

**STUDIES ON LARVAL AND ADULT ECHINOSTOMES
(TREMATODA: DIGENEA) IN MALABAR,
KERALA**

Thesis submitted to the
University of Calicut for the Degree of
DOCTOR OF PHILOSOPHY IN ZOOLOGY

By

VENUGOPALAN NAMBIAR. M

PARASITOLOGY LABORATORY
DEPARTMENT OF ZOOLOGY, UNIVERSITY OF CALICUT
KERALA, 673 635, INDIA
2001

To My Teachers

DEPARTMENT OF ZOOLOGY
UNIVERSITY OF CALICUT



Dr. K.P. Janardanan
Professor

Phone: Office 401144*
345
Calicut University P.O.
673 635, Kerala

Date: 10 - 12 - 2001

CERTIFICATE

This is to certify that this thesis is an authentic record of the research work done by **Mr. Venugopalan Nambiar, M.** from December 1995 to December 2001, under my supervision and guidance, that no part thereof has been presented before for any other degree and that he has passed the Ph.D. Preliminary Qualifying Examination of the University of Calicut, held in December 1997.

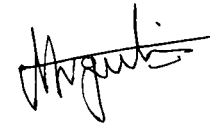
Dr. K.P. Janardanan

DECLARATION

I 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,

10 - 12 - 2001.



Venugopalan Nambiar, M.

ACKNOWLEDGEMENTS

I have great pleasure in expressing my sincerest gratitude to Dr. K.P. Janardanan, Professor, Department of Zoology, University of Calicut for his guidance and encouragement throughout the course of this investigation and also for his support and constructive criticism in the preparation of this thesis.

I am grateful to Dr. U.V.K, Mohamed, Professor and Head, Department of Zoology, University of Calicut for providing me all the necessary facility to carry out this work.

I would like to record my sincere thanks to Dr. N.V. Subha Rao, Zoological survey of India, Culcutta for the identification of the snails used during the course of this work and the Head of the Department of Life Science, University of Calicut for providing experimental animals.

I offer my heartfelt thank to Mr. Vasandakumar, Mr. Chandrasekharan Nambiar, and my friends Mr. Satheeshkumar, Mr. Sanil, Mr. Vijayan and Anandakrishnan for their unstinted support and co-operation in the course of this work.

I am thankful to the University Grants Commission, New Delhi for the financial assistance received in the form of Junior and Senior Research fellowships. I am deeply indebted to my mother and sisters for their whole-hearted support, understanding and sacrifices without which I could not have made this work a reality.

Finally, I would like to tender my appreciations to Mr. Santhosh and Mr. Azeez for their technical assistance in photography, Mr. Jabir and Ms. Surabhi for typing the manuscript and to all others whose generous assistance at one stage or other have made this work a success.

CONTENTS

	Page
Introduction	1
Historical Review	3
Materials and Methods	21
Results	32
<i>Echinostoma revolutum</i> (Frölich, 1802) Dietz, 1909	32
<i>Echinostoma</i> sp.I n.sp	41
<i>Echinostoma</i> sp.II n.sp	56
<i>Echinostoma malayanum</i> Leiper, 1911	68
<i>Echinostoma</i> sp. III n. sp	77
<i>Echinoparyphium</i> sp. I n. sp.	86
<i>Paryphostomum giganticum</i> Rai and Agarwal, 1961	100
<i>Cercariae indicae</i> XX Sewell, 1922	101
<i>Cercaria</i> sp. VI Kerala Mohandas, 1981	105
<i>Cercaria</i> sp.I Malabar n.sp.	110
<i>Cercaria</i> sp.II Malabar n.sp	114
<i>Cercaria</i> sp.III Malabar n.sp	121
<i>Cercaria</i> sp.IV Malabar n.sp	127
<i>Cercaria</i> sp.V Malabar n.sp	133
<i>Cercaria</i> sp.VI Malabar n.sp	139
Discussion	145
Summary	157
References	160
Appendix	

INTRODUCTION

Echinostomes are a group of digenetic parasites characterised by the presence of well-developed collar and collar spines. They parasitize mostly reptiles, birds and mammals, but they have also been reported from fishes. Kerala, with its tropical climate, evergreen vegetation, heavy annual rainfall and various aquatic bodies, is well-known for the richness and diversity of vertebrate and molluscan fauna. Preliminary studies revealed that these vertebrates and molluscs act as hosts for a number of adult and larval echinostomes. Fairly vast information is available on digenetic trematodes of India. A voluminous literature, mostly devoted to descriptions of new species of adults and larval stages from different regions of the country, has accumulated over the years. A perusal of the literature shows that comprehensive studies dealing with echinostome cercariae and their adults are lacking. Published accounts on echinostomes in Kerala are regrettably meagre. The available information is restricted to the descriptions of 9 species of cercariae and life cycles of 3 species of adults (Sewell, 1922; Mohandas, 1971, 1973, 1981; Nambiar and Janardanan, 2001). The present investigation has been undertaken with a view to throwing more light on the larval and adult echinostomes in Malabar, Kerala.

During the present study as many as 15 species of echinostome cercariae were collected from 4 species of freshwater snails in Malabar. Complete life cycles were elucidated for 4 new ^{species} using cercaria as the starting point. They comprised 3 species belonging to the genus *Echinostoma*, and one species belonging to *Echinoparyphium*. Observations were made on further course of development of 3 species of cercariae of previously known life histories; these are *E. revolutum*, *E. malayanum* and *Paryphostomum giganticum*. Cercaria of known life histories are referred to by their adult names. The new species are described as genus sp. I n. sp., sp. II n. sp. and so on. The remaining 8 cercarial species encountered in the present study could not be developed into their adults. Of these, 6 are new and 2 are known species. The new species of cercariae are designated as *Cercaria* sp. I Malabar series, after the area of collection. Detailed descriptions are furnished for all these new forms and the known species are redescribed. Data on prevalence of infections by various cercariae are presented. Host-specificity, mixed infections and other aspects of host-parasite relations of echinostomes have been discussed in the light of available information.

HISTORICAL REVIEW

Fifteen species of echinostome cercariae have been recovered from four species of freshwater snails in Malabar during the course of the present investigation. Of these, seven species of cercariae have been developed into their adults. These adults belong to the genera *Echinostoma*, *Echinoparyphium* and *Paryphostomum*. The remaining 8 cercarial species could not be developed into their adults even after consistent attempts. A brief review of literature of echinostome cercariae and adults belonging to the above mentioned 3 genera is presented here.

CERCARIA

Siebold (1837) described the cercaria of an echinostome from Germany and designated it *Cercaria echinata*; this forms the first record of an echinostome cercaria. Later, La Valette (1855) reported two echinostome cercariae, *Cercaria echinifera* and *C. spinifera* from Europe. De Fillipi (1857) redescribed *C. echinifera* with additional details, renamed it as *C. echinatoides* (La Valette, 1855) and added one more cercaria, *C. coronata*. Lühe (1909) in his admirable work on trematodes placed all the four echinostome cercariae under the Leptocercous group characterising it as follows: "Distome cercarien mit ungegabelten und borstenlosem

Ruderschwaze dessen Breit auch in Kontrahierten Zustends wesentlich hinter derdes Körpers Zurakbleibt". The Leptocercous group was divided into three subgroups, one of which was the echinostome sub group. Cort (1915) established the group of Megalurous cercariae to accommodate 3 forms, *Cercaria distomatosa* Sonsino, 1892, *C. megalura* Cort, 1914 and *Cercaria* sp. Lutz, 1893, that stood out separately from echinostome cercariae and included it under Gymnocephala. De Fillipi (1859) reported *C. agilis* in which the collar spines developed only in the metacercarial stage and ascribed it to the echinostome group. Lühe in his scheme of classification placed *C. agilis* De Fillipi, 1859 in Gymnocephala subgroup of the Leptocercous cercariae.

Taking Lühe's 3 forms, *Cercaria echinata*, *C. echinatoides* and *C. coronata* as types of 3 divergent groups, Sewell (1922) in his monumental work 'Cercariae indicae' created 3 groups, Echinata, Echinatoides and Coronata and included *C. spinifera* under the second group along with his species *Cercariae indicae* XLVIII. He included *C. indicae* XII and *C. indicae* XXIII described by him under Echinata group and *C. indicae* XX under Coronata group. Further, he included Megalurous group under echinostomes and shifted *Cercaria agilis* to the newly created Agilis group along with *Cercariae indicae* XLI Sewell, 1922. Similarly, *Cercaria reflexae* Cort, 1914 which Cort (1915) had provisionally placed

under the echinostomes, was put under Reflexae group by Sewell. The Agilis and Reflexae groups were, however, not included under the echinostomes.

Faust (1924) proposed a different form of classification based on flame cell formula. According to this classification he included 9 groups under echinostomes. While accepting the Echinatoides and Coronata groups of Sewell, he placed Agilis and Reflexae groups under echinostomes. Besides, the groups like Complexa, Cristacantha, Circumstricta, Pseudoechinostoma and Echinochasmida were also placed under Echinostomes.

Dubois (1928) and Wesenberg-Lund (1934) did not agree with Sewell in his division of echinostome cercariae into small natural groups. Miller (1936) also did not agree with Sewell's scheme because characters applied to each subgroup were applicable to all subgroups. Later, it was found that two of the cercariae which had been separated from echinostomes on the plea that they lack spines, were found to develop spines after encystment.

Mukherjee (1992) while revising the classification of echinostome cercariae known from India, accepted Sewell's division and added a new subgroup called Megacaudata for broad-tailed echinostome cercariae.

There are several workers who have contributed much to the knowledge of echinostome cercariae. Those who made significant contributions from abroad are Bisseru (1953, 1967), Khan (1960), Lie (1963 a, 1963 b, 1964, 1965) Lie and Umathevy (1965 a, 1966) Lie and Basch (1967), Hsu *et al.* (1968), Nasir (1960), Haseeb and Khan (1983), Kanev and Odening (198), Kanev (1994), Kanev *et al.* (1995), Fried *et al.*⁽¹⁹⁹⁸⁾ and others.

Among the Indian workers who added much to the knowledge of echinostome cercariae, Sewell (1922), Peter (1955), Ganapati and Rao (1968), Srivastava (1968), Pandey (1973), Baugh (1975), Mukherjee and Ghosh (1977), Agrawal and Sharma (1980), Mohandas (1981), Pandey and Singh (1982), Choubisa and Sharma (1984), Singh and Bali (1991) and Srivastava and Saxena (1992) are prominent.

The echinostomatid cercariae are characterised by a collar, with or without collar spines. The number of spines varies from species to species and very rarely within the same species. The number and disposition of the collar spines are of importance in differentiating species although at times different species possess the same number of spines. Eyespots are invariably absent, digestive system well developed and body dense with cystogenous glands. Excretory system with anterior and posterior secondary

ducts and in almost all cases the main ducts contain excretory concretions.

Development takes place in collared redia within the host.

A list of echinostome cercariae reported from Indian freshwater snails is presented in table I.

Table I. List of echinostome cercariae reported from freshwater snails in India

Sl. No	Name	No. of collar spines	Host (s)	Locality	Reference
1	2	3	4	5	6
1.	Cercaria of <i>Echinostoma revolutum</i> (Frölich, 1802) Dietz, 1909	37	<i>Lymnaea auricularia</i> , <i>L. luteola</i> , <i>L. acuminata</i>	Madras (Tamil Nadu), Palghat, Trichur, and Trivandrum (Kerala), Sambalpur and Bhubaneswar (Orissa)	Peter (1955), Patnaik and Rai (1966), Mohandas (1973)
2.	Cercaria of <i>Echinoparyphium flexum</i> . (Linton, 1892) Dietz, 1910	45	<i>L. luteola</i>	Ludhiana (Punjab)	Singh and Bali (1991)
3	Cercaria of <i>Echinostoma malayanum</i> Leiper, 1911	43-45	<i>Indoplanorbis exustus</i> , <i>L. luteola</i>	Trivandrum (Kerala), Ropar (Punjab)	Mohandas (1971), Singh and Bali (1991)
4.	<i>Cercariae indicae</i> XII Sewell, 1922	43	<i>Gyraulus euphraticus</i> , <i>I. exustus</i>	Calcutta (West Bengal)	Sewell (1922)
5.	<i>C. indicae</i> XX Sewell, 1922	29	<i>I. exustus</i>	Calcutta (West Bengal)	Sewell (1922)

1	2	3	4	5	6
6.	<i>C. indicae</i> XXIII Sewell, 1922	43	<i>L. acuminata</i> , <i>L. ovalis</i> , <i>L. luteola</i>	Calcutta (West Bengal), Madras (Tamil Nadu)	Sewell (1922), Rao (1933)
7.	<i>C. indicae</i> XLVIII Sewell, 1922	43	<i>I. exustus</i>	Mananthody, Sultan's Battery, (Kerala), Lucknow (Uttar Pradesh)	Sewell (1922), Thapar (1969)
8.	<i>Cercaria</i> of <i>Echinostoma</i> <i>cinetorchis</i> Ando and Ozaki, 1923	37	<i>L. luteola</i>	Gurdaspur (Panjab)	Singh and Bali (1991)
9.	<i>Cercaria mehrai</i> Faruqui, 1930	43	<i>I. exustus</i>	Lucknow and Allahabad (Uttar Pradesh), Madras (Tamil Nadu), Ropar (Panjab)	Faruqui (1930), Chatterji (1933), Peter (1955), Jain (1960), Singh and Bali (1991)
10.	<i>Cercaria</i> of <i>Echinochasmus</i> <i>bagulai</i> Verma, 1935	24	<i>Amnicola</i> <i>travancorica</i>	Madras (Tamil Nadu), Visakhapatanam (Andra Pradesh)	Ramalingam (1960), Dhanumkumari <i>et al.</i> (1991)
11.	<i>Cercaria</i> of <i>Protechinostoma</i> <i>macronisertulatum</i> Beaver, 1943	-	<i>I. exustus</i>	Ropar (Punjab)	Singh and Bali (1991)
12.	<i>Cercaria nairi</i> Peter, 1955	41	<i>I. exustus</i>	Madras (Tamil Nadu)	Peter (1955)
13.	<i>Cercaria</i> of <i>Echinoparyphium</i> . <i>baquali</i> Jain, 1960	42	<i>L. auricularia</i> , <i>L. luteola</i>	Ludhiana, Hoshiarpur (Panjab)	Jain (1960), Singh and Bali (1991)
14.	<i>Cercaria</i> of <i>Paryphostomum</i> <i>giganticum</i> Rai and Agarwal, 1961	47	<i>L. luteola</i>	Kannur, Kozhikode (Kerala)	Nambiar and Janardanan (2001)

1	2	3	4	5	6
15.	<i>Cercaria rithorensis</i> Mukherjee, 1963	19	<i>G. convexisculus</i>	Bareilly	Mukherjee (1963)
16.	<i>C. mainpurensis</i> Pandey, 1965	48	<i>I. exustus</i>	Mainpuri (Uttar Pradesh)	Pandey (1965)
17.	<i>C. tetraglandulata</i> Srivastava, 1968	42	<i>I. exustus</i>	Lucknow (Uttar Pradesh)	Srivastava (1968)
18.	<i>C. andraensis</i> Ganapathi and Rao, 1969	33	<i>Pila globosa</i>	Waltair (Andra Pradesh)	Ganapathi and Rao (1969)
19.	<i>C. oviglandulata</i> Pandey, 1973	28	<i>L. stagnalis</i>	Lucknow (Uttar Pradesh)	Pandey (1973)
20.	<i>C. megaglandulata</i> Agrawal, 1974	24	<i>I. exustus</i>	Durg (Madhya Pradesh)	Agrawal (1974)
21.	<i>Cercaria</i> of <i>Echinoparyphium</i> <i>hymani</i> Singh, 1975	43	<i>L. luteola</i>	Durg (Madhya Pradesh)	Singh (1975 a)
22.	<i>Cercaria</i> of <i>E.</i> <i>lanceolatum</i> Singh, 1975	45	<i>L. luteola</i>	Durg (Madhya Pradesh)	Singh (1975 b)
23.	<i>Cercaria granulosa</i> Baugh, 1975	43	<i>L. acuminata</i>	Lucknow (Uttar Pradesh)	Baugh (1975)
24.	<i>C. triglandulata</i> Baugh, 1975	41	<i>I. exustus</i>	Lucknow (Uttar Pradesh)	Baugh (1975)
25.	<i>C. megacauda</i> Baugh, 1975	22	<i>G. convexiusculus</i>	Lucknow (Uttar Pradesh)	Baugh (1975)
26.	<i>Cercaria</i> of <i>E.</i> <i>vitellocompactum</i> Singh, 1976	45	<i>L. luteola</i>	Durg (Madhya Pradesh)	Singh (1976)
27.	<i>Cercaria beaveri</i> Pandey and Agrawal, 1977	40	<i>Bellamyia</i> <i>bengalensis</i>	Lucknow (Uttar Pradesh)	Pandey and Agrawal (1977)

1	2	3	4	5	6
28.	<i>C. dharmatallansis</i> Mukherjee and Ghosh, 1977	32	<i>L. luteola</i>	Calcutta (West Bengal)	Mukherjee and Ghosh (1977)
29.	<i>C. dietzi</i> Singh, 1977	37	<i>L. luteola</i>	Durg (Madhya Pradesh)	Singh (1977)
30.	<i>C. itoi</i> Pandey and Agrawal, 1977	32	<i>L. auricularia</i>	Lucknow (Uttar Pradesh)	Pandey and Agrawal (1977)
31.	<i>C. komiyai</i> Pandey and Agrawal, 1977	32	<i>B. bengalensis</i>	Lucknow (Uttar Pradesh)	Pandey and Agrawal (1977)
32.	<i>C. chinahatensis</i> Srivastava, 1978	26	<i>I. exustus</i>	Lucknow (Uttar Pradesh)	Srivastava (1978)
33.	<i>C. chillavanensis</i> Agrawal and Sharma, 1980	28	<i>L. acuminata</i>	Behsa, Lucknow (Uttar Pradesh)	Agrawal and Sharma (1980)
34.	<i>Cercaria</i> sp. VI Kerala Mohandas, 1981	47	<i>L. luteola</i>	Trivandrum (Kerala)	Mohandas (1981)
35.	<i>Cercaria</i> sp. VII Kerala Mohandas, 1981	45	<i>L. luteola</i>	Trichur, Palghat and Ernakulam (Kerala)	Mohandas (1981)
36.	<i>Cercaria</i> sp. VIII Kerala Mohandas, 1981	51	<i>L. luteola</i>	Palghat and Trivandrum (Kerala)	Mohandas (1981)
37.	<i>Cercaria</i> <i>unnaoensis III</i> Pandey and Lal, 1982	37	<i>B. bengalensis</i> , <i>L. auricularia</i> , <i>I. exustus</i>	Unnao (Uttar Pradesh)	Mukherjee (1992)
38.	<i>C. unnaoensis IV</i> Pandey, Singh and Lal, 1982	27	<i>L. auricularia</i>	Unnao (Uttar Pradesh)	Mukherjee (1992)
39.	<i>C. unnaoensis V</i> Pandey, Singh and Lal, 1982	24	<i>I. exustus</i>	Unnao (Uttar Pradesh)	Mukherjee (1992)

1	2	3	4	5	6
40	<i>C. tandani</i> Pandey and Singh, 1982	32	<i>I. exustus</i>	Sultanpur (Uttar Pradesh)	Pandey and Singh (1982)
41	<i>C. narindapurensis</i> Pandey and Singh, 1984	58	<i>I. exustus</i>	Sultanpur (Uttar Pradesh)	Pandey and Singh (1984)
42	<i>C. nawabganjensis</i> Pandey and Singh, 1984	32	<i>I. exustus</i>	Unnao (Uttar Pradesh)	Pandey and Singh (1984)
43	<i>C. spinosa</i> Pandey and Singh, 1984	37-38	<i>B. bengalensis</i>	Unnao (Uttar Pradesh)	Pandey and Singh (1984)
44	<i>C. tewarii</i> Choubisa and Sharma, 1984	18	<i>I. exustus</i>	Udaipur (Rajasthan)	Choubisa and Sharma (1984)
45	<i>Cercaria</i> sp. XI Punjab Singh and Bali, 1991	32	<i>L. luteola</i>	Faridkot (Panjab)	Singh and Bali (1991)
46	<i>Cercaria</i> sp. XII Punjab Singh and Bali, 1991	28	<i>L. luteola</i>	Ludhiana (Punjab)	Singh and Bali (1991)
47	<i>C. bellamyaii</i> Srivastava and Saxena, 1992	38	<i>B. bengalensis</i>	Lucknow (Uttar Pradesh)	Srivastava and Saxena (1992)
48	<i>C. banglabazarensis</i> I sp. Srivastava and Soloman, 1992	30	<i>I. exustus</i>	Lucknow (Uttar Pradesh)	Srivastava and Soloman (1992)
49	<i>C. banglabazarensis</i> II sp. Srivastava and Solomon, 1992	22	<i>I. exustus</i>	Lucknow (Uttar Pradesh)	Srivastava and Soloman (1992)

ADULT

An adult trematode belonging to echinostome group was first reported in Germany by Goeze (1782) as *Planaria laticula* from *Falco milvus*. The description given by Goeze was inadequate. The first detailed description of an echinostome fluke was given by Frölich in 1802 based on the specimen collected in 1798 from wild ducks, *Anas boschas fereae*, but he named it *Fasciola revoluta*. Later, La Valette (1855), Linstow (1873) and Ercolani (1881) added more species to the group of collar spined worms. Stossich (1895) gave a list of species under the genus title *Echinostomum* Rudolphi, 1809 in which he included 5 species from mammals, 22 species from birds and one from reptile.

After analysing the collection of echinostomes from birds, which were preserved in the major Zoological Museums in Europe, Dietz (1909) in his classical work 'Die Echinostomiden der Vogel', established the family Echinostomatidae, divided the genus *Echinostomum* into several independent genera, uniting all of them under the new family and renamed the genus *Echinostomum* as *Echinostoma*. Lühe (1909) added 3 more genera to the family Echinostomatidae.

Other workers who contributed much to the taxonomy of the collar-spined worms include Odhner (1911), Travassos (1922) Poche (1926) and

Skryabin and Shul'ts (1937). Yamaguti (1971) revised the characters of the family Echinostomatidae and listed 10 subfamilies and 42 genera under it.

During the present investigation, I could raise adults of 7 species belonging to *Echinostoma*, *Echinoparyphium* and *Paryphostomum*, from 7 cercarial species. As the review of literature of all the 42 genera under Echinostomatidae has no relevance in the present context, I am presenting here the review of only *Echinostoma* Dietz, 1909, *Echinoparyphium* Dietz, 1909 and *Paryphostomum* Dietz, 1909.

Genus *Echinostoma* Dietz, 1909

Frölich (1802) described *Fasciola revoluta* from wild ducks *Anas boschas fereae*, but Rudolphi (1809) shifted this species to the genus *Distoma* Retzius, 1786, called it *D. revoluta* and considered it as conspecific to *Distoma echinatum* Zeder, 1803. Dietz (1909) in his systematic reorganization of the collar-spined worms of the family Echinostomatidae established the genus *Echinostoma*, restored the validity of *F. revoluta*, renamed it *Echinostoma revolutum* and declared *Distoma echinatum* as a synonym of *E. revolutum*. The present day knowledge of *E. revolutum* is based mainly on the descriptions and illustrations published in over 500 works. However, only conflicting information was available on the validity, identity, synonymy and other aspects of *E. revolutum*. By extensive

comparative experimental studies, Kanev (1994) resolved the matter and treated *E. paraulum* Dietz, 1909, *E. audyi* Lie and Umathevy, 1965, and *E. ivaniosi* Mohandas, 1973 as synonyms of *E. revolutum*.

Till date more than 130 species of *Echinostoma* have been reported; these include 7 species described from India by Mogh (1932), Verma (1936), Lal (1939), Dube (1964), Mohandas (1973) and Ghosh and Chauhan (1977). Life cycles of a few species have been described in detail. The important among them are the contributions made by Patnaik and Ray (1966) and Mohandas (1971, 1973).

The characters of the genus *Echinostoma* Dietz, 1909, as given by Mehra (1980), are: Body elongate. Head collar well-developed with single or double dorsally uninterrupted row of spines. Acetabulum near anterior extremity. Testes post-equatorial, tandem. Cirrus sac anterior to acetabulum or somewhat overlapping it. Genital pore post bifurcal. Ovary median or slightly sub-median, pre-testicular, usually equatorial or pre-equatorial. Vitellaria surrounding caeca in hind body reaching almost posterior margin of acetabulum, confluent or not in post-testicular region. Uterus long, coiled, intercaecal, between ovary and acetabulum. Excretory vesicle Y-shaped, arms and side branches often distended with concretions;

collecting duct turning on itself at anterior end of body, with ciliary flames on inner face of its wall. Parasitic in reptiles, birds and mammals.

A list of *Echinostoma* species reported from India is presented here.

List of *Echinostoma* spp. reported from India

No.	Parasite	Hosts	Locality	Reference
1.	<i>Echinostoma revolutum</i> (Frölich, 1802) Dietz, 1909	Ducks, geese, fowl, crow, muskrat, domestic ducks	Uttar Pradesh; Kerala	Verma (1936); Dube (1964); Mohandas (1973)
2.	<i>E. govindum</i> Moghe, 1932	<i>Philomachus pugnax</i> , <i>Corvus splendens</i>	Maharashtra, Uttar Pradesh	Mogh (1932); Verma (1936)
3.	<i>E. bhattacharyai</i> Verma, 1936	Black-winged stilt	Uttar Pradesh	Verma (1936)
4.	<i>E. chasma</i> Lal, 1939	<i>Querquedula ciria</i>	Uttar Pradesh	Lal (1939)
5.	<i>E. eduardoi</i> Ghosh and Chauhan, 1977	<i>Bandicota bengalensis</i>	West Bengal	Ghosh and Chauhan (1977)
6.	<i>E. minimum</i> (Verma, 1936) Srivastava, 1982	<i>Cygnus atratus</i>	Bihar	Verma (1936); Srivastava (1982)

Genus *Echinoparyphium* Dietz, 1909

The genus *Echinoparyphium* was proposed by Dietz in 1909 with *E. elegans* (Looss, 1899) as its type species. However, *E. cinctum* (Rudolphi, 1803) is the oldest species of the genus, the adults and larvae of

which were reported with different species and generic names. The important names are given here. *Fasciola cincta* Rudolphi, 1803 was the first and original name. *Distoma (Echinostoma) cincta* (Rudolphi, 1803) was another name used by Rudolphi (1809) for the same worm. Rudolphi (1819), Cobbold (1860), Dietz (1909) and Kanev *et al.* (1994) considered *Distoma (Echinostoma) cincta* and *F. cincta* identical. *Echinostoma cincta* (Rudolphi, 1803) was the next name used by Cobbold (1860) for *F. cincta*. *Echinoparyphium cinctum* (Rudolphi, 1803) was the last name offered for *F. cincta* by Bashkirova (1941). Kanev *et al.* (1994) agreed with her and treated *Echinoparyphium cinctum* as the valid name. Thus according to the rule of priority *E. cinctum* becomes the type species of the genus *Echinoparyphium*. A large number of species of echinostomes have been attributed to the genus *Echinoparyphium*, since its establishment by Dietz (1909). In reviews of the genus *Echinoparyphium* by Mendheim (1943), Dollfus (1953), Skryabin (1956) and Buscher (1978) many of the incorrectly placed species have been removed. Kanev (1990) published a checklist of the genus *Echinoparyphium*, containing 151 species names. The current knowledge of many species are problematic and open for discussion.

The characters of the genus *Echinoparyphium* Dietz, 1909, as given by Buscher (1978), are: Body slender, attenuated posteriorly. Head collar well developed, reniform, with double dorsally uninterrupted row of spines.

Spines in aboral row larger than spines in oral row. Acetabulum rounded, situated in anterior half of body. Testes, regularly oval, tandem, median, at or near middle of hind body. Cirrus pouch anterior or antero-dorsal to acetabulum, not extending beyond posterior border of acetabulum. Pars prostatica present or absent. Seminal vesicle undivided or bipartite. Ovary round or oval, median or sub-median, a little in front of anterior testis. Uterine seminal receptacle present or absent. Uterus short, egg comparatively large, not numerous. Vitellaria extending in lateral fields to near acetabulum, confluent or not posterior to testes. Gastro-intestinal parasites of birds and mammals. This generic diagnosis is a modification of the one given by Yamaguti (1958).

So far more than 250 species (Kanve, 1994) have been reported under the genus *Echinoparyphium*. The Indian reports are limited to the descriptions of 9 species from their natural hosts by Verma (1936), Agarwal (1959), Rai (1962), Dube (1964), Nath (1973), Gupta and Jahan (1976) and Chishti and Tanveer (1992), and 3 species from experimental hosts by Singh (1975a, 1975b, 1976). Life cycles of 3 Indian species have been elucidated (Singh 1975a, 1975b, 1976).

A list of *Echinoparyphium* species reported from India is given here.

List of *Echinoparyphium* spp. reported from India

No.	Parasite	Host	Locality	Reference
1.	<i>Echinoparyphium recurvatum indiana</i> Verma, 1936	Common snipe, Brahmany duck	Uttar Pradesh	Verma (1936)
2.	<i>E. longicirrus</i> (Verma, 1936) Mehra, 1980	Mute Swan	Bihar	Verma (1936); Mehra (1980)
3.	<i>E. gizzardai</i> Verma, 1936	Black Swan	West Bengal	Verma (1936)
4.	<i>E. dollfusi</i> Agarwal, 1959	<i>Anhinga melanogaster</i>	Madhya Pradesh	Agarwal (1959)
5.	<i>E. indicum</i> Rai, 1962	<i>Neophron percnopterus</i>	Madhya Pradesh	Rai (1962)
6.	<i>E. clerici</i> Skryabin, 1915	<i>Anas platyrhynchos platyrhynchos domesticus</i>	Uttar Pradesh	Jain (1960); Dube (1964)
7.	<i>E. flexum</i> (Linton, 1892) Dietz, 1910	Albinorat; chick, pigeon; domestic duck	Uttar Pradesh	Nath (1973)
8.	<i>E. hymani</i> Singh, 1975	Ducklings	Madhya Pradesh	Singh (1975 a)
9.	<i>E. lanceolatum</i> Singh, 1975	Ducklings	Madhya Pradesh	Singh (1975 b)
10.	<i>E. vitellocompactum</i> Singh, 1976	Ducklings	Madhya Pradesh	Singh (1976)
11.	<i>E. magniovum</i> Gupta and Jahan, 1976	<i>Falco jugger</i>	Uttar Pradesh	Gupta and Jahan (1976)
12.	<i>E. kashmirensis</i> Chishti and Tanveer, 1992	<i>Gallus gallus domesticus</i>	Jammu and Kashmir	Chishti and Tanveer (1992)

Genus *Paryphostomum* Dietz, 1909

Dietz (1909) established the genus *Paryphostomum* to receive *P. radiatum* (Dujardin, 1845) as its type species. Subsequently, 10 species have been added to the genus by several workers all over the world (Yamaguti, 1971; Mehra, 1980; Srivastava, 1982). Mukherjee and Ghosh (1968) reviewed the status of the genus and synonymised it with *Echinostoma*, but Yamaguti (1971) revived the generic name *Paryphostomum*.

Life cycles of 3 species have been established: *Paryphostomum radiatum* by Johnston and Angel (1942), *P. segregatum* by Lutz (1924) and Lie and Basch (1967), and *P. giganticum*.

The characters of the genus *Paryphostomum* Dietz, 1909, as given by Mehra (1980), are: Body elongate, spinulate or unspinulate, small, under-middle sized to middle sized. Head collar reniform with 27-47 spines in single or double dorsally uninterrupted rows; 2-5 groups in each (corner) end group. Acetabulum large, near anterior extremity. Testes tandem, strongly lobed or branched, rosette-shaped, clover leaf-shaped, antler-shaped or of other typical type. Cirrus sac small, preacetabular or antero-dorsal to acetabulum. Seminal vesicle saccular, bipartite. Cirrus coiled when not protruded. Ovary about midway between acetabulum and anterior testis or

posterior testis. Uterus with a few or many coils. Vitellaria lateral, from acetabulum to posterior extremity, approaching mesially or becoming confluent in post-testicular region. Eggs a few or numerous. Excretory vesicle Y-shaped with long median stem bifurcating behind posterior testis. Parasitic in intestine of birds.

As far as is known, only 5 species have been reported from India. These are, *Paryphostomum testitriifolium* Gogate, 1934; *P. pentalobum* Verma, 1936; *P. horai* Baugh, 1950; *P. dollfusi* Agarwal, 1958 and *P. giganticum* Rai and Agarwal, 1961.

A list of *Paryphostomum* species known from India is given here.

A List of *Paryphostomum* spp. reported from India

No.	Parasite	Host	Locality	Reference
1.	<i>Paryphostomum pentalobum</i> Verma, 1936	Snipes	Uttar Pradesh,	Verma (1936)
2.	<i>P. horai</i> Baugh, 1950	<i>Anas poecilorhyncha</i>	Uttar Pradesh	Baugh (1950)
3.	<i>P. dollfusi</i> Agarwal, 1959	<i>Bubulcus ibis</i>	Madhya Pradesh	Agarwal (1959)
4.	<i>P. giganticum</i> Rai and Agarwal, 1961	<i>Porphyrio poliocephalus</i>	Madhya Pradesh	Rai and Agarwal (1961)
5.	<i>P. testitriifolium</i> Gogate, 1934	<i>Phalacrocorax niger</i>		Srivastava (1982)

MATERIALS AND METHODS

Collection and Maintenance of snail hosts

Freshwater snails belonging to 9 genera and 11 species, collected from their natural habitats such as paddy fields, ponds, rivulets and streams in Kasargod, Kannur, Kozhikode, Wayanad, Malappuram and Palakkad districts of Malabar, Kerala, were used during the course of this investigation. Malabar region of Kerala (Fig. 1) is situated in the western coast of India at its peninsular region (Lat. $10^{\circ} 20'$ N and $12^{\circ} 48'$ N and Long. $74^{\circ} 52'$ E and $76^{\circ} 54'$ E). Collections were made from March 1996 through May 1999. Different species of snails (Plates I and II) were collected by hand picking. A classified list of snails examined, with their locality, period of collection and number examined is presented in Table 2.

Table 2. List of host specimens examined

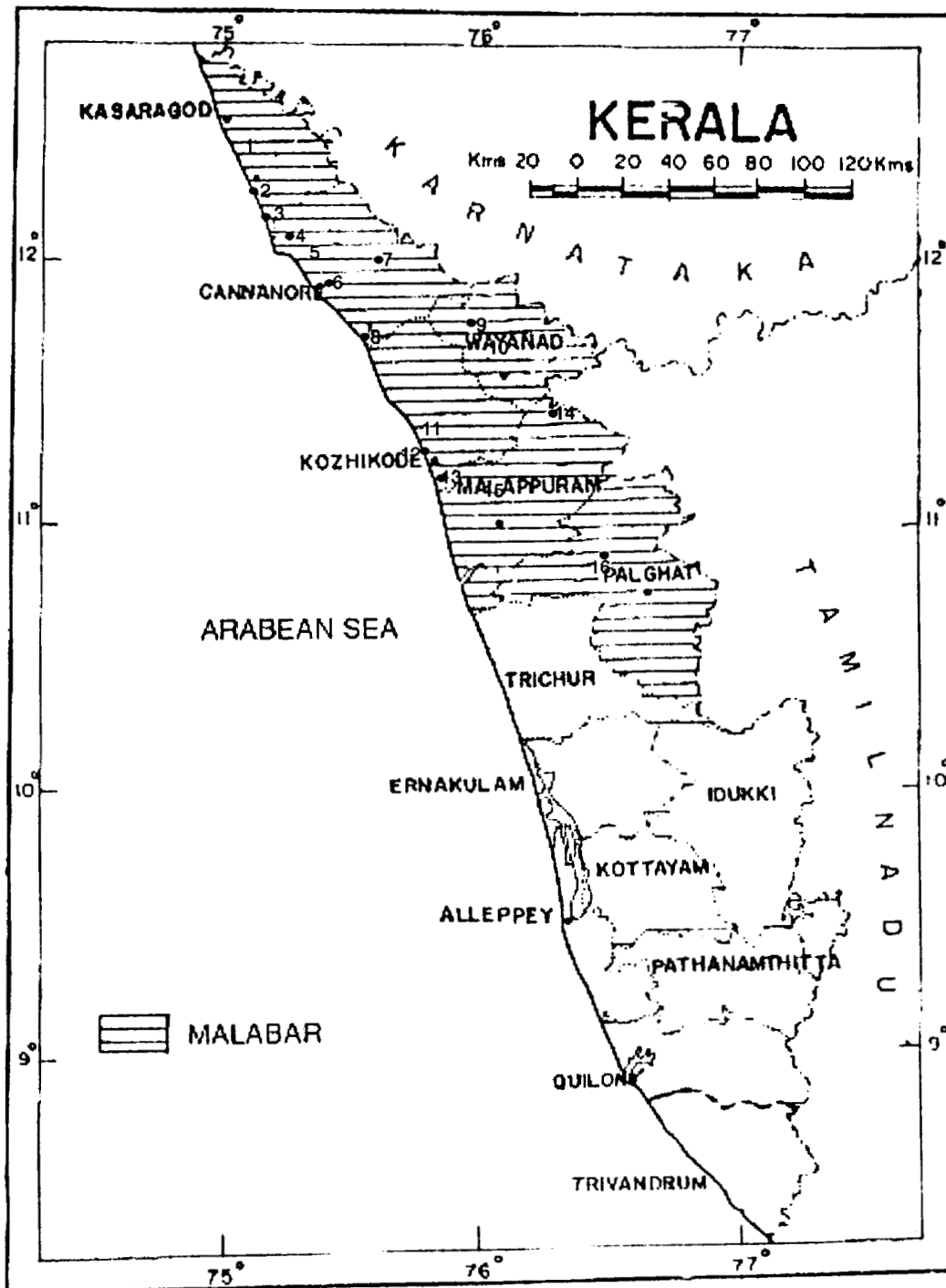
Classification and name of hosts	No. of host specimens examined	Locality of collection	Period of collection
1	2	3	4
Phylum : Mollusca Class : Gastropoda Subclass: Prosobranchia Order : Mesogastropoda Family : Viviparidae			
<i>Bellamyia bengalensis</i> (Lamarck, 1822)	138	Payangadi, Ezhome and Taliparamba in Kannur district; Chorode and Feroke in Kozhikode district; Nilambur in Malappuram district	May to October 1996; May to August 1997; July to November 1998; February 1999
<i>Bellamyia dissimilis</i> (Mueller, 1774)	3827	Payyanur, Cherukunnu and Thazhechovva in Kannur district; Kundayithode and Feroke in Kozhikode district; Idimuzhikkal, Kadakkattupara, Calicut University Campus and Kizhisseri in Malappuram district; Malampuzha in Palakkad district	March 1996 to December 1997; February and June 1998; October to December 1998; January to May 1999

1	2	3	4
Family: Pilidae			
<i>Pila virens</i> (Lamarck, 1822)	2230	Kanhangad and Trikaripur in Kasargod district; Payyanur, Cherukunnu, Payangadi, Thazhechovva, Thottada, Pinarayi, Kanhileri, Kuttimakool, Karetta, Koothuparamba and Mattanur in Kannur district; Chorode, Devagiri, Ramanattukara, Kundayithode and Feroke in Kozhikode district; Idimuzhikkal, Kadakkattupara, Nedugattumedu, Calicut University Campus, Nilambur and Kizhisseri in Malappuram district; Malampuzha in Palakkad district	June 1996 to October 1997; January to March 1998; October to December 1998; February and April 1999
Family: Bithyniidae			
<i>Digoniostoma pulchella</i> (Benson, 1836)	56	Sultan Bathery and Chundale in Wayanad district; Malampuzha in Palakkad district	March to May 1996; February and October 1997; May 1998
Family: Thiaridae			
<i>Thiara scabra</i> (Mueller, 1774)	670	Thottada and Pinarayi in Kannur district; Sultan Bathery in Wayanad district; Malampuzha in Palakkad district	July 1996 to August 1997; February to May 1998; March to May 1999

1	2	3	4
<i>Thiara tuberculata</i> (Mueller, 1774)	6165	Theerthankara and Cheruvathoor in Kasargod; Payyanur, Payangadi, Cherukunnu, Kanhileri and Kuttimakool in Kannur district; Sultan Bathery and Vythiri in Wayanad district; Malampuzha in Palakkad district	October to December 1996; February to May 1997; December 1997 to February 1998; October to December 1998; March to May 1999
<i>Sulcospira huegeli</i> (Philippi, 1841)	288	Karetta and Kelakam in Kannur district; Chundale in Wayanad district; Nilambur in Malappuram district; Malampuzha in Palakkad district	October and November 1997; January to March and December 1998
<i>Paludomous tanschauricus</i> (Gmelin, 1771)	3504	Kanhileri and Karetta in Kannur district; Chundale and Vythiri in Wayanad district; Kizhisseri and Nilambur in Malappuram district; Malampuzha in Palakkad district	March and April 1996; April 1998; July to September 1998; January to March 1999
Subclass: Pulmonata			
Order: Basommatophora			
Family: Lymnaeidae			
<i>Lymnaea luteola</i> (Lamarck, 1822)	2850	Kanhangad and Trikaripur in Kasargod district; Payyanur, Payangadi, Cherukunnu, Thazhechovva, Thottada and Pinarayi in Kannur district; Chorode, Feroke, Ramanattukara and Kundayithode in Kozhikode district; Sultan Bathery,	March and April 1996; July 1996 to October 1997; January to June 1998; November and December 1998; February to May 1999

1	2	3	4
		Vythiri and Mananthavady in Wayanad district; Idimuzhikkal, Kadakkattupara, Olippuramkadavu, Nedugattumedu, Calicut University Campus, Nilambur and Kizhisseri in Malappuram district; Malampuzha in P'alakkad district	
Family : Planorbidae			
<i>Indoplanorbis exustus</i> (Deshayes, 1834)	4056	Theerthankara, Kanhangad and Trikaripur in Kasargod district; Payyanur, Cherukunnu, Thazhechovva, Melechovva, Kanhileri and Kuttimakool in Kannur district; Feroke, Ramanattukara, Kundayithode and Kadalundi in Kozhikode district; Sultan Bathery, Vythiri, Mananthavady and Chundale in Wayanad district; Idimuzhikkal, Olippuramkadavu, Kadakkattupara, Nedugattumedu, Calicut University Campus, Kizhisseri in Malappuram district; Malampuzha in Palakkad district	March 1996 to December 1997; February and March 1998; August to November 1998; January to April 1999
<i>Gyraulus convexiusculus</i> (Hutton, 1849)	12	Mattanur in Kannur district; Malampuzha in Palakkad district	November 1998; March and May 1999

Fig. 1 Important Collection Sites



1. Kanhangad
2. Trikaripur
3. Payyannur
4. Pazhayangadi
5. Thazhechovva
6. Thalassery
7. Mattannur
8. Chorode

9. Sulthan Bathery
10. Vythiri
11. Ramanattukara
12. Feroke
13. Calicut University Campus
14. Nilambur
15. Kizhisseri
16. Malampuzha



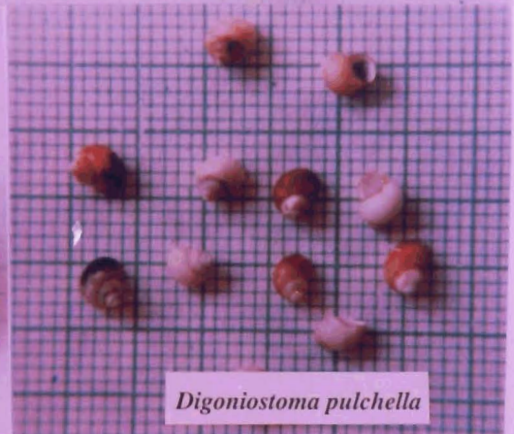
Bellamyia bengalensis



Bellamyia dissimilis



Pila virens



Digoniostoma pulchella



Thiara scabra



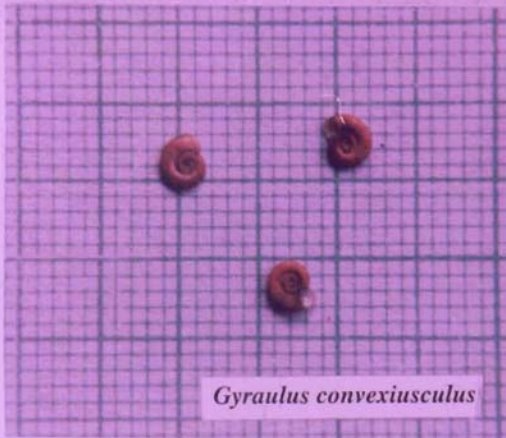
Thiara tuberculata



Sulcospira huegeli



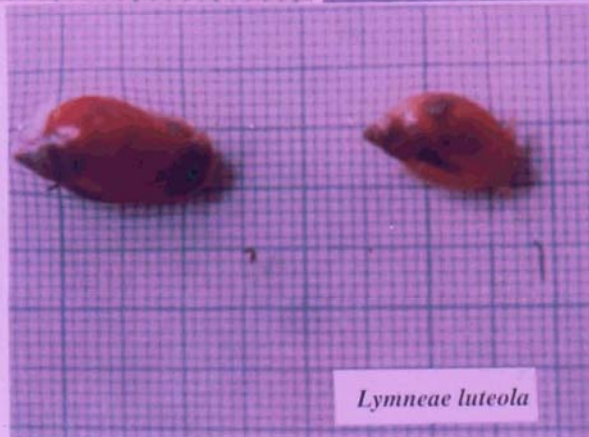
Indoplanorbis exustus



Gyraulus convexiusculus



Paludomous tanschauricus



Lymnaea luteola

The snails were brought alive to the laboratory in suitable containers and kept in groups of 2-10 in beakers containing well-water. The mouths of the beakers were covered with nylon 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 occasionally with biscuit crumbs and/or aquatic plants. As far as possible, the natural habitat was provided for the snails. The water in the beakers was checked daily for cercariae, and on detection of cercariae the snails which shed cercariae were isolated and individually kept in separate beakers.

Study of cercaria

Fresh and naturally emerged cercariae were used for detailed studies on their morphology and behaviour. To study the swimming movements, the cercariae were transferred to a watch glass containing water and observed under a binocular dissecting microscope. Viability of cercariae was determined by keeping them at room temperature (26° – 28°C). The cercariae were transferred on to a glass slide containing a few drops of water, stained supravitaly with neutral red or Nile blue sulphate, covered with a No.1 cover glass and observed under a Leitz Diaplan phase-contrast microscope to study their structural details. Gradual removal of water from beneath the cover glass with a piece of blotting paper inhibited vigorous

movements of the larvae and made their internal organization more clear. The use of stain mixture containing equal proportions of neutral red and Nile blue sulphate 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 (0.1%). 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 dilute Nile blue sulphate in 0.67% saline and refrigerated for 30 minutes. Cercariae placed in 1% urea solution also helped the study of excretory system. Genital primordia were observed in lacto-aceto-carmin-stained cercariae. Permanent mounts of cercariae were prepared after staining them in alum carmine, following the procedure outlined by Cantwell (1981).

Study of intramolluscan stages

The snails, which shed cercariae were later dissected, their body tissues macerated, smeared on slides and examined for intramolluscan stages of development of cercariae. The stages were studied under a microscope with or without vital staining. Permanent preparations of the stages were made by staining them with alum carmine.

Studies on life cycles

Cercariae emerged from naturally infected snails were used in experimental infection studies. Natural habitats of infected snails were searched for second intermediate hosts. The host snails, other snails of the habitat, freshwater fishes, tadpoles (of *Rana tigrina*, *R. cyanophlyctis* and *Bufo bufo*) and crabs (*Paratelphusa hydrodromous* and *Sesarma quadratum*) were used as experimental second intermediate hosts. The uninfected hosts were exposed to freshly released cercariae, and examined at intervals to follow the development of metacercariae. The metacercariae were studied alive with or without vital staining.

A few species of animals were used as experimental definitive hosts. These included one-day-old white leg-horn chicks bought from Government Poultry Farm at Mundayad in Kannur and Regional Poultry Farm at Chathamangalam in Kozhikode, and about one-month-old albino-rat and mice received from the Department of Life Science, University of Calicut. The experimental hosts were force-fed with metacercariae obtained from snails, fishes or tadpoles and kept in individual cages under adequate diet. The host specimens were dissected and examined at various intervals to follow the development of adult flukes. Faecal examination of a few experimental hosts was done regularly to determine the prepatent period of

flukes, and once this was determined, the hosts were autopsied to recover the gravid flukes. Flukes recovered were mounted on slides and studied under a phase-contrast microscope. Permanent slides of the flukes were made by staining them in alum carmine.

The gravid flukes, collected from experimental hosts, were used to obtain eggs by tearing uterus. The eggs were maintained in water at room temperature (26° to 28°C) to study the development and release of miracidia.

The miracidia were studied alive after staining them with vital dyes. Silver impregnation technique of Lynch (1933) was very useful to study the number and pattern of arrangement of epidermal plates. In order to establish the life cycles experimentally, laboratory-raised clean host snails were exposed to miracidia and examined at various intervals to study the intramolluscan stages of development of the parasite. The cercariae shed by the snails were compared with those shed by the same snail species infected naturally.

Natural definitive hosts

Birds such as jungle crow (*Corvus macrorhychos*), house crow (*Corvus splendens*), pond heron (*Ardeola grayii*), cattle egret (*Bubulcus ibis*), whitebreasted waterhen (*Amaurornis phoenicurus*) and blue kingfisher

(*Alcedo atthis*), collected from different localities, were examined for adult echinostomes. Only *A. phoenicurus* was found to be positive for adult flukes.

Measurements and sketches

Measurements were made with the aid of a calibrated ocular micrometer. At least 20 specimens were measured from each stage of the life cycle. Measurements are in micrometres (μm); the range is followed by the mean values in parantheses. Sketches were made with the aid of a prism type camera lucida and details added free hand from observations made on live specimens.

RESULTS

Echinostoma revolutum (Frölich, 1802) Dietz, 1909

Cercaria (Figs. 2a-b; Table 3)

The characters of the present cercaria agreed fully with the descriptions of the cercaria of the *Echinostoma revolutum* given by Patnaik and Rai (1966), Mohandas (1973, 1981), Mukherjee (1992) and Kanev (1994). All the life cycles stages of this fluke have been established in the laboratory. The stages were identical with those reported by Patnaik and Rai (1966) Mohandas (1973) and Kanev (1994). Brief descriptions of the different stages in the life cycle of the worm are presented here.

Forty-three out of 627 *Lymnaea luteola*, collected from Kanhangad in Kasargod, Payyanur in Kannur, Chorode, Feroke and Ramanattukara in Kozhikode and Malampuzha in Palakkad districts of Kerala, were infected by the cercariae of *Echinostoma revolutum*. The infection was prevalent throughout the year.

Behaviour: Cercariae emerged from snails during the morning hours. They were negatively phototactic and remained alive for about 8 h in well-water.

Description: Body elongate-ovoid, spinose, measured 312-402 (365) x 150-176 (161) in size. Collar distinct, 130-149 (138) wide, with 37 spines, 8.5-11.8 (10.2) long. Spine arrangement characteristic: five corner spines on each

ventral lappet, 3 oral and 2 aboral; six laterals on each side in single row; 15 dorsals, 8 oral and 7 aboral. Oral sucker oval, measured 39-53 (47) x 42-55 (51) in size. Acetabulum post-equatorial, protrusible, measured 66-83 (74) in diameter. Tail aspinose, with 8-10 sensory hairs at anterior half; measured 426-478 (458) x 61-86 (72). Tip of tail finger-like, capable of independent contraction and expansion. Tail finfolds 7 in number, 2 dorsal, 2 ventral, 2 small ventro-lateral and one very small ventral just anterior to narrow tip.

Prepharynx 26-33 (31) long, with a prepharyngeal body. Pharynx oval, muscular, 27-31 (29) x 21-26 (24) in size. Oesophagus solid, 90-112 (107) long; consisting of 7 cells. Caeca reach to anterior margin of excretory bladder; each caecum with 7 cells and a median cell at the point of bifurcation.

Penetration glands 4 pairs, along oesophagus, opening on dorsal lip of oral sucker. Paraoesophageal gland-cells present; 14-16 open around oral sucker and 4-6 open in pharyngeal region. Cystogenous glands numerous throughout body, contain ovoid bodies.

Genital primordia represented by two masses of cells, one at anterior margin of acetabulum, the other between acetabulum and base of tail, and connected by a string of cells passing dorsal to acetabulum. Excretory system stenostomate; excretory bladder bipartite, at posterior end of body; main collecting ducts distended between collar and acetabulum, each containing 50-80 excretory concretions, upto 11 in diameter. Caudal excretory duct extends

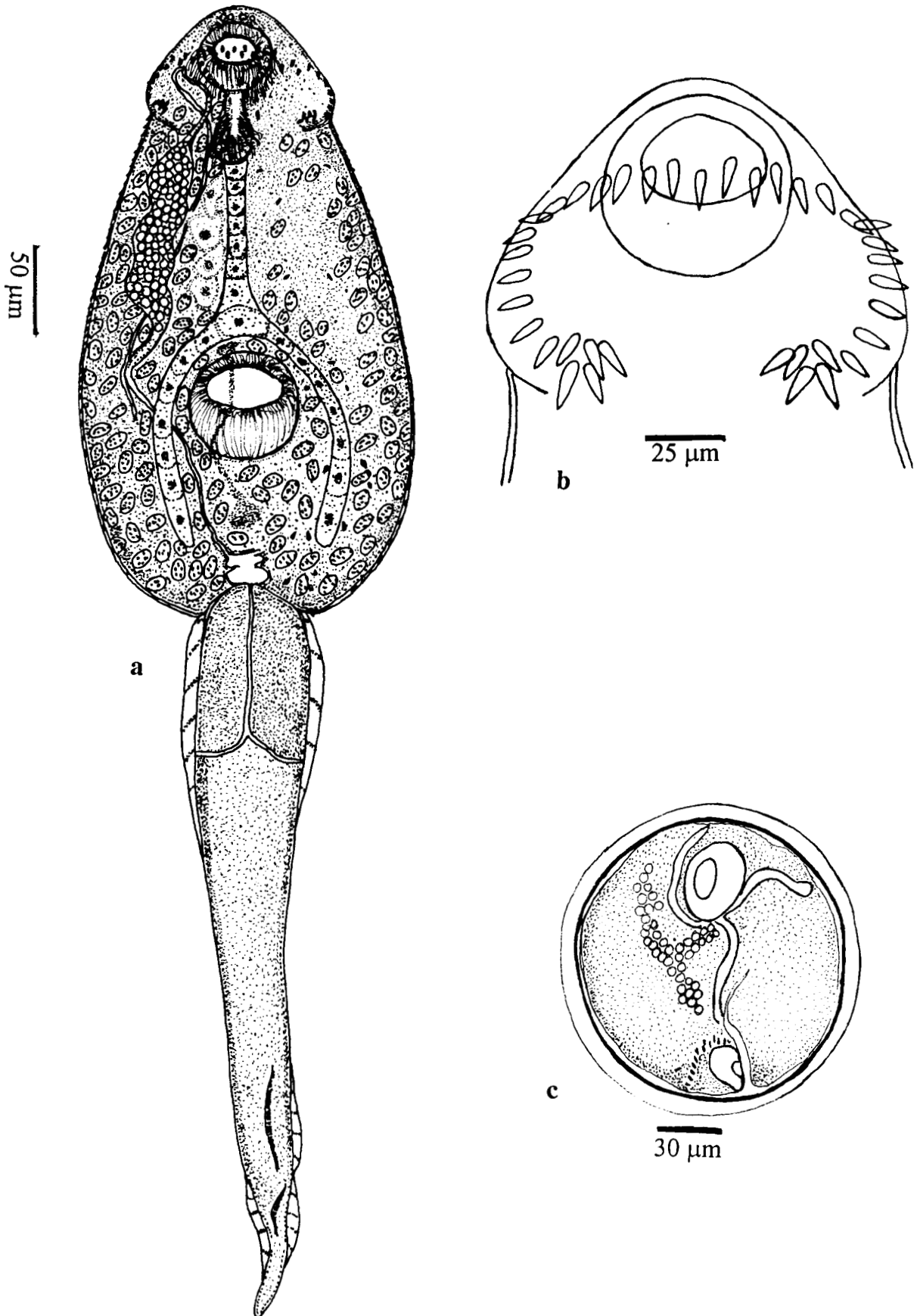


Fig. 2. *Echinostoma revolutum* (Frölich, 1802) Dietz, 1909.
a. Cercaria; b. Collar with collar spines; c. Encysted metacercaria.

Table 3. Measurements of cercariae of *Echinostoma revolutum* (Frölich, 1802) Dietz, 1909

Character	Range	Mean
Body	312-402 x 150-176	365 x 161
Collar width	130-149	138
Collar spines (length)	8.5-11.8	10.2
Oral sucker	39-53 x 42-55	47 x 51
Acetabulum	66-83	74
Tail	426-478 x 61-86	458 x 72
Prepharