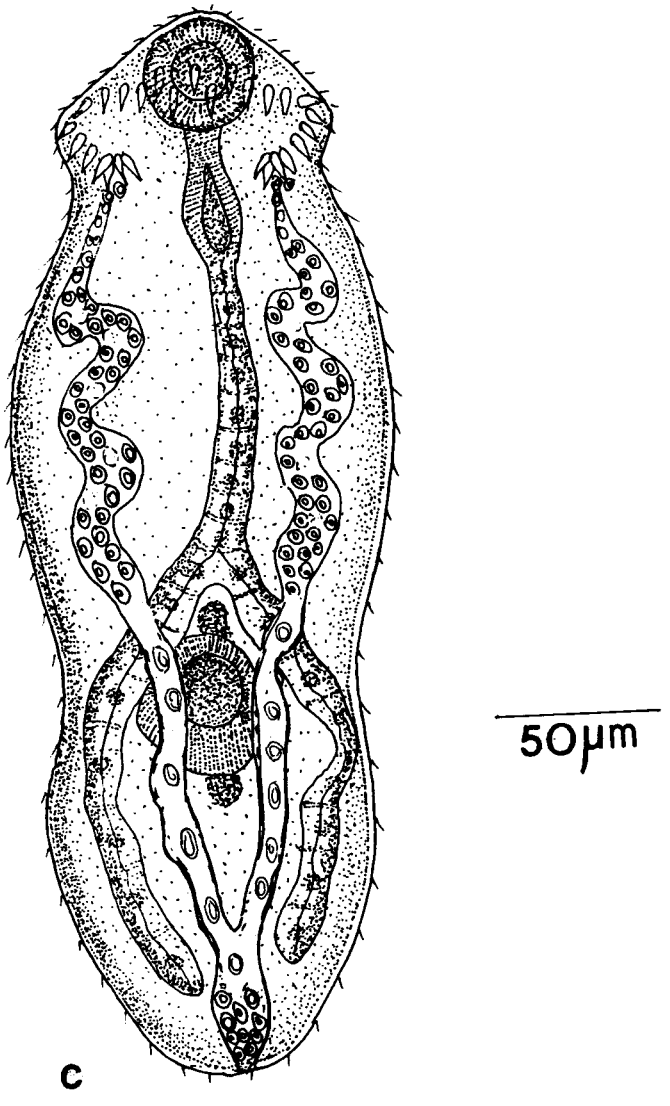


Fig. 13. Echinostome metacercaria

c. Excysted metacercaria

Table 19. Measurements (μm) of echinostome metacercaria

Character	Range	Mean
Encysted metacercaria	148-254 x 145-231	195 x 170
Excysted metacercaria	315-462 x 112-146	346 x 125
Collar width	89-146	108
Oral sucker	24-54 x 33-46	37 x 39
Acetabulum	38-61	49
Distance between suckers	147-223	195
Pre-pharynx	15-30	19
Pharynx	29-49 x 16-23	34 x 20
Oesophagus	89-158	121
Caeca	154-285	180

of two groups of cells, one located in front of acetabulum and the other slightly behind it, both connected by a string of cells. Excretory bladder 'Y'-shaped, filled with numerous refringent granules; excretory pore terminal.

Experimental attempts to develop adult flukes by feeding metacercarial cysts to *Ardeola grayii, Bubulcus ibis,* white rats and one-day-old domestic chicks were unsuccessful.

Echinostome cercaria with 27 collar spines emerged from the snail *Indoplanorbis exustus*, and exposed to *Esomus barbatus* and *Horadandia atukorali,* developed into the present metacercaria. *I. exustus* were collected from Kanhilari and Kuttimakkul in Kannur and Feroke in Kozhikode districts of Kerala.

Cercaria (Fig. 13d; Table 20)

Cercariae emerged during night and morning hours. Cercariae are negatively phototactic, swimming easily and rapidly through water while curving the body ventrally; performs leech-like movements on the bottom of the container.

Body oval, spinose, with variable number of setae in anterior region; measured 316-409 x 154-277 (399 x 198) in size. Collar 66-115 (97) wide, with 27 spines, arranged as in metacercaria. Tail 254-400 x 53-77 (363 x 60), narrowing at the tip. Oral sucker subterminal, round, 46-62 (57) in diameter. Acetabulum protrusible, post-equatorial, 46-62 x 69-92 (56 x 78). Distance between suckers 184-208 (197). Pre-pharynx 19-40 (30) long; pre-pharyngeal body conspicuous, consisting of two triangular refractile bodies. Pharynx muscular, 26-31 x 10-15 (30 x 13) in size. Oesophagus 115-

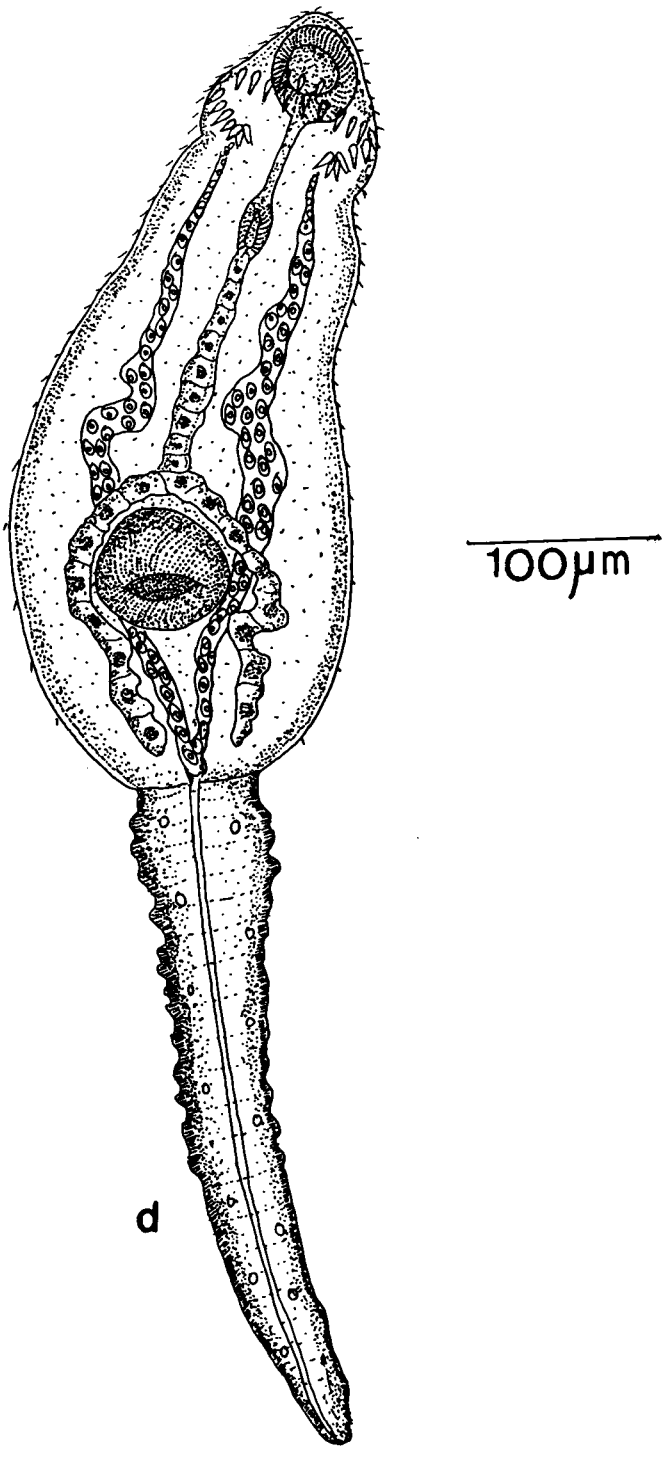


Fig. 13 d. 27-spined echinostome Cercaria

Table 20. Measurements (μm) of echinostome cercariae

Character	Range	Mean
Body size	316-409 x 154-277	399 x 198
Collar width	66 - 115	97
Tail size	254-400 x 53-77	363 x 60
Oral sucker	46-62	57
Acetabulum	46-62 x 69-92	56 x 78
Distance between suckers	184 - 208	197
Pre-pharynx	19-40	30
Pharynx	26-31 x 10-15	30 x 13
Oesophagus	115-162	137
Caeca	138-200	163

162 (137) long, solid, consisting of 9 rectangular cells with distinct nuclei. Caecal bifurcation anterior to acetabulum. Caeca with 8 cells each, 138-200 (163) long, reaching to the level of excretory bladder. Penetration glands three pairs, occur along oesophagus with gland ducts opening on dorsal lip of oral sucker. Genital primordia consist of two groups of cells, one located in front of acetabulum and the other slightly behind it. Cystogenous cells present throughout body, only a few in region of oral sucker and pharynx. These cells contained rod-shaped contents, arranged in parallel bundles. Excretory bladder 'Y'-shaped, at posterior end of body; main collecting tubes distended between acetabular and pharyngeal levels, each containing 30-40 excretory concretions, 15 to 19 in size. Flame cells incospicuous, number not ascertained.

Redia (Fig. 13e)

Rediae developed in the digestive gland of the snail host. Body long, slender, brown, measured 1060-2099 x 230-296 (1731 x 282) in size. Mouth terminal; pharynx globular, muscular, 39-73 in diameter. Collar 200-241 from anterior end. Birth pore dorsal and immediately posterior to collar. Gut elongated, extends to middle of body; 702-1357 x 31-128 (1091 x 65) in size. Rediae contained up to 20 cercariae in addition to numerous germ balls.

Fish host : *Esomus barbatus* Jerdon
Horadandia atukorali Deraniyagala
Mystus malabaricus (Jerdon)
Puntius parrah Day

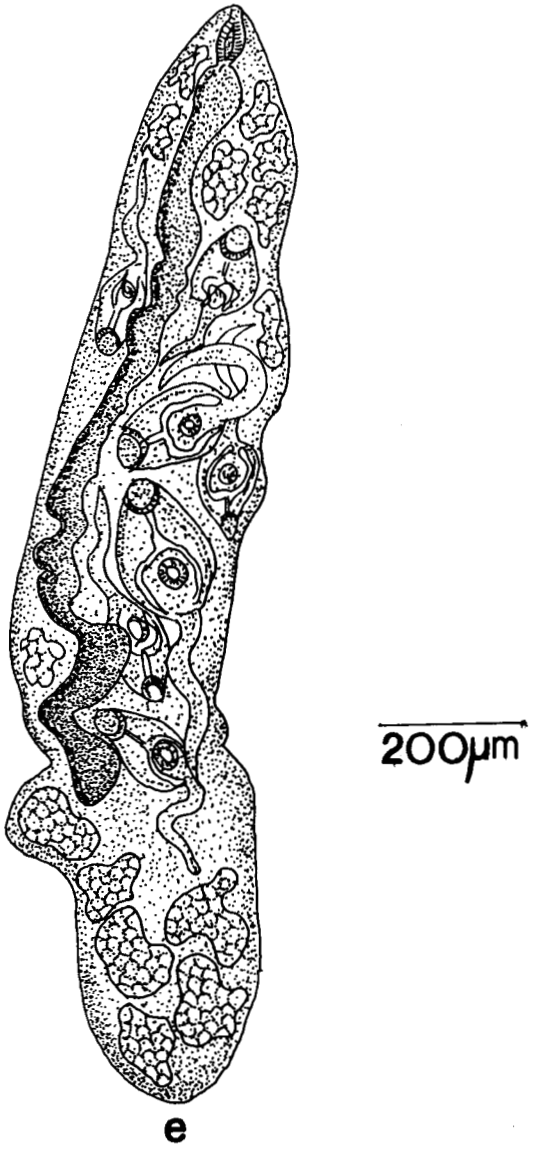


Fig. 13 e. 27-spined echinostome Redia

		Heteropneustes fossilis (Bloch)
		Channa punctatus (Bloch)
Site of infection	:	Base of gill filaments and gill arches.
Locality	:	Kuttimakkul, Kanhilari and Karetta in Kannur district; Feroke and Puthukode in Kozhikode,and Meenangadi and Sultan Bathery in Wayanad districts of Kerala.
Period of collection	:	June to September 1995, January to November 1997.
Prevalence of infection	:	122 of 360 (33.89%) *E. barbatus* examined
	:	133 of 162 (82.1%) *H. atukorali* examined
	:	30 of 420 (7.14%) *P. parrah* examined
	:	3 of 10 (30%) *M. malabaricus* examined
	:	2 of 38 (5.26%) *H. fossilis* examined
	:	3 of 22 (13.63%) *C. punctatus* examined
Intensity of infection	:	5 to 38
Snail host	:	*Indoplanorbis exustus* (Deshayes)
Site of infection	:	Hepatopancreas
Locality	:	Kanhilari and Kuttimakkul in Kannur,and Feroke in Kozhikode districts of Kerala.
Prevalence of infection	:	300 of 751 (39.94) snails examined.

Remarks:

The present metacercaria with 27 collar spines infects the gill filaments and gill arches of *Esomus barbatus, Horadandia atukorali, Mystus malabaricus, Puntius parrah,Heteropneustes fossilis* and *Channa punctatus*. The

27-spined echinostome cercaria recovered from *Indoplanorbis exustus* during this investigation developed into the present metacercaria in *E. barbatus* and *H. atukorali*. This cercaria agrees fully with *Cercaria beaveri* reported by Peter (1955) from the same host in Bareilly and, therefore, both are identical. *C. beaveri* was found encysting in the gill chambers of tadpoles. Nath (1973) reported a similar metacercaria with 27 collar spines from *Channa punctatus* and *Rana cyanophlyctis* in Uttar Pradesh. Several echinostome species with 27 collar spines were reported from Indian birds and mammals. These belonged to the genera *Petasiger* Dietz, 1909; *Paryphostomum* Dietz, 1909; *Euparyphium* Dietz, 1909, and *Chaunocephalus* Dietz, 1909 (Mehra, 1980; Srivastava, 1982). Experimental attempts to develop adult flukes by feeding infective metacercarial cysts to one-day-old chicks, white rats, *Ardeola grayii* and *Bubulcus ibis*, were unsuccessful. The present metacercaria may be expected to develop into a species belonging to one of these genera.

Family: Echinostomatidae (Looss, 1902) Poche, 1926

Genus: *Echinochasmus* Dietz, 1909

Echinochasmus megavitellus Lal, 1939

Metacercaria

Natural infections by this metacercaria were found in *Labeo rohita* collected from Malampuzha in Palakkad district of Kerala, during November of 1996 and 1997. The prevalence of infection was 12.9% (8 of 62 fishes). The intensity of infection varied from 8 to 13. Mixed infections

with the present metacercaria and metacercariae of *Centrocestus formosanus* and *Diplostomulum* sp. were found in *L. rohita*.

Description:

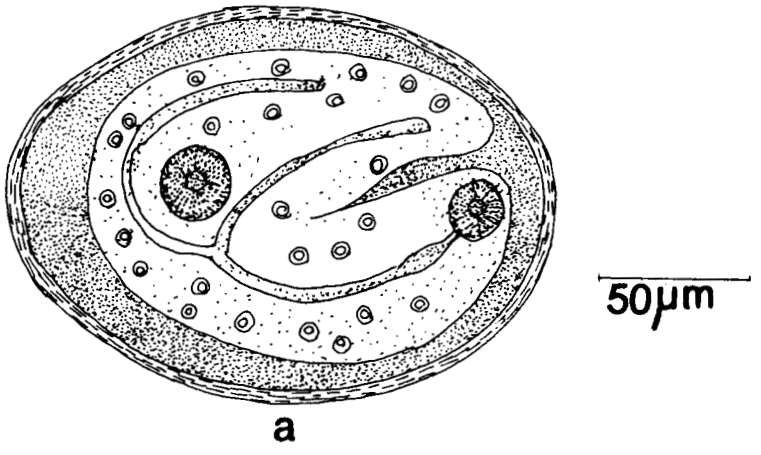
Cysts small, round to oval, transparent with double-layered wall; measured 121-201 x 100-185 (153 x 130). The larva remains folded inside the cyst wall (Fig. 14a; Table 21).

Excysted metacercaria (Fig. 14b; Table 21)

Body elongate-oval, spinose with prominent collar at anterior end; measured 385-539 x 100-169 (471 x 122) in size. Collar reniform, beset with a dorsally interrupted single row of 24 collar spines. Oral sucker subterminal, ovoid, 30-54 x 21-46 (41 x 30) in size. Acetabulum equatorial, larger than oral sucker; measured 38-73 x 30-61 (53 x 38) in size. Pre-pharynx 23-38 (32) long; pharynx muscular, oval, 38-58 x 16-31 (46 x 23) in dimensions. Oesophagus long, measured 80-140 (129), bifurcating immediately anterior to acetabulum into two, long, narrow caeca terminating near posterior end of body. Caeca measured 154-231 (195) in length. Excretory bladder 'Y'-shaped, with the cornua extending to the level of pharynx. Excretory pore terminal.

Adult

Natural infections by adult flukes were found in the pond heron, *Ardeola grayii* collected from Kadakkattupara in Malappuram district. Experimental infection was successfully established in one-day-old chicks fed with infective metacercariae.



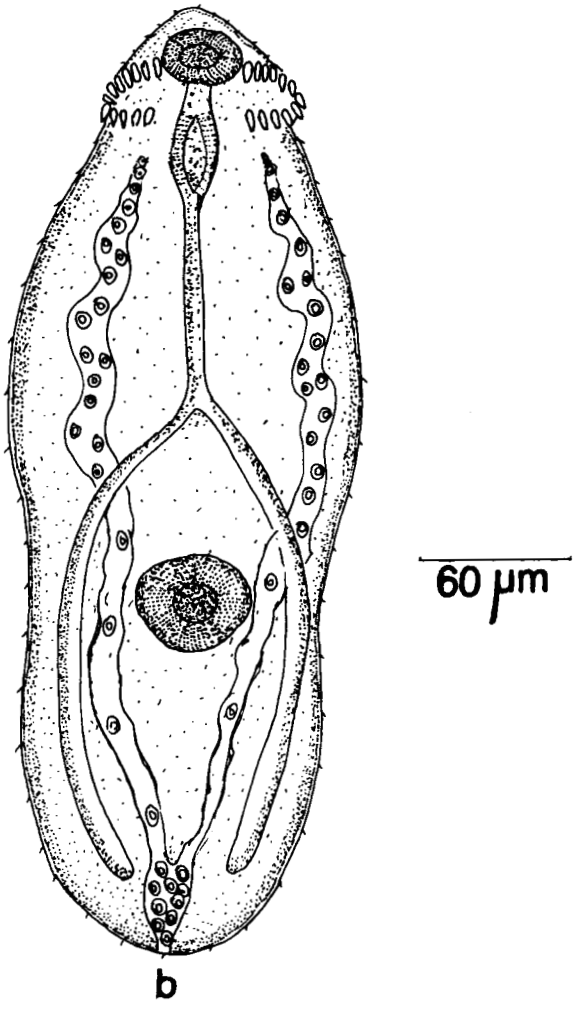


Fig. 14. *Echinochasmus megavitellus* Lal, 1939

a. Encysted metacercaria; b. Excysted metacercaria

Table 21. **Measurements (μm) of metacercariae of *Echinochasmus megavitellus* Lal, 1939**

Character	Range	Mean
Encysted metacercaria	121-201 x 100-185	153 x 130
Excysted metacercaria	385-539 x 100-169	471 x 122
Oral sucker	30-54 x 21-46	41 x 30
Acetabulum	38-73 x 30-61	53 x 38
Pre-pharynx	23 - 38	32
Pharynx	38-58 x 16-31	46 x 23
Oesophagus	80 - 140	129
Caeca	154-231	195

Flukes recovered 2 days after exposure were identical to excysted metacercariae except that they showed slight increase in body size and their reproductive structures were in an advanced stage of development.

Flukes recovered 7-days post-feeding were very much similar to that collected from natural infections of *A. grayii*.

Description of mature adult (Fig. 14c; Table 22)

Body elongate, spinose, 1586-1803 x 343-546 (1682 x 473) in size. Collar well developed, kidney-shaped, armed with dorsally interrupted single row of 24 spines (Fig. 14d). Oral sucker subterminal, 62-94 x 62-109 (73 x 75) in size. Acetabulum slightly pre-equatorial, measured 202-281 x 187-280 (237 x 235) in size. Pre-pharynx very short; pharynx elliptical, muscular, measured 93-117 x 78-125 (95 x 83). Oesophagus 78-148 (108) long, bifurcating immediately anterior to acetabulum into long, narrow caeca terminating near posterior end of body; caeca 795 - 1498 (1443) long. Testes intercaecal, tandem, post-equatorial. Anterior testis transversely oval, 109-218 x 171-389 (146 x 329) in size; posterior testis slightly larger than anterior one; measured 140-343 x 179-388 (213 x 361). Cirrus sac large, thick walled, pre-acetabular, slightly curved antero-medially, encloses a bipartite seminal vesicle and a short cirrus; measured 49-234 x 46-124 (153 x 94) in size. Ovary transversely oval, pre-testicular, 78-125 x 85-156 (102 x 132) in dimensions. Uterine coils short, pre-testicular containing 1-30 eggs. Vitelline follicles numerous commencing from posterior margin of acetabulum, extend along lateral fields to posterior end of body, filling post-testicular space. Eggs oval, operculated, measured 66-78 x 42-46 (70 x 44) (Fig. 14e).

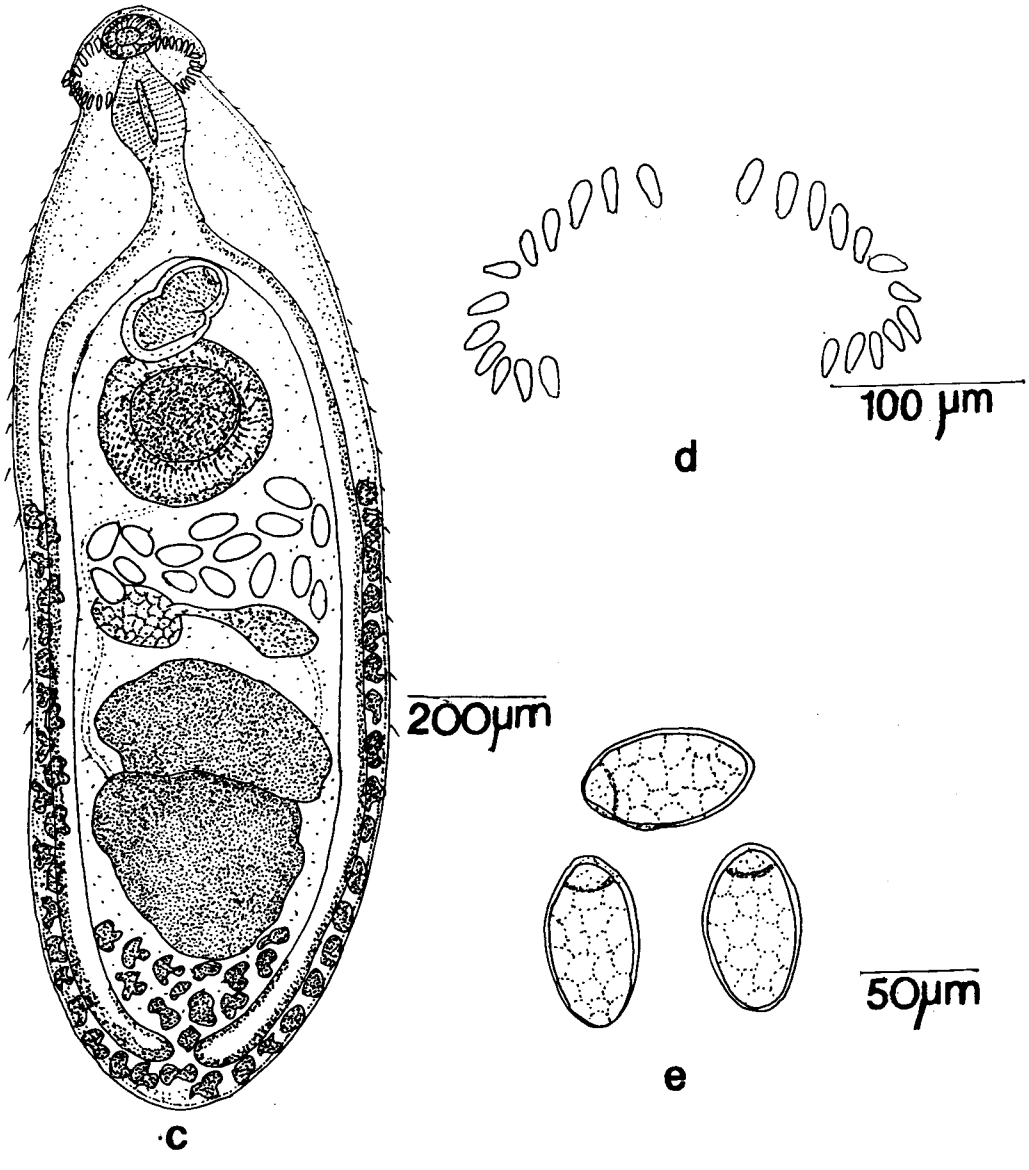


Fig. 14. *Echinochasmus megavitellus* Lal, 1939

c. Adult; d. Head collar showing spines; e. Eggs

Table 22. **Measurements (μm) of adults of *Echinochasmus megavitellus* Lal, 1939**

Character	Range	Mean
Body size	1586-1803 x 343-546	1682x473
Oral sucker	62-94 x 62-109	73 x 75
Acetabulum	202-281 x 187-280	237x235
Pharynx	93-117 x 78-125	95 x 83
Oesophagus	78-148	108
Caeca	795-1498	1443
Anterior testis	109-218 x 171-389	146 x 329
Posterior testis	140-343 x 179-388	213 x 361
Cirrus sac	49-234 x 46-124	153 x 94
Ovary	78-125 x 85-156	102 x 132
Egg	66-78 x 42-46	70x 44

Fish host	:	*Labeo rohita* (Hamilton)
Site of infection	:	Gills
Locality	:	Malampuzha in Palakkad district of Kerala.
Period of collection	:	November of 1996 and 1997.
Prevalence of infection	:	8 of 62 (12.9%) fishes examined
Intensity of infection	:	8 to 13
Natural definitive host	:	*Ardeola grayii* (Sykes)
Site of infection	:	Intestine
Locality	:	Kadakkattupara in Malappuram district of Kerala.
Experimental host	:	One-day-old chicks.

Remarks:

The present fluke recovered from the intestine of *Ardeola grayii* has (1) dorsally interrupted, single row of 24 collar spines, (2) caeca reaching to posterior extremity, (3) post-equatorial tandemly placed testes and (4) pre-acetabular cirrus sac. Based on these characters, the present fluke resembles the genus *Echinochasmus* Dietz, 1909 of the family Echinostomatidae Poche, 1925. As far as is known, only 10 species of this genus have been reported from India. Of these, only 5 species have 24 collar spines which are dorsally interrupted. These are *E. bagulai* Verma, 1935; *E. ruficapensis* Verma, 1935; *E. narayani* Mudaliar, 1938; *E. megavitellus* Lal, 1939, and *E. antigonus* Gupta, 1953. Among these, *E.bagulai* has collar spines arranged in alternate rows, where as in the remaining 4 species the spines are arranged in single row. The present fluke also has single row of spines and, therefore, deserves comparison with the four species. A

comparative study of characters revealed that it is identical to *E. megavitellus* described by Lal (1939) from *Ardeola grayii* in Lucknow, in the ratio of suckers, and in having inwardly curved caecal ends and bipartite seminal vesicle. A variety of fishes are known to act as second intermediate hosts of the different species of *Echinochasmus* (Nath and Pande, 1970a; Madhavi, 1980; Dhanumkumari *et al.*, 1991). Neither the cercaria nor the metacercaria of *E. megavitellus* have so far been reported. The present metacercariae recovered from *Labeo rohita* developed into the adults of *E. megavitellus* in *Ardeola grayii* and one-day-old chicks. Therefore, it forms the first report of *Echinochasmus megavitellus* metacercaria. Finding of adult fluke in *A. grayii* at Kadakkattupara of Malappuram district represents the first record of *E. megavitellus* from Kerala.

Family: Renicolidae Dollfus, 1939

Renicolid metacercaria

Twenty of 295 (6.78%) *Danio aequipinnatus*, collected from Kanhangad in Kasargod, Nirmalagiri and Kanhilari in Kannur and Noolpuzha in Wayanad districts of Kerala during May 1996, September-November 1997 and February 1998, were positive for this metacercaria. Eight of 122 (6.56%) *Puntius melanampyx melanampyx*, collected from Kanhangad and Nirmalagiri, during May 1996 and November 1997, were also positive for this infection. The intensity of infection varied from 12 to 29.

Description:

Cysts elliptical, translucent, measured 423-456 x 154-187 (434 x 172), provided with elastic cyst wall. The larva remains folded inside the cyst wall (Fig. 15a, Table 23).

Excysted metacercaria (Fig. 15b; Table 23)

Body elongate-oval, spinose, measured 462-686 x 161-235 (577 x 208) in size. Oral sucker subterminal, 72-78 (74) in diameter. Acetabulum post-equatorial, 46-72 x 52-70 (52 x 61) in size. Distance between suckers 223-274 (226). Pre-pharynx absent; pharynx globular, muscular, measured 23-63 x 32-46 (45 x 38). Oesophagus absent. Caeca 435-599 (439) long. Excretory bladder 'Y'-shaped, with a long stem, which bifurcates just below the level of acetabulum into two long arms reaching near oral sucker. The bladder gives off lateral branches.

Fish host	:	*Danio aequipinnatus* McClelland *Puntius melanampyx melanampyx* (Day)
Site of infection	:	Liver
Locality	:	Kanhangad in Kasargod, Nirmalagiri and Kanhilari in Kannur, and Noolpuzha in Wayanad districts of Kerala
Period of collection	:	May 1996, September-November 1997, February 1998.
Prevalence of infection	:	20 of 295 (6.78%) *D. aequipinnatus* examined 8 of 122 (6.56%) *P. melanampyx melanampyx* examined
Intensity of infection	:	12 to 29.

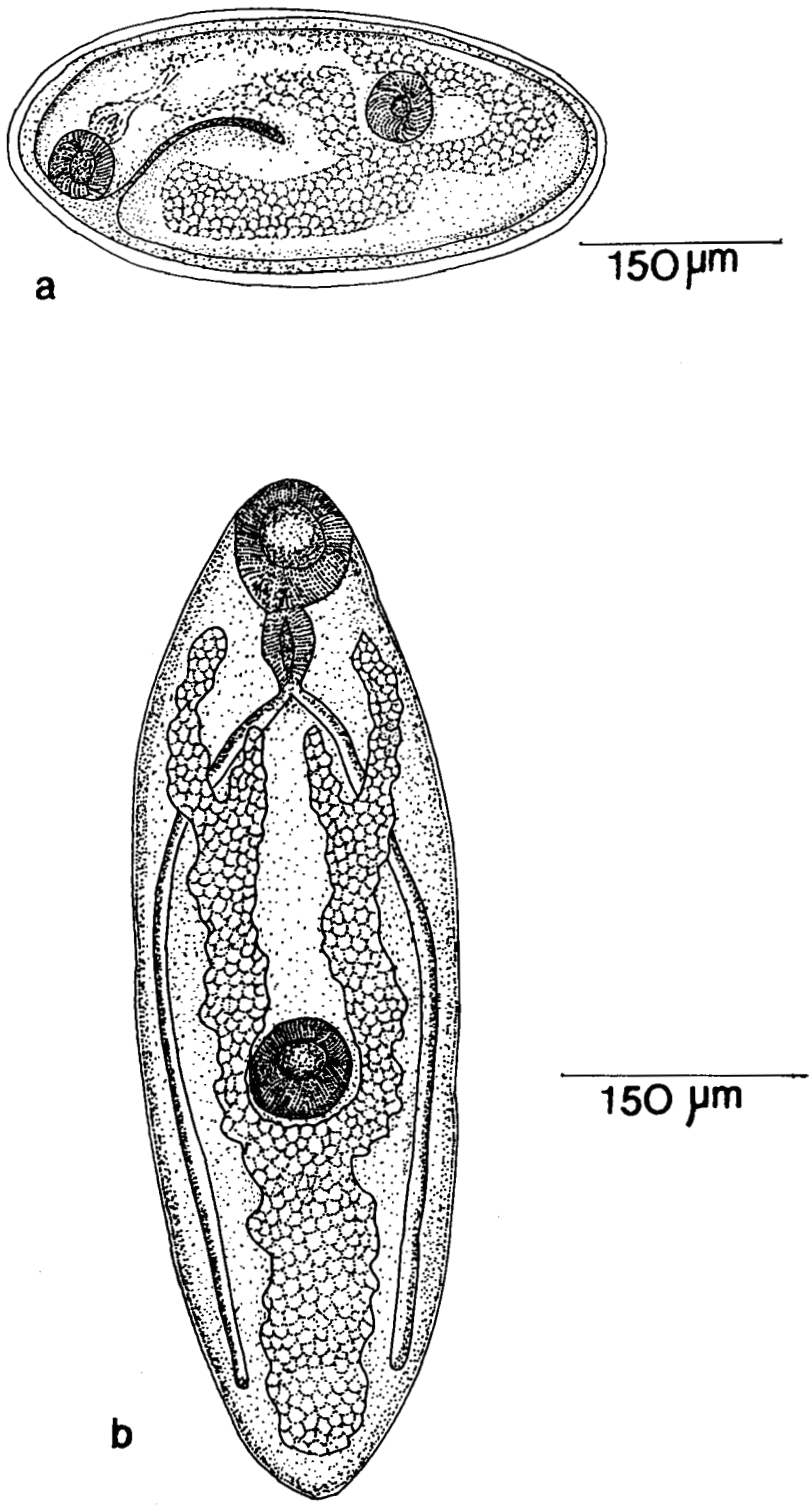


Fig. 15. Renicolid metacercaria

a. Encysted metacercaria; b. Excysted metacercaria

Table 23. Measurements (μm) of renicolid metacercariae

Character	Range	Mean
Encysted metacercaria	423-456 x 154-187	434 x 172
Excysted metacercaria	462-686 x 161-235	577 x 208
Oral sucker	72-78	74
Acetabulum	46-72 x 52-70	52 x 61
Distance between suckers	223-274	226
Pharynx	23-63 x 32-46	45 x 38
Caeca	435-599	439

Remarks:

The present metacercaria recovered from the liver of *Danio aequipinnatus* and *Puntius melanampyx melanampyx* is comparable to the metacercariae assigned to the family Renicolidae Dollfus, 1939, in having spined tegument, and 'Y'-shaped excretory bladder with lateral branches. Madhavi (1980) reported similar metacercariae from *Aplocheilus melastigma*. As far as is known, neither the cercariae nor the adults of renicolid metacercariae have been reported from India. Further studies are required to reveal the identity of the metacercaria. It is, therefore, tentatively reported here as a renicolid metacercaria.

Family: Plagiorchiidae (Lühe, 1901) Ward, 1917

Genus: *Encyclometra* Baylis and Cannon, 1924

***Encyclometra colubrimurorum* (Rudolphi, 1819) Dollfus,1929**

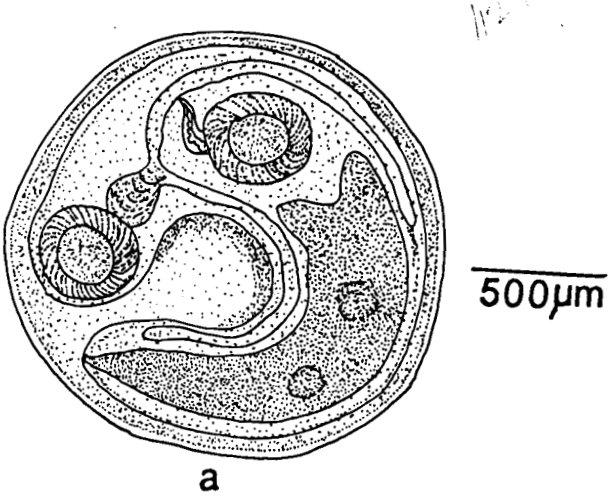
This metacercaria was recovered from 11 *Channa orientalis*, collected from Sultan Bathery in Wayanad district, during May 1997 and February 1998. The prevalence of infection was 26.19% in a total of 42 specimens examined. The intensity of infection varied from 3 to 9. Mixed infections with the adult of *Genarchopsis goppo*, metacercariae of *Euclinostomum heterostomum*, *Encyclometra colubrimurorum* and *Centrocestus formosanus* were frequently observed in *C. orientalis*.

Description:

Cysts oval to round with thin, transparent wall; measured 1450-1638 x 1420-1685 (1552 x 1482). The larva remains curled up inside the cyst wall (Fig. 16a; Table 24).

Excysted metacercaria (Fig. 16b; Table 24):

Body fusiform, aspinose, 2560-3610 x 768-1129 (2975 x 947) in size. Oral sucker oval, subterminal, 320-435 x 307-486 (389 x 386) in size. Acetabulum pre-equatorial, oval to round, larger than oral sucker, and measuring 332-563 x 384-538 (452 x 439)in size. Distance between suckers 500- 635 (582). Mouth subterminal; pre-pharynx 27-54 (40) long; pharynx oval to round, muscular, measuring 115-256 x 128-256 (174 x 205) in size. Oesophagus very short. Caeca long, asymmetrical, extend almost up to posterior end of body. Left caecum 1869-2726 (2273) long; right caecum 1702-2368 (2002) long. Testes well developed, oval, tandem, in the posterior half of body. Anterior testis, measured 102-205 x 89 - 128 (154 x 112); posterior testis 102 - 205 x 89 - 128 (154 x 118). Distance between testes 166-240 (198). Cirrus sac crescentic, immediately above anterior border of acetabulum, and measured 287-384 x 89-141 (349 x 119). It contained coiled seminal vesicle which leads into pars prostatica and protrusible cirrus. Genital pore opens close to the anterior part of left caecum. Ovary small, oval, median, just behind acetabulum and measured 70-90 x 70-76 (74 x 72) in size. Excretory bladder large, saccular, filled with concretions; extending from posterior end to just below acetabulum; measured 1331-1868 x 250 - 439 (1600 x 378). Excretory pore terminal.



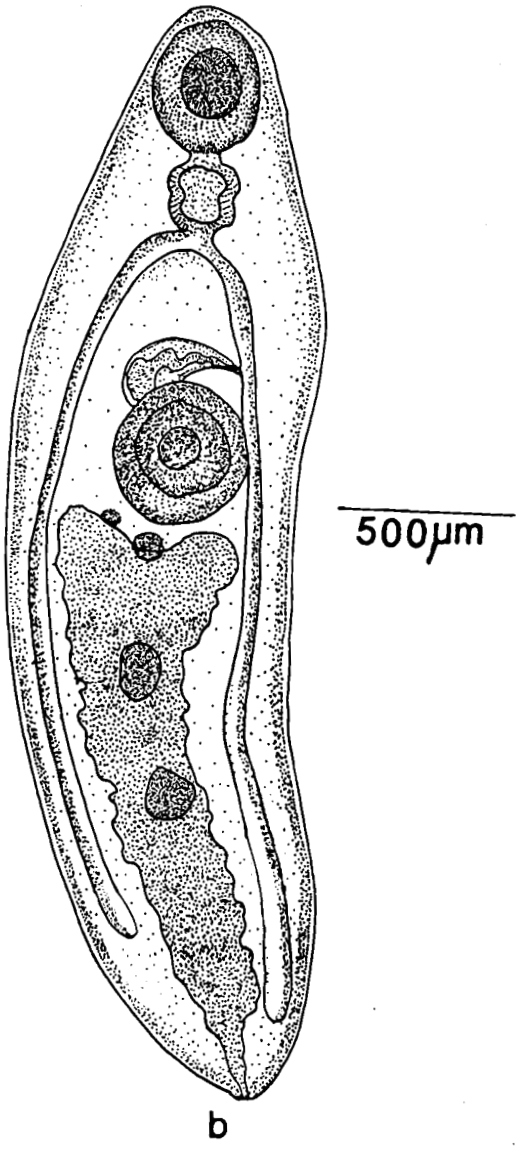


Fig. 16. *Encyclometra colubrimurorum* (Rudolphi, 1819) Dollfus, 1929

a. Encysted metacercaria; b. Excysted metacercaria

Table 24. Measurements (μm) of metacercariae of *Encyclometra colubrimurorum* (Rudolphi, 1819) Dollfus, 1929

Character	Range	Mean
Encysted metacercaria	1450-1638 x 1420-1685	1552 x 1482
Excysted metacercaria	2560-3610 x 768-1129	2975 x 947
Oral sucker	320-435 x 307-486	389x386
Acetabulum	332-563 x 384-538	452 x 439
Distance between suckers	500-635	582
Pre-pharynx	27-54	40
Pharynx	115-256 x 128-256	174 x205
Left caecum	1869-2726	2273
Right caecum	1702-2368	2002
Anterior testis	102-205 x 89-128	154 x 112
Posterior testis	102-205 x 89-128	154 x 118
Distance between testes	166-240	198
Cirrus sac	287-384 x 89-141	349 x 119
Ovary	70-90 x 70-76	74 x 72
Excretory bladder	1331-1868 x 250-439	1600 x 378

Fish host	: *Channa orientalis* (Schneider)
Site of infection	: Muscles
Locality	: Sultan Bathery in Wayanad district of Kerala.
Period of collection	: May 1997 and February 1998.
Prevalence of infection	: 11 of 42 (26.19%) fishes examined
Intensity of infection	: 3 to 9

Remarks:

The present metacercaria recovered from the muscles of *Channa orientalis* has asymmetrical caeca, sac-like excretory bladder, tandemly placed testes and crescentic cirrus sac situated above the anterior border of acetabulum. Based on these characters, the present form is included under the genus *Encyclometra* Baylis and Cannon, 1924 of the family Plagiorchiidae (Lühe, 1901) Ward, 1917. As far as is known, only 3 species of the genus *Encyclometra* have been reported: *E. colubrimurorum* (Rudolphi, 1819) Dollfus, 1929; *E. asymmetrica* Wallace, 1936, and *E. bungara* Srivastava and Ghosh, 1968. Adults of all the three species have been reported from India (Mehra 1931; Bhalerao, 1926, 1936; Gupta, 1954; Srivastava and Ghosh, 1968; Dwivedi and Chauhan, 1970). The life-cycle of the genus has not so far been worked out. Metacercariae of *E. colubrimurorum* and *E. asymmetrica* have been reported by Yamaguti (1936) and Chiang (1951) respectively; there is no report of *Encyclometra* metacercaria from India. The present metacercaria shows close resemblance to the metacercaria of *E. colubrimurorum* in most respects and is, therefore, tentatively reported here as *E. colubrimurorum* metacercaria. Recovery of this metacercaria from *C.*

orientalis forms the first record of an *Encyclometra* sp. metacercaria from India.

Family: Heterophyidae Odhner, 1914

Genus: *Centrocestus* Looss, 1899

***Centrocestus formosanus* (Nishigori, 1924) Price, 1932**

Metacercariae of *Centrocestus formosanus* were recovered from 13 species of fish hosts collected from various localities in Kerala. The number of each host species examined, their locality of collection, and prevalence and intensity of infection by the metacercaria are summarised in Table 25. *Channa orientalis* frequently carried mixed infections with the metacercariae of *C. formosanus, Encyclometra colubrimurorum* and *Euclinostomum heterostomum,* and the adult of *Genarchopsis goppo.* Mixed infections with the metacercariae of *Diplostomulum* sp., *Echinochasmus megavitellus* and *C. formosanus* were observed in *Labeo rohita.* The metacercariae of *Haplorchoides mehrai* and of *C. formosanus* were frequently found in *Puntius melanampyx melanampyx, P. parrah, P. sophore, Mystus malabaricus* and *Heteropneustes fossilis. P. parrah* was found infected by the metacercariae of 27- spined echinostome and *C. formosanus,* and *Danio aequipinnatus* by *Tetracotyle* metacercaria sp. I and the present metacercaria. The metacercariae of *C. formosanus* and *Acanthostomum burminis* were frequently found in *P. parrah* and *Channa orientalis.*

Table 25. List of host species examined, their locality of collection and prevalence, and intensity of infection by metacercariae of *Centrocestus formosanus* (Nishigori, 1924) Price, 1932

Name of fish	No. of fishes		Prevalence of infection	Intensity of infection	Locality
	Examined	Infected			
(1)	(2)	(3)	(4)	(5)	(6)
Danio aequipinnatus McClelland	295	30	10.17	10 to 16	Karetta and Kanhilari in Kannur district; Nedungottumedu in Malappuram district
D. spinosus Day	15	3	20.00	2 to 3	Karetta in Kannur district; Calicut University Campus in Malappuram district
Puntius melanampyx melanampyx (Day)	122	30	24.59	10 to 13	Kanhangad in Kasargod district; Nirmalagiri, Kuttimakkul and Karetta in Kannur district; Thamarassery, Kundayithode and Puthukode in Kozhikode district; Nedungottumedu, Kadakkattupara and Calicut University Campus in Malappuram district
P. parrah Day	420	302	71.90	11 to 38	Kuttimakkul and Karetta in Kannur district; Feroke, Puthukode and Ramanattukara in Kozhikode district; Pakkam, Vythiri, Meenangadi and Sultan Bathery in Wayanad district; Nedungottumedu and Thalapara in Malappuram district
P. sophore (Hamilton)	60	15	25.00	2 to 6	Thalapara in Malappuram district
Labeo rohita (Hamilton)	62	24	38.71	9 to 24	Malampuzha in Palakkad district
Mystus malabaricus (Jerdon)	10	4	40.00	5 to 11	Kuttimakkul and Karetta in Kannur district; Nedungottumedu in Malappuram district

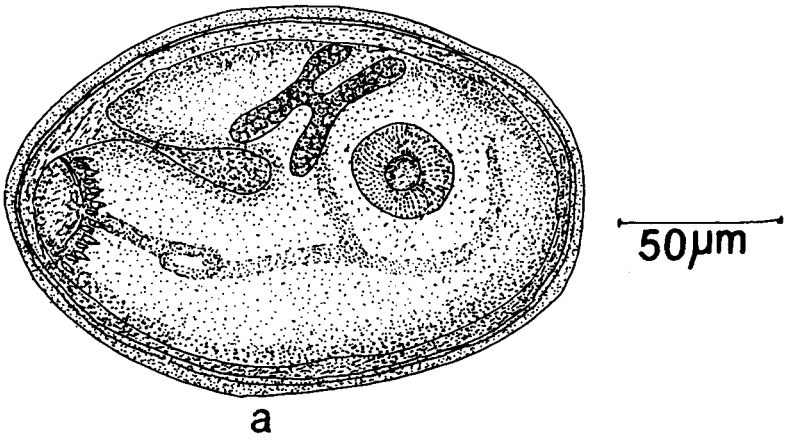
(1)	(2)	(3)	(4)	(5)	(6)
Heteropneustes fossilis (Bloch)	38	14	36.84	8 to 19	Kanhangad in Kasargod district; Kuttimakkul and Karetta in Kannur district; Thamarassery in Kozhikode and Nedungottumedu in Malappuram districts
Aplocheilus panchax (Hamilton)	120	62	51.67	4 to 14	Kuttimakkul and Karetta in Kannur district, Puthukode, Nallur, Feroke Ramanattukara, Devagiri and Kundayithode in Kozhikode district; Vythiri and Sultan Bathery in Wayanad district
Channa orientalis (Schneider)	42	36	85.71	8 to 39	Feroke and Puthukode in Kozhikode district; Nedungottumedu in Malappuram; Sultan Bathery in Wayanad district
C. punctatus Bloch	22	18	81.82	6 to 21	Kanhilari and Kuttimakkul in Kannur district; Feroke and Puthukode in Kozhikode district; Sulttan Bathery in Wayanad district; Nedungottumedu in Malappuram district
Etroplus maculatus (Bloch)	25	4	16.00	2 to 6	Kuttimakkul in Kannur district; Ramanattukara and Kundayithode in Kozhikode district
Macropodus cupanus Valenciennes	301	49	16.28	4 to 11	Kuttimakkul, Kanhilari and Karetta in Kannur district; Kundayithode, Feroke, Ramanattukara, Nallur and Devagiri in Kozhikode district; Nedungottumedu and Thalapara in Malappuram district

Description:

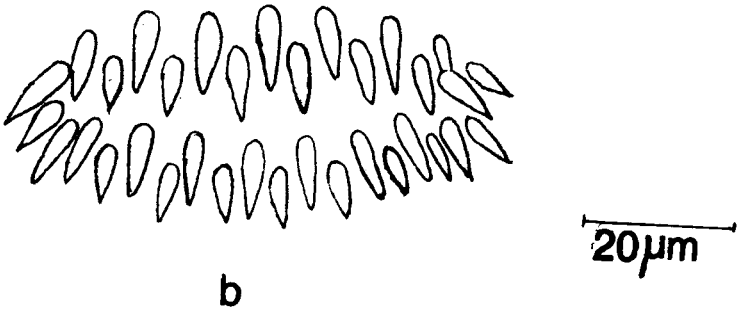
Cysts oval or ellipsoidal, transparent, found attached to gill filaments in close association with blood vessels; measured 154-252 x 105 -165 (188 x 143) in size. Cyst wall double-layered. The larva remains curled up inside the cyst wall (Fig. 17a; Table 26).

Excysted metacercaria (Fig. 17c; Table 26):

Body elongate, flask-shaped, spinose, measured 200-401 x 106-172 (273 x 130). Body spines larger, prominent and densely distributed in the anterior half of body, becoming progressively smaller and sparse toward posterior end. Oral sucker funnel-shaped, terminal, 30-58 x 36-53 (47 x 48), provided with 34 circum-oral spines arranged in two alternating rows (Fig. 17b). Spines in the inner row measured 10-16 (14) in length, that in the outer row measured 8-13(11). Acetabulum post-equatorial, measured 33-43 x 33-46 (38 x 43). Pre-pharynx short, 19-28 (25) in length. Pharynx muscular, 33-48 x 15-33 (42 x 29) in size. Oesophagus short. Caeca long, measured 73-131 (96) in length, terminate just in front of excretory bladder; filled with disc-shaped, colourless bodies. Testes round to oval, situated symmetrically at posterior end of body, enclosed by arms of 'X'-shaped excretory bladder. Right testis measured 26-36 x 36-46 (31 x 44); left testis 29-40 x 38-54 (35 x 46). Ovary oval, lies just in front of the right arm of excretory bladder, and measured 23-31 x 33-38 (26 x 32) in dimensions. Excretory bladder 'X'-shaped, lies in between testes; filled with excretory concretions.



a



b

Fig. 17. *Centrocestus formosanus* (Nishigori, 1924) Price, 1932

a. Encysted metacercaria; b. Circum-oral spines

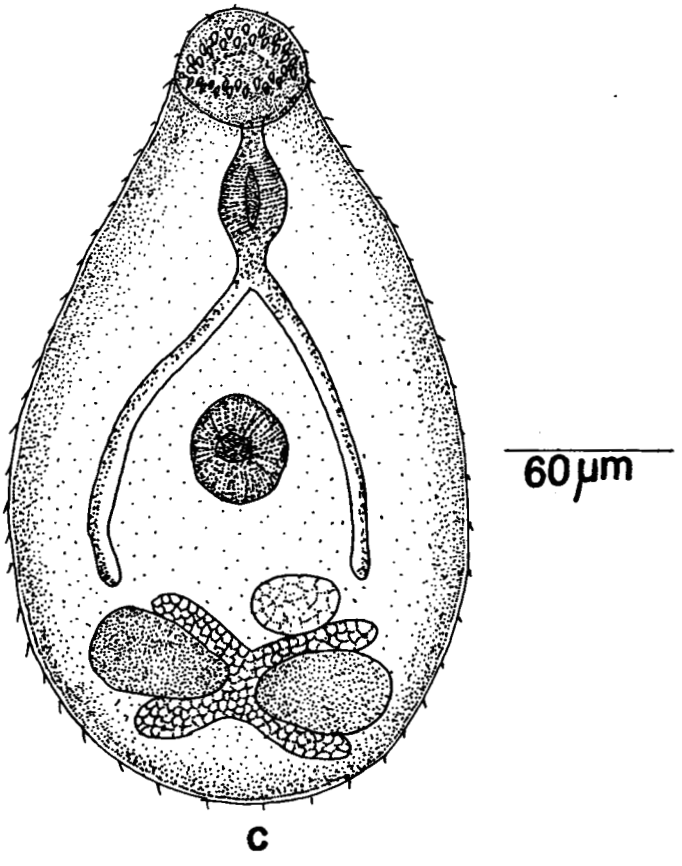


Fig. 17. *Centrocestus formosanus* (Nishigori, 1924) Price, 1932

c. Excysted metacercaria

Table 26. **Measurements (μm) of metacercariae of *Centrocestus formosanus* (Nishigori, 1924) Price, 1932**

Character	Range	Mean
Encysted metacercaria	154-252 x 105-165	188 x143
Excysted metacercaria	200-401 x 106-172	273 x 130
Oral sucker	30-58 x 36-53	47 x 48
Acetabulum	33-43 x 33-46	38 x 43
Pre-pharynx	19-28	25
Pharynx	33-48 x 15-33	42 x 29
Caeca	73-131	96
Right testis	26-36 x 36-46	31 x 44
Left testis	29 - 40 x 38-54	35 x 46
Ovary	23-31 x 33-38	26 x 32

Life-Cycle:

A search for the adult of this species in a few birds of the locality revealed that *Ardeola grayii* was infected by the adult of *C. formosanus*. Large number of flukes were recovered from anterior part of small intestine of the bird. In one instance as many as 189 flukes were recovered from a single bird. The link between the metacercaria and the adult has been successfully established by feeding infection-free *A. grayii* or one-day-old chicks with the infective metacercariae. Several egg-bearing adults were obtained after 5 days of infection. Attempts made to recover the cercaria of this fluke in the snails of the present locality were futile. A brief description of the adult is given below (Fig. 17d; Table 27).

Body elongate to oval, spinose; spines densely distributed at anterior half of body; measured 416-579 x 207-257 (479 x 235). Oral sucker terminal, 56-77 x 59-66 (61 x 64), with two alternating rows of 34 circum-oral spines; inner spines long, and outer spines short. Acetabulum measured 44-62 x 46-66 (50 x 58) in size. Distance between suckers 210-264 (248). Pre-pharynx long, measured 30-32 (31); pharynx globular, 46-62 (53) in diameter. Oesophagus short, bifurcating about one-third of body length from anterior end; caeca 190-240 (220) in length, reaching in front of ovary.

Testes oval, smooth, symmetrical, on either side of excretory bladder. Right testis measured 46-53 x 77-94 (49 x 86), and left testis 46-54 x 84-93 (49 x 88) in size. Seminal vesicle large, sac-like, opens in front of acetabulum. Ovary transversely oval, 46-69 x 43-63 (56 x 51) in size, with smooth margins, in front of right testis. Seminal receptacle well developed, median, pre-testicular, measured 46-55 x 25-30 (47 x 29).

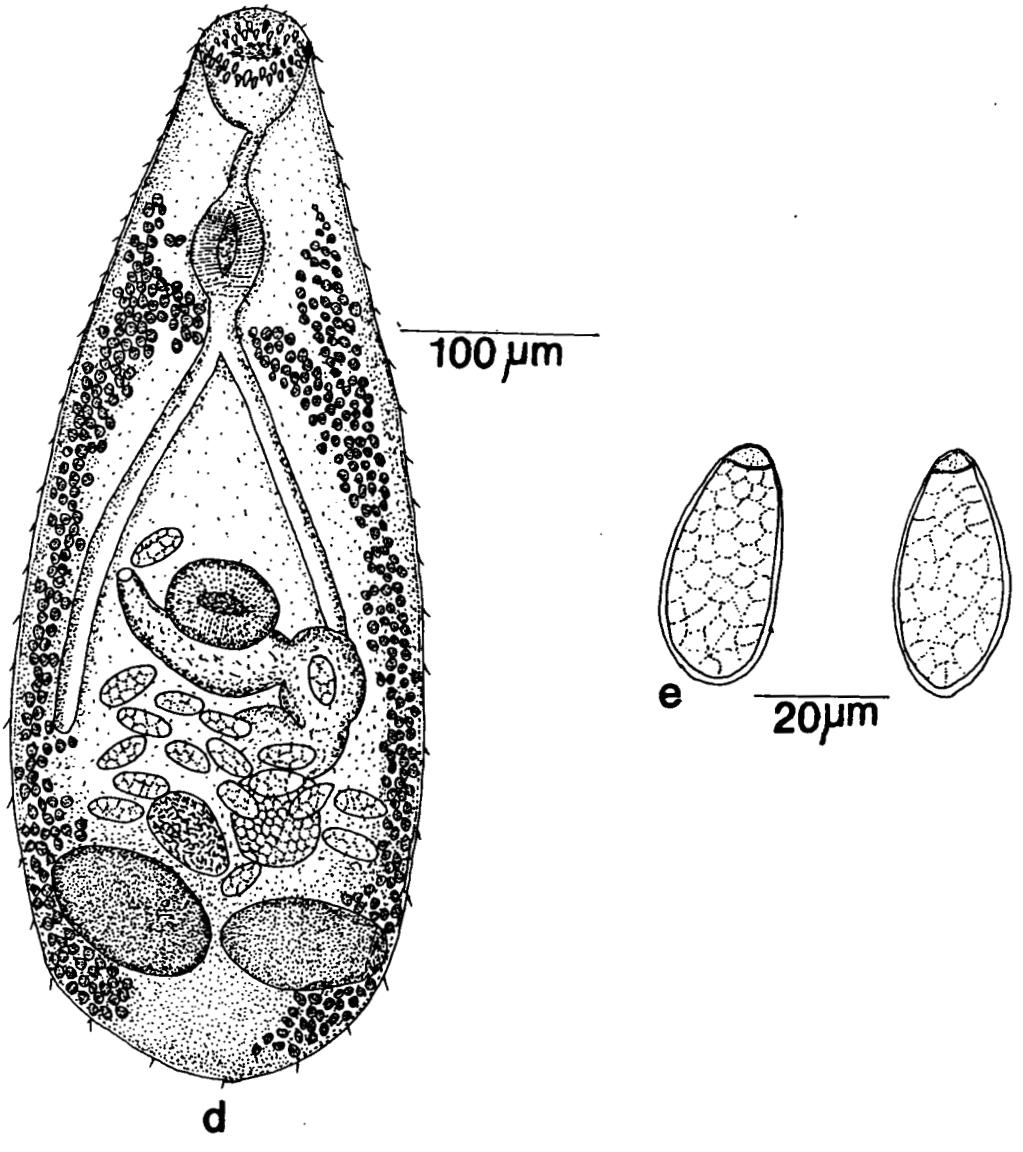


Fig. 17. *Centrocestus formosanus* (Nishigori, 1924) Price, 1932

d. Adult; e. Eggs

Table 27. **Measurements (μm) of adults of *Centrocestus formosanus* (Nishigori, 1924) Price, 1932**

Character	Range	Mean
Body size	416-579 x 207-257	479 x 235
Oral sucker	56-77 x 59-66	61 x 64
Acetabulum	44-62 x 46-66	50 x 58
Distance between suckers	210-264	248
Pre-pharynx	30-32	31
Pharynx	46-62	53
Caeca	190-240	220
Right testis	46-53 x 77-94	49 x 86
Left testis	46-54 x 84-93	49 x 88
Ovary	46-69 x 43-63	56 x 51
Seminal receptacle	46-55 x 25-30	47 x 29
Egg	36-40 x 18-20	38 x 19

Vitellaria consist of round to oval follicles, extending along lateral body margins from pharyngeal level to post-testicular space and tend to meet at the caecal bifurcation. Uterus thin-walled, occupying the space between acetabulum and testes. Eggs oval, yellowish brown, operculate, 36-40 x 18-20 (38 x 19) in size (Fig. 17e). Number of eggs varied from 18-42. Excretory bladder 'X'-shaped, lies between testes.

Fish hosts, locality, and prevalence and intensity of infection	:	Table 25
Site of infection	:	Gill filaments
Period of collection	:	March-December 1995; February-December 1996; July-December 1997; February-April 1998.
Natural definitive host	:	*Ardeola grayii* (Sykes)
Site of infection	:	Anterior part of small intestine
Experimental hosts	:	One-day-old chicks and *A. grayii*

Remarks:

The characters of the present metacercaria and its adult suggest that this fluke belongs to the genus *Centrocestus* Looss, 1899. Of the 3 valid species of *Centrocestus*, only *C. formosanus* (Nishigori, 1924) Price, 1932 has been reported from India. A comparative study of characters of the present form with that of *C. formosanus* revealed that it undoubtedly belongs to *C. formosanus*. The life-history of this fluke from India was established by Dhanumkumari *et al.* (1993). Metacercariae of *C. formosanus* have previously been reported from the gills of several freshwater fishes in India. Nath and Pande (1970b) recorded it from *Cirrhina rebe, Amblypharyngodon*

mola, Labeo bata and *Puntius* sp; Pande and Shukla (1972) from *Esomus danricus, P. sophore, P. chola, P. ticto, Nandus nandus, Osteobrame cotio, Xenentodon cancilla, Channa punctatus, Notopterus notopterus, Chela laubuca, Oxygaster phulo, O. bacaila* and *Mastacembelus puncalus,* and Madhavi (1980) from *Aplocheilus panchax* and *A. melastigma.* Rekharani and Madhavi (1985) recovered the metacercariae from the gills of brackishwater fishes, *Mugil cephalus, Liza macrolepis* and *Valamugil cunnesius.* During the present investigation, 13 species of fishes were found infected by the metacercaria of *C. formosanus* (Table 25). The adults were recovered from *Ardeola grayii.* Recovery of the metacercaria from *Danio aequipinnatus, D. spinosus, Puntius melanampyx melanampyx, Labeo rohita, Mystus malabaricus, Heteropneustes fossilis, Channa orientalis, Etroplus maculatus* and *Macropodus cupanus* from Kerala forms new host and geographical records for this species.

Family : Acanthostomidae Poche, 1926

Genus : *Acanthostomum* Looss, 1899

***Acanthostomum burminis* (Bhalerao, 1926) Bhalerao, 1936**

Natural infections by the metacercaria were found in 9 species of fish hosts collected from various localities in Kerala during January-May and August-December 1995; March-November 1996; February-August and November-December 1997, and February 1998. The number of host species examined, their locality of collection and site, prevalence and intensity of metacercarial infection are summarised in the Table 28. Mixed infections by this metacercaria and the metacercaria of *Centrocestus formosanus* were frequently found in *Puntius parrah* and *Channa orientalis.* Similarly a few

Table 28. List of host species examined, their locality of collection, and site, prevalence, and intensity of infection by metacercariae of *Acanthostomum burminis* (Bhalerao, 1926) Bhalerao, 1936

Name of fish	Site of infection	No. of fishes		Prevalence of infection	Intensity of infection	Locality
		Examined	Infected			
(1)	(2)	(3)	(4)	(5)	(6)	(7)
Rasbora daniconius (Hamilton)	Fins	622	228	36.66	16 to 34	Kanhangad in Kasargod; Kuttimakkul, Kanhilari and Karetta in Kannur; Feroke, Puthukode and Ramanattukara in Kozhikode; Meenangadi and Sultan Bathery in Wayanad; Nedungottumedu, Calicut University Campus, Kadakkattupara and Thalapara in Malappuram districts of Kerala
Puntius melanampyx melanampyx (Day)	Fins	122	41	33.61	18 to 28	Kanhangad in Kasargod; Kuttimakkul, Kanhilari and Karetta in Kannur; Puthukode in Kozhikode; Sultan Bathery in Wayanad; Nedungottumedu, Calicut University Campus and Kadakkattupara in Malappuram districts of Kerala

(1)	(2)	(3)	(4)	(5)	(6)	(7)
P. parrah Day	Fins	420	193	45.95	18 to 36	Kuttimakkul and Karetta in Kannur; Feroke, Puthukode and Ramanattukara in Kozhikode; Pakkam, Vythiri, Meenangadi and Sultan Bathery in Wayanad, and Nedungottumedu and Thalapara in Malappuram districts of Kerala
Garra mullya (Sykes)	Fins	9	1	11.11	18 to 21	Nedungottumedu in Malappuram district of Kerala
Mystus malabaricus (Jerdon)	Fins	10	2	20.00	16 to 23	Kuttimakkul and Karetta in Kannur; Puthukode in Kozhikode, and Nedungottumedu in Malappuram districts of Kerala
M. oculatus (Valenciennes)	Fins	31	13	41.94	16 to 24	Thenhipalam in Malappuram district of Kerala
Heteropneustes fossilis (Bloch)	Fins	38	11	28.95	18 to 25	Kanhangad in Kasargod; Kuttimakkul and Karetta in Kannur, and Nedungottumedu in Malappuram districts of Kerala

(1)	(2)	(3)	(4)	(5)	(6)	(7)
Channa orientalis (Schneider)	Fins	42	20	47.62	20 to 33	Feroke and Ramanattukara in Kozhikode; Sultan Bathery in Wayanad, and Nedungottumedu in Malappuram districts of Kerala
Etroplus maculatus (Bloch)	Fins	25	9	36.00	17 to 25	Kuttimakkul in Kannur, and Ramanattukara in Kozhikode districts of Kerala

specimens of *P. parrah, P. melanampyx melanampyx* and *R. daniconius* were found simultaneously infected by the metacercariae of *A. burminis* and *Haplorchoides mehrai.* The life-cycle of *Acanthostomum burminis* has already been worked out and published (Acta Parasitologica, 1998, 43(4), 189-193). A copy of the publication is included under Appendix I.

DISCUSSION

Digenetic trematodes infecting freshwater fishes of Kerala have not been the subject of any extensive investigation. The present investigation has been undertaken with a view to studying the systematics and biology of digenetic trematodes infecting some freshwater fishes in Malabar. This study is significant in view of its scientific and economic importance. Fish forms the best and easily available source of protein in the diet of Keralites and, therefore, information on fish parasites has economic importance. The results reported here have bearing on the control strategies of digenean infections in fish.

Thirty species of freshwater fishes representing 20 genera, collected from various water bodies in Malabar, were examined for their digenean fauna during the course of this investigation. Of these, 22 species were found infected by 5 species of adult trematodes and 14 species of metacercariae. The 5 species of adults have been recovered from 4 species of fishes. These are, *Transversotrema patialense* (Soparker, 1924) Crusz and Sathananthan, 1960 from beneath the scales of *Rasbora daniconius*; *Caballeroia bhavani* (Achan, 1956) Devraj, 1972, and *Neopodocotyle dayali* Pandey, 1973 from the intestine of *P. sarana sarana*; *Haplorchoides mehrai* Pande and Shukla, 1976 from the intestine of *Mystus malabaricus*, and *Genarchopsis goppo* Ozaki, 1925 from the stomach of *Channa orientalis*.

The 14 species of metacercariae have been recovered from 21 fish species. These include *Tetracotyle* sp.I from the muscles near vertebral

column of *Danio aequipinnatus; Tetracotyle* sp. II from the mesentry of visceral organs of *Etroplus suratensis; Diplostomulum* sp. from the muscles of *Labeo rohita; Neascus* sp. I from the liver of *Xenentodon cancila* and *Aplocheilus panchax; Neascus* sp. II from the liver of *Macropodus cupanus; Diplostomum ketupanense* Vidyarthi, 1937 from the muscles of *Rasbora daniconius; Euclinostomum heterostomum* (Rudolphi, 1809) Travassos, 1928 from the muscles of *Channa orientalis* and *Etroplus maculatus;* an echinostome metacercaria from the base of gill filaments and gill arches of *Esomus barbatus, Horadandia atukorali, Puntius parrah, Mystus malabaricus, Heteropneustes fossilis* and *Channa punctatus; Echinochasmus megavitellus* Lal, 1939 from the gills of *L. rohita;* a renicolid metacercaria from the liver of *D. aequipinnatus* and *Puntius melanampyx melanampyx; Encyclometra colubrimurorum* (Rudolphi, 1819) Dollfus, 1929 from the muscles of *C. orientalis; Centrocestus formosanus* (Nishigori, 1924) Price, 1932 from the gill filaments of 13 species of fishes (Table 25); *Haplorchoides mehrai* Pande and Shukla, 1976 from different sites of 11 fish species (Table 6), and *Acanthostomum burminis* (Bhalerao 1926) Bhalerao, 1936 from the fins of 9 species of fishes (Table 28).

The following are new host records for adult digeneans: *Transversotrema patialense* in *Rasbora daniconius; Caballeroia bhavani* in *Puntius sarana sarana; Haplorchoides mehrai* in *Mystus malabaricus,* and *Genarchopsis goppo* in *Channa orientalis.*

The following are new host records for metacercariae: *Diplostomum ketupanense* in *Rasbora daniconius; Euclinostomum heterostomum* in *Etroplus maculatus; Centrocestus formosanus* in *Danio aequipinnatus, D. spinosus, Puntius melanampyx melanampyx, Labeo rohita, Mystus malabaricus,*

Heteropneustes fossilis, C. orientalis, E. maculatus and *Macropodus cupanus,* and *Haplorchoides mehrai* in *Esomus barbatus, Horadandia atukorali, R. daniconius, P. filamentosus, Mystus oculatus* and *H. fossilis.*

The metacercariae of *Encyclometra colubrimurorum* and *Echinochasmus megavitellus* are reported for the first time from India, and the adults of *C. bhavani, N. dayali* and *G. goppo* and adults and metacercariae of *H. mehrai,* and metacercariae of *Tetracotyle* sp.I, *Tetracotyle* sp.II, *Diplostomulum,* sp., *Neascus* sp.I, *Neascus* sp.II, *Diplostomum ketupanense, Euclinostomum heterostomum,* renicolid species and *Centrocestus formosanus* for the first time from Kerala.

Discussions on the systematics and life-cycles of individual species of parasites have been made while describing them in the thesis. However, a general discussion on biology and certain aspects of host-parasite relations of digeneans recovered during the present investigation is presented here.

The life-cycles of *Diplostomum ketupanense, Transversotrema patialense, Haplorchoides mehrai* and *Acanthostomum burminis* have been successfully established in the laboratory. Their natural hosts have also been recovered.

The life-cycle of *D. ketupanense,* worked out during the present study forms the first report on an Indian *Diplostomum* sp. The furcocercous cercariae liberated by the snail, *Indoplanorbis exustus,* encysted in the muscles of the fish, *Rasbora daniconius,* and the metacercariae developed into adult flukes in the intestine of the pond heron, *Ardeola grayii.* Only the metacercariae and miracidia of *D. ketupanense* have previously been reported (Ganapati and Rao, 1954; Abraham and Anantaraman, 1955; Ganapati and Rao, 1962). The present metacercariae are identical to those

described already. Besides, 5 species of metacercariae have been described under the larval genus *Diplostomulum* (Kaw, 1950; Pandey *et al.*, 1964; Thapar, 1967; Pandey, 1970). It is expected that discovery of the adults of these metacercariae will result in the loss of species status to some of these metacercariae.

The transversotrematid cercaria, emerged from the snail, *Thiara tuberculata*, and exposed to *Rasbora daniconius*, developed into adult of *T. patialense*. Crusz *et al.* (1964) first experimentally demonstrated the life-cycle of *T. patialense* showing that *Thiara tuberculata* is the snail host. Additional observations on life-cycle of this species were made by Rao and Ganapati (1967), and Murty and Rao (1968). The life-cycle revealed during the present investigation is identical with that reported by the above workers. Crusz *et al.* (1964) preferred to call the stages recovered from fishes as 'mature metacercariae'. Rao and Ganapati (1967) opined that the cercariae attacked the fishes, and directly developed into adults without an intervening metacercarial stage. The present observation lends support to the view of Rao and Ganapati.

The life-cycle of *H. mehrai* was illustrated by Shameem and Madhavi (1988). It is very similar to that presented in the thesis except for the differences in the second intermediate and definitive hosts utilised. Information on life-cycles of the genus is available for two other species, *H. cahirinus* and *H. vacha*, also (El-Naffar, 1980; Agrawal and Agrawal, 1981). The pattern of life-cycles shown by the two species is similar to that exhibited by the present species. Metacercariae of *H. mehrai* have previously been reported from several fishes (Pande and Shukla, 1976;

Shameem and Madhavi, 1988). I could recover the metacercariae from 11 species of freshwater fishes (Table 6), showing that *H. mehrai* exhibits lack of specificity in the selection of second intermediate hosts.

The life-cycle of *Acanthostomum burminis* infecting the water snake, *Xenochrophis piscator* was established during the present study. The metacercariae have previously been reported from various freshwater fishes in India (Madhavi, 1980; Rekharani and Madhavi, 1985). A paper on the life-cycle was published by Roopa and Janardanan (1998). A copy of the paper is included under Appendix I.

The cercarial stage of 27-spined echinostome metacercaria, and the adults of *Echinochasmus megavitellus* and *Centrocestus formosanus* have been recovered and reported in the thesis. The link between 27-spined echinostome cercaria emerged from *Indoplanorbis exustus* and the 27-spined echinostome metacercaria has been successfully established by experiments involving exposure of *Esomus barbatus* and *Horadandia atukorali* to cercariae. Experimental attempts to develop its adults in one-day-old chicks, white rats, the pond heron, *Ardeola grayii* and the cattle egret, *Bubulcus ibis* and to find the adult flukes in piscivorous birds of the locality were unsuccessful. Thus the adult stage is still not known. As adults could not be raised from the metacercariae, it is not possible to assign the present metacercaria to any genus.

Natural infections by the metacercariae of *E. megavitellus* were found in the gills of *Labeo rohita.* Experimental infections by adults were successfully established in one-day-old chicks fed with infective metacercariae. Natural infection by adult flukes was found in the intestine

of *A. grayii*. The life-cycle stages of *E. megavitellus* recovered during the present study resemble that reported for other species in the genus (Dhanumkumari *et al.*, 1991; Ditrich *et al.*, 1996). The parasite possesses reniform collar with dorsally interrupted single row of 24 spines, inwardly curved caecal ends and bipartite seminal vesicle, and these characters make *E. megavitellus* distinct from other species in the genus. The genus *Echinochasmas* seems to have zoonotic significance in that, 2 of its species, *E. japonicus* Tanabe, 1926, and *E. perfoliatus* (V. Ratz, 1908) Dietz, 1909, have been recorded from man (Sprent, 1967; Uchida, 1977; Lin *et al.*, 1981). Since the metacercariae of *E. megavitellus* develop on the gills of *L. rohita* there is every possibility of man contracting the infection by eating fish.

The metacercariae of *Centrocestus formosanus* were recovered from 13 species of freshwater fishes (Table 25). Natural infections by the adult flukes were observed in the intestine of *A. grayii*. The link between metacercariae and adults has been successfully established by feeding infection-free *A. grayii* and one-day-old chicks with infective metacercariae. The life-cycle stages illustrated in the thesis is in accordance with that given by earlier works (Nishigori, 1924b; Chen, 1942, 1948; Zhang *et al.*, 1985; Dhanumkumari *et al.*, 1993). The metacercariae as well as adults obtained possess 34 circum-oral spines, and this character distinguishes *Centrocestus formosanus* from other species in the genus. This species shows lack of specificity in the selection of second intermediate and definitive hosts under natural conditions (Nath and Pande, 1970 b; Nath, 1972a; Pande and Shukla, 1972; Nath 1974; Premvati and Pande, 1974; Madhavi, 1980; Rekharani and Madhavi, 1985). However, the metacercariae show site

specificity in that they occur on gills of fishes. Because of the occurrence of this metacercaria on the gills of several food fishes, there is a potential danger of man getting infection by eating fish. Human cases of infection by this fluke were reported by Nishigori (1924a) in Formosa and Vasquez-Colet and Africa (1938, 1939, 1940) in Philippines. As far as is known, no cases of human infection have so far been reported from India. However, there is a need to control the metacercarial infection in fishes.

The digenetic trematodes recovered during the course of present investigation, their hosts, sites and prevalence of infection are summarised in Table 29. An analysis of the data reveals that all the species of adult digeneans recovered and reported are species specific, each restricted to a specific fish host. The 14 species of metacercariae exhibited different levels of host specificity, ranging from narrow to wide. The metacercariae of *Tetracotyle* sp. I, *Tetracotyle* sp. II, *Diplostomulum* sp., *Neascus* sp. II, *Diplostomum ketupanense, Echinochasmas megavitellus* and *Encyclometra colubrimurorum* are species specific, each having its own fish as host. The metacercariae of *Neascus* sp. I, renicolid species and *Euclinostomum heterostomum* infected 2 species of fishes. The remaining metacercariae were found to be generalists, being adapted to infect a wide range of hosts. The echinostome metacercariae were found in 6 fish species, while the metacercariae of *Acanthostomum burminis, Haplorchoides mehrai* and *Centrocestus formosanus* were found to infect 9, 11 and 13 fish species respectively. There are several examples in which one species of digenean is found infecting either a single host species or more than one species of host. This type of specificity has been previously reported by several

workers (Nath and Pandey, 1970; Pande and Shukla, 1972; Madhavi, 1980; Rekharani and Madhavi, 1985; Dhanumkumari *et al.*, 1993). Examples of one species of host getting infected by more than one digenean species are not rare. Such instances were reported by Skryabin (1947), Yamaguti (1971), Mehra (1980), Srivastava (1982) and others. The factor(s) responsible for the different levels of host-specificity exhibited by digeneans recovered during the present study are not understood.

All the 22 infected fish species are not equally preferred by the digenetic trematodes. The most preferred host was *Channa orientalis.* Examination of 42 specimens from various localities revealed it to be a host for 5 species of digeneans including *Genarchopsis goppo,* and metacercariae of *Euclinostomum heterostomum, Encyclometra colubrimurorum, Centrocestus formosanus* and *Acanthostomum burminis.* Of 42 *C. orientalis* examined, only 3 (7.14%) fish were found infection free. Next to this was 5 fish species infected by 4 digenean species each. *Puntius parrah* had 96.19%, *P. melanampyx melanampyx* 91.8%, *Heteropneustes fossilis* 81.57%, *Mystus malabaricus* 80% and *Rasbora daniconius* 74.91%. Three fish species, *Labeo rohita* (77.42%), *Etroplus maculatus* (76%) and *Danio aequipinnatus* (37.96%), were found infected by 3 digenean species each. There were 9 species of fishes which exhibited infection by 2 species of trematodes each. The percentage of infection in each case is, *Puntius sarana sarana* 100%, *Channa punctatus* 95.45%, *Horadandia atukorali* 87.65%, *Puntius sophore* 86.66%, *Aplocheilus panchax* 89.16%, *Esomus barbatus* 72.22%, *Mystus oculatus* 48.38%, *Garra mullya* 33.33% and *Macropodus cupanus* 17.6%. Only 4 fish species carried infection by one digenean species each. *Xenentodon cancila* showed a

prevalence of 94.2%, *Etroplus suratensis* 90.91%, *Puntius filamentosus* 76.19% and *Danio spinosus* 20%. Because of the variations in the number of individual host species examined, no justifiable explanation could be offered for this pattern of host preference. This is a profitable area for future investigations.

Table 29. Summary of digenetic trematodes recovered, their fish hosts, sites and prevalence of infection

Parasite	Host	Site	No. of Fishes		Prevalence (%)
			Examined	Infected	
(1)	(2)	(3)	(4)	(5)	(6)
ADULTS					
Family : Transversotrematidae Yamaguti, 1954					
Transversotrema patialense (Soparker,1924) Crusz and Sathananthan, 1960	*Rasbora daniconius* (Hamilton)	Beneath scales	622	8	1.29
Family : Paramphistomidae Fischoeder, 1901					
Caballeroia bhavani (Achan,1956) Devraj, 1972	*Puntius sarana sarana* (Hamilton)	Intestine	2	2	100.00
Family: Opecoelidae Ozaki, 1925					
Neopodocotyle dayali Pandey, 1973	*P. sarana sarana*	Intestine	2	2	100.00
Family: Heterophyidae Odhner, 1914					
Haplorchoides mehrai Pande and Shukla, 1976	*Mystus malabaricus* (Jerdon)	Intestine	10	6	60.00
Family: Hemiuridae Lühe, 1901					
Genarchopsis goppo Ozaki, 1925	*Channa orientalis* (Schneider)	Stomach	42	15	35.71

(1)	(2)	(3)	(4)	(5)	(6)
METACERCARIAE					
Family: Strigeidae Railliet, 1919					
Tetracotyle sp.I	*Danio aequipinnatus* McClelland	Muscles near vertebral column	295	70	23.73
Tetracotyle sp.II	*Etroplus suratensis* (Bloch)	Mesentery of visceral organs	22	20	90.91
Diplostomulum sp.	*Labeo rohita* (Hamilton)	Muscles	62	41	66.13
Neascus sp.I	*Xenentodon cancila* Hamilton	Liver	69	65	94.20
	Aplocheilus panchax (Hamilton)	Liver	120	45	37.50
Neascus sp.II	*Macropodus cupanus* Valenciennes	Liver	301	4	1.33
Family: Diplostomidae Poirier, 1886					
Diplostomum ketupanense Vidyarthi, 1937	*Rasbora daniconius* (Hamilton)	Muscles	622	420	67.52
Family: Clinostomatidae Lühe, 1901					
Euclinostomum heterostomum (Rudolphi, 1809) Travassos, 1928	*Channa orientalis* (Schneider)	Muscles	42	9	21.43

(1)	(2)	(3)	(4)	(5)	(6)
	Etroplus maculatus (Bloch)	Muscles	25	6	24.00
Family: Echinostomatidae (Looss, 1902) Poche, 1926					
Echinostome metacercaria	*Esomus barbatus* (Jerdon)	Base of gill filaments and gill arches	360	122	33.89
	Horadandia atukorali Deraniyagala	Base of gill filaments and gill arches	162	133	82.10
	Puntius parrah Day	Base of gill filaments and gill arches	420	30	7.14
	Mystus malabaricus (Jerdon)	Base of gill filaments and gill arches	10	3	30.00
	Heteropneustes fossilis (Bloch)	Base of gill filaments and gill arches	38	2	5.26
	Channa punctatus Bloch	Base of gill filaments and gill arches	22	3	13.64
Echinochasmus megavitellus Lal, 1939	*Labeo rohita* (Hamilton)	Gills	62	8	12.90

(1)	(2)	(3)	(4)	(5)	(6)
Family: Renicolidae Dollfus, 1939					
Renicolid metacercaria	*Danio aequipinnatus* McClelland	Liver	295	20	6.78
	Puntius melanampyx melanampyx (Day)	Liver	122	8	6.56
Family: Plagiorchiidae (Luhe, 1901) Ward, 1917					
Encyclometra colubrimurorum (Rudolphi, 1819) Dollfus, 1921	*Channa orientalis* (Schneider)	Muscles	42	11	26.19
Family: Heterophyidae Odhner, 1914					
Centrocestus formosanus (Nishigori, 1924) Price, 1932	*Danio aequipinnatus* McClelland	Gill filaments	295	30	10.17
	D. spinosus Day	Gill filaments	15	3	20.00
	Puntius melanampyx melanampyx (Day)	Gill filaments	122	30	24.59
	P. parrah Day	Gill filaments	420	302	71.90
	P. sophore (Hamilton)	Gill filaments	60	15	25.00
	Labeo rohita (Hamilton)	Gill filaments	62	24	38.71
	Mystus malabaricus (Jerdon)	Gill filaments	10	4	40.00
	Heteropneustes fossilis (Bloch)	Gill filaments	38	14	36.84
	Aplocheilus panchax (Hamilton)	Gill filaments	120	62	51.67

(1)	(2)	(3)	(4)	(5)	(6)
	Channa orientalis (Schneider)	Gill filaments	42	36	85.71
	C. punctatus Bloch	Gill filaments	22	18	81.82
	Etroplus maculatus (Bloch)	Gill filaments	25	4	16.00
	Macropodus cupanus Valenciennes	Gill filaments	301	49	16.28
Haplorchoides mehrai Pande and Shukla, 1976	*Esomus barbatus* (Jerdon)	Fins and gills	360	138	38.33
	Horadandia atukorali Deraniyagala	Fins, muscles and gills	162	120	74.07
	Rasbora daniconius (Hamilton)	Fins, muscles and gills	622	451	72.51
	Puntius filamentosus (Valenciennes)	Fins, scales, muscles and gills	21	16	76.19
	P. melanampyx melanampyx (Day)	Scales, fins and muscles	122	83	68.03
	P. parrah Day	Scales, fins, muscles, gills, kidney and liver	420	392	93.33
	P. sophore (Hamilton)	Scales, fins, muscles and gills	60	42	70.00
	Garra mullya (Sykes)	Fins	9	2	22.22

(1)	(2)	(3)	(4)	(5)	(6)
	Mystus malabaricus (Jerdon)	Fins	10	4	40.00
	M. oculatus (Valenciennes)	Fins	31	2	6.45
	Heteropneustes fossilis (Bloch)	Fins and gills	38	12	31.58
Family: Acanthostomidae Poche, 1926					
Acanthostomum burminis (Bhalerao, 1926) Bhalerao, 1936	*Rasbora daniconius* (Hamilton)	Fins	622	228	36.65
	Puntius melanampyx melanampyx (Day)	Fins	122	41	33.61
	P. parrah Day	Fins	420	193	45.95
	Garra mullya (Sykes)	Fins	9	1	11.11
	Mystus malabaricus (Jerdon)	Fins	10	2	20.00
	M. oculatus (Valenciennes)	Fins	31	13	41.94
	Heteropneustes fossilis (Bloch)	Fins	38	11	28.95
	Channa orientalis (Schneider)	Fins	42	20	47.62
	Etroplus maculatus (Bloch)	Fins	25	9	36.00

SUMMARY

The present investigation on the digenean fauna of freshwater fishes in Malabar, carried out for a period of 3 years and 7 months (January 1995 to July 1998), was aimed at studying the systematics and biology of digenetic trematodes infecting some freshwater fishes in Malabar. A total of 2708 freshwater fishes belonging to 20 genera and 30 species, collected from different water bodies in Malabar, were subjected to parasitological studies. Altogether 18 species of digeneans were encountered in 22 species of fishes. All the digeneans recovered have been described and illustrated in the thesis.

The 18 species include 4 species of adults, *Transversotrema patialense* from *Rasbora daniconius; Caballeroia bhavani* and *Neopodocotyle dayali* from *Puntius sarana sarana,* and *Genarchopsis goppo* from *Channa orientalis,* and the adult of *Haplorchoides mehrai* from *Mystus malabaricus* and its metacercaria from *M. malabaricus* and 10 other species of fishes (Table 29), and 13 species of metacercariae, *Tetracotyle* sp. I from *Danio aequipinnatus; Tetracotyle* sp. II from *Etroplus suratensis; Diplostomulum* sp. from *Labeo rohita; Neascus* sp. I from *Xenentodon cancila* and *Aplocheilus panchax; Neascus* sp. II from *Macropodus cupanus; Diplostomum ketupanense* from *R. daniconius; Euclinostomum heterostomum* from *C. orientalis* and *Etroplus maculatus;* an echinostome species from *Esomus barbatus, Horadandia atukorali, Puntius parrah, Mystus malabaricus, Heteropneustes fossilis* and *Channa punctatus; Echinochasmus megavitellus* from *L. rohita;* a renicolid species from *D. aequipinnatus* and *Puntius melanampyx melanampyx; Encyclometra*

colubrimurorum from *C. orientalis; Centrocestus formosanus* from 13 species of fishes (Table 29), and *Acanthostomum burminis* from 9 species of fishes (Table 29).

Life-cycles of 4 species of digenetic trematodes have been successfully established in the laboratory during the course of present investigation, and their natural hosts have also been recovered. These include *Diplostomum ketupanense, Transversotrema patialense, Haplorchoides mehrai* and *Acanthostomum burminis.*

Diplostomum ketupanense has a 3 host life-cycle: the furcocercous cercaria liberated by *Indoplanorbis exustus,* encysted in the muscles of *R. daniconius,* and the metacercaria developed into adult fluke in the intestine of *Ardeola grayii.* The life-cycle described in the thesis forms the first report on an Indian *Diplostomum* sp.

The transversotrematid cercaria, emerged from the snail, *Thiara tuberculata,* and exposed to *Rasbora daniconius,* developed into the adult of *T. patialense* without an intervening metacercarial stage.

The cercaria of *H. mehrai,* released by *T. tuberculata,* and exposed to *R. daniconius* and *Puntius parrah,* developed into metacercaria. Natural infections by the metacercariae were found in 11 species of fishes. The adults were obtained from the intestine of *Mystus malabaricus.*

The life-cycle of *A. burminis,* infecting the water snake, *Xenochrophis piscator,* was established and published in 1998 (Acta Parasitologica, 1998, 43(4), 189-193).

The cercarial stage of 27-spined echinostome metacercariae, and adults of *Echinochasmus megavitellus* and *Centrocestus formosanus* have been recovered. The 27-spined echinostome cercaria, shed by *I. exustus,* and exposed to *Esomus barbatus* and *Horadandia atukorali,* developed into an unidentified echinostome metacercaria. Natural infections by the metacercariae were found in 6 species of fishes.

Infections by the metacercariae of *E. megavitellus* were found in the gills of *L. rohita,* and adults in the intestine of *A. grayii.* The link between metacercaria and adult has been successfully established by feeding one-day-old chicks with infective metacercariae. *E. megavitellus* metacercaria is reported for the first time from India.

The metacercariae of *C. formosanus* were found in 13 fish species. Experimental infections by adults were successfully established by feeding infection-free *A. grayii* and one-day-old chicks with infective metacercariae.

Data on prevalence and intensity of infection have been presented. Host-specificity exhibited by digeneans infecting freshwater fishes has been briefly discussed. New host and geographical records have also been presented in the thesis.

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The life cycle of *Acanthostomum burminis* (Trematoda, Acanthostomidae)

T. M. Roopa and K. P. Janardanan

Parasitology Laboratory, Department of Zoology, University of Calicut, Kerala 673 635, India

Abstract. The life cycle of *Acanthostomum burminis* (Bhalerao, 1926) Bhalerao, 1936 (Trematoda, Acanthostomidae) infecting *Xenochrophis piscator* has been elucidated. Life cycle stages were successfully reared in the laboratory. The life cycle, from cercaria to adult, took more than two months to complete. Pleurolophocercous cercariae were found in the thiarid snail *Thiara tuberculata*. Metacercariae occurred in fin rays of several species of freshwater fishes. Snakes became infected when fed on fishes containing metacercariae. The prepatent period is 28 days. Successive stages in the development of metacercariae and adults were followed.

Key words: *Acanthostomum burminis*, life cycle, *Xenochrophis piscator*, cercaria, metacercaria, *Thiara tuberculata*, fish, trematoda

Introduction

The genus *Acanthostomum* was established by Looss in 1899 with *A. spiniceps* from *Bagrus bayad* as its type species. Altogether 27 species of *Acanthostomum* have been reported: 9 from fishes and 18 from reptiles (Yamaguti 1975). As far as is known, only two valid species of *Acanthostomum* have been reported from India: *A. burminis* (Bhalerao, 1926) Bhalerao, 1936 from *Tropidonotus piscator* and *A. indicum* Sinha, 1942 from a crocodile. *A. burminis* was first described by Bhalerao (1926) as *Acanthochasmus burminis*, and in 1936 he shifted it to *Acanthostomum*. In 1940 he proposed a new genus *Atrophecaecum* to accomodate this species, but Mehra (1980) and Srivastava (1982) suppressed this genus and considered *Acanthostomum burminis* as valid. Further, they treated *Acanthostomum simhai* Khalil, 1963; *Atrophecaecum hindustanensis* Baugh, 1956; *A. indicum* Simha, 1958; and *Haplocaecum proctoporum* Dwivedi, 1966 as synonyms of *A. burminis*. Of the 27 species of *Acanthostomum*, life cycles of only 3 species have been elucidated: *A. imbutiforme* by Maillard (1973), *A. gnerii* by Ostrowski de Nunez (1991), both parasitizing fishes, and *A. brauni*, infecting reptiles, by Ostrowski de Nunez (1987). The complete life cycle of *A. burminis* has not been elucidated; the only available information is a report on its metacercariae from a few freshwater fishes in Andhra Pradesh by Madhavi (1980) and Madhavi and Rekharani (1985).

During study on the cercarial fauna of Kerala, we recovered a pleurolophocercous cercaria from the thiarid snail, *Thiara tuberculata* in the paddy fields, ponds and rivulets at Sultan Bathery in Wayanad, Thalassery in Kannur, Ramanattukara in Kozhikode and Nedungattumadu in the Malappuram districts of Kerala. The cercariae encysted in the fins of *Rasbora daniconius, Puntius parrah, P. malanampyx melanampyx, Mystus oculatus, M. malabaricus, Heteropneustus fossilis, Channa orientalis, Etroplus maculatus* and *Garra mullya*. The metacercariae, fed to the water snake, *Xenochrophis piscator*, developed into adults of *A. burminis*. We could establish experimentally the life cycle of this species in the laboratory. This is the first report on the life cycle of an Indian *Acanthostomum*; it is also the fourth report on the life cycle of *Acanthostomum* spp.

Materials and methods

The thiarid snails, *Thiara tuberculata* were collected from paddy fields, ponds and rivulets in Wayanad, Kozhikode, Kannur and Malappuram districts of Kerala from December 1994 to March 1996. The infected snails were isolated, kept in separate beakers and the cercariae emerging from them were studied alive. Cercariae mounted in 0.7% saline solution were observed under the oil immersion objective of a phase-contrast microscope to study the flame cell pattern. Genital primordia were observed in acetic orcein-stained cercariae. Five infected snails were

crushed and examined for intramolluscan stages. Measurements of cercariae were made on heat-killed specimens.

Rasbora daniconius and *Puntius parrah* collected from a tank in the Calicut University Campus and virtually free of natural infections with *A. burminis* metacercariae were exposed to cercariae. The fishes were dissected at various intervals post-exposure and the development of the metacercariae was followed. Metacercariae were recovered also from naturally infected fishes collected from Kozhikode, Wayanad, Kannur and Malappuram districts of Kerala. In order to obtain adults, the infected fishes were fed to *X. piscator* free from parasites as determined by faecal investigations for 4 months. The fed snakes were dissected at various intervals, and the flukes at different stages of development were recovered. Flukes were found also in naturally infected snakes. The cercariae, metacercariae and adults were studied alive with or without vital staining. Metacercariae and adults used for permanent preparations were fixed in AFA and stained with Mayer's carmalum (Cantwell 1981).

Measurements are given in micrometres. Figures were drawn with the aid of a camera lucida and details added free-hand from observations made on living specimens.

Results

Egg

Eggs small, oval, yellowish brown, operculate, measured 36–40 × 14–16. Attempts made to induce hatching of miracidia were futile.

Redia (Figs. 1 and 2)

Sporocysts were not observed. Mother rediae and daughter rediae were recovered from the hepatopancreas of *Thiara tuberculata*. The mother redia contains 1–3 daughter rediae and many germ balls. Mother rediae are comparatively small, measuring 310–325 × 95–116; their guts extend to 1/3–1/2 of the total length of the body (Fig. 1). Body of daughter rediae is long, almost cylindrical, narrowing posteriorly, measuring 315–654 × 100–246. Mouth terminal; pharynx subglobular, 23–39 × 13–38, followed by a short gut, ending blindly. Each daughter redia encloses a number of germ balls and 15–20 cercariae in different stages of development (Fig. 2).

Cercaria (Fig. 3)

Natural infections with the cercariae were found in 210 of 600 (35%) *T. tuberculata* collected from December 1994 to March 1996. Infected snails were present throughout the year, but infections were prevalent during October – March, when 43.5% of the snails examined were infected. Cercariae were positively phototactic and emerged throughout the day with peak emergence during noon hours. They appeared as dark-brown, opaque larvae showing characteristic swimming bahaviour, with prolonged resting periods alternating with brief swimming activity. While resting, the cercaria remains suspended in the water column in a typical tobacco pipe form with the body downward and the tail upward.

Description. Pleurolophocercous cercaria belonging to the opisthorchioid group. Body elongate, 261–392 × 69–123 in size, densely pigmented. Body tegument spiny, spines in anterior one-fourth arranged in regular rows. Tail slender, muscular, longer than body, 377–577 × 30–46 large, inserted in a socket at posterior end of body, provided with two lateral fin folds in anterior third and a long dorso-ventral fin fold covering posterior two-third on dorsal side and posterior one-fourth on ventral side. Ten pairs of sensory cilia occur on body margins: one pair at the level of oral sucker and the rest in post-equatorial region. Two prominent eyespots, X- or Y-shaped, at a distance of 69–79 from anterior end.

Oral sucker terminal, large, muscular, protrusible, 46–49 × 36–46 large. Acetabulum absent. Mouth terminal. Preoral lobe with 18 forwardly directed spines and pores of penetration glands. Pharynx globular; prepharynx rudimentary; oesophagus and caeca not discernible.

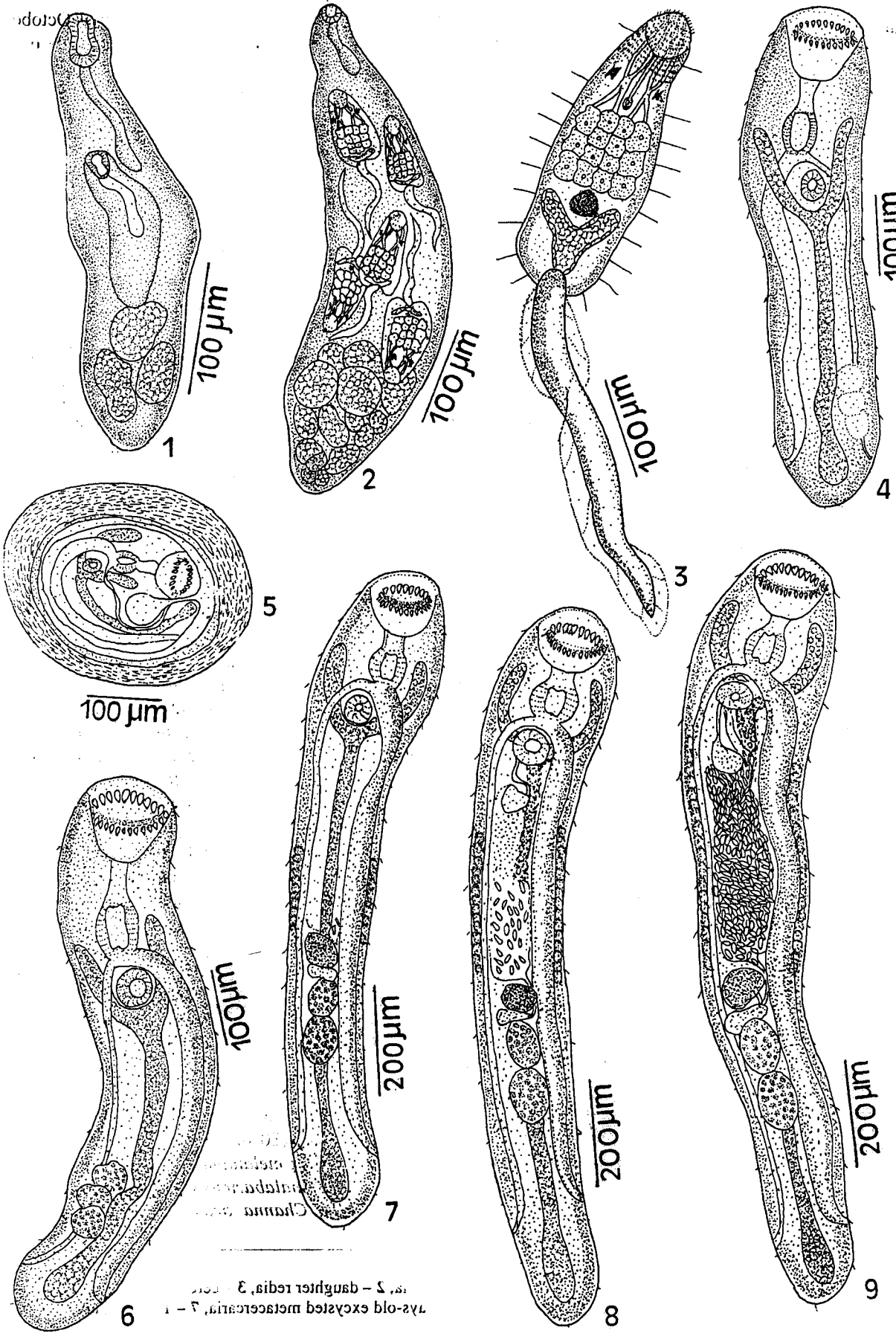
Penetration glands 14 in number, arranged in four rows, situated between pharynx and excretory vesicle; two outer rows contain 3 glands each and two inner rows 4 glands each. Glands are large, roughly rectangular with rounded nuclei and granular cytoplasm. Ducts of penetration glands from each row run forward in 4 separate bundles which join at the level of eyespots into 2 bundles, then again divide into 4 bundles of enlarged ducts near posterior margin of oral sucker and open out side through 4 groups of pores arranged according to 3:4:4:3 pattern in preoral lobe. Cystogenous glands small, numerous, distributed in lateral fields of body.

Genital rudiments triangular, in front of excretory bladder. Excretory bladder Y-shaped, lined by large epithelial cells and filled with granular concretions. Flame cell formula 2[(3+3+3) + (3+3+3)] = 36.

Metacercaria (Figs. 4–6)

In natural infection metacercariae were found in fin rays of *Rasbora daniconius* (80 of 248), *Puntius parrah* (91 of 243), *P. melanampyx melanampyx* (4 of 21), *Mystus oculatus* (7 of 12), *M. malabaricus* (2 of 10), *Heteropneustus fossilis* (5 of 7), *Channa orientalis* (1 of 3), *Etroplus*

Figs. 1–9. Stages in the life cycle of *Acanthostomum burminis*: 1 – mother redia, 2 – daughter redia, 3 – cercaria, 4 – 14-days-old excysted metacercaria, 5 – encysted metacercaria from natural infection, 6 – 22-days-old excysted metacercaria, 7 – 12-days-old adult, 8 – 22-days-old adult, 9 – 28-days-old adult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macilatus (3 of 7) and *Garra mullya* (1 of 9) collected from paddy fields, ponds and rivulets in various localities of North Kerala. Experimental infection has been successfully established in *R. daniconius* and *P. parrah* exposed to a large number of cercariae. All the fishes exposed were infected. The metacercariae developed in fin rays. As many as 17 cysts were obtained from a single fish exposed to cercariae. Snails were resistant to metacercarial infection.

In experimentally infected fishes, free cercarial bodies were observed in fin rays after 1 h of exposure. Metacercariae enclosed in thin cyst wall were observed 6 h post exposure; they were similar in structure to cercaria, but their penetration and cystogenous glands were not visible. In fishes examined 7-days post exposure, the metacercariae were surrounded by an outer, thick, fibrous wall over the inner, thin, delicate layer; pharynx was visible (31 × 26). In 9-day-old metacercaria, caeca reached up to the middle of the body. Metacercariae recovered 10-days post exposure showed conspicuous acetabulum, slightly enlarged pharynx and eyespots starting to disintegrate. On 14th day after exposure, the eyespots disappeared completely and a crown of 23 circumoral spines appeared. The caeca extended to hind body and opened out laterally; the left caecum was well developed while the right one was atrophied. Primordia of reproductive organs were visible in the hind body (Fig. 4). Metacercariae completed the development within 22 days, and became infective to the definitive host.

Description. Cysts small, almost spherical, with an outer wall thick, fibrous and an inner one thin, delicate; they measured 146–431 × 107–323. Metacercarial body measured 547–1001 × 77–207. Body spines larger in anterior region and arranged in regular rows. Oral sucker terminal, funnel-shaped, measured 92–138 × 82–171. Circumoral spines 24–26, arranged in a single row. Dorsal spines larger (22) than ventral spines (16.5); mid ventral spine is the smallest (13.2). Acetabulum small, measured 46–69 × 39–77. Prepharynx 38–77 long. Pharynx muscular, 61–100 long and 38–77 wide. Oesophagus short. Caeca long, asymmetrical, open out through anal pores on lateral body margins near posterior end; 377–438 long; right caecum atrophied. Excretory vesicle Y-shaped, with a long stem and two short cornua reaching near prepharynx and filled with small concretions; primordia of ovary and testes arranged in tandem in posterior part of body. Ovary measured 30–61 × 38–46. Anterior testis measured 36–53 × 38–61 and posterior testis 38–69 × 38–61.

Adults

Adult flukes were obtained from the small intestine of *Xenochrophis piscator* collected from the water bodies from where infected snails and fishes were collected. All the snakes collected were infected. The flukes were identical to *Acanthostomum burminis* (Bhalerao, 1926) Bhalerao, 1936 reported from the same host in Burma. The snakes fed with fishes containing naturally infected metacercariae or those carrying experimentally reared 22-day-old metacercariae yielded adult flukes in 28 days.

The flukes recovered from the snake after 24 h post infection were structurally similar to excysted metacercariae. Three-day-old flukes measured 792–898 × 185–234; their oral sucker, acetabulum and pharynx increased in size.

On the 7th day post infection the flukes measured 918–983 × 175–209. Testes and ovary well developed; a sac-like receptaculum seminis developed between ovary and anterior testis. Follicular vitellaria became visible in the extracaecal field at the level of the ovary. Uterus began to develop, and contained 2 embryonic eggs.

The 12-day-old specimens measured 1170–1262 × 173–216. Anterior testis measured 68–107 × 45–61 and posterior testis 96–103 × 43–64 (Fig. 7). Receptaculum seminis enlarged in size and measured 43–49 × 59–63. Vitellaria extended from the level of ovary to the middle of body; uterus contained 8–12 eggs. Seminal vesicle developed in 22-days-old flukes; convoluted uterine coils were filled with 20–30 eggs (Fig. 8).

The flukes recovered on the 28th day were mature, measured 1325–1460 × 208–274; uterine coils were filled with numerous eggs. Vitellaria extended from ovary to seminal vesicle (Fig. 9). Flukes recovered after 40 days post infection resembled the original *A. burminis* (Bhalerao, 1926) Bhalerao, 1936 in body dimensions and structural details.

Discussion

The genus *Acanthostomum* Looss, 1899 belongs to subfamily Acanthostominae Nicoll, 1914. Of the 27 species of *Acanthostomum*, life cycles of only three species have been elucidated: *A. imbutiforme, A. brauni* and *A. gnerii*. The life cycle of *A. burminis* is similar to that of the above three species in that it uses prosobranch snails as the first and fish as the second intermediate hosts, but differs in having unrelated definitive hosts. In the case of *A. burminis*, water snakes serve as the definitive host while fishes are the definitive host of *A. imbutiforme* and *A. gnerii*, and turtles and alligators are the definitive hosts of *A. brauni*. Further, there are differences in the morphology and morphometry of adults and larval stages.

Acanthostomum burminis has a three-host life cycle. Cercariae emerge from the thiarid snail, *Thiara tuberculata*, and encyst in the fin rays of several freshwater fish species. The metacercariae became infective in 22 days. The metacercariae develop into mature adults during 28 days in the intestine of *Xenochrophis piscator*.

Cercaria of *A. burminis* agrees fully with *Cercariae indicae* VIII Sewell, 1922, reported from *Melania tuberculata* of Calcutta, Bombay and Nilambur (in Kerala). Mohandas (1976) recorded the same cercaria from the same host in Palghat, Ernakulam and Trivandrum districts

of Kerala. The molluscan host of *C. indicae* VIII and of the present cercaria is the same. (*Thiara tuberculata* is the valid name of *Melania tuberculata*). Therefore, in all probability the present cercaria and that described by Sewell (1922) are identical.

The metacercariae of *A. burminis* have been previously reported from *Aplocheilus panchax, Mugil cephalus, Liza macrolepis* and *Valamugil cunnesius* in Andhra Pradesh (Madhavi 1980, Madhavi and Rekharani 1985). Occurrence of this metacercaria in *Rasbora daniconius, Puntius parrah, P. melanampyx melanampyx, Mystus oculatus, M. malabaricus, Heteropneustus fossilis, Channa orientalis, Etroplus maculatus* and *Garra mullya* in Kerala constitutes new hosts and geographical record for this parasite.

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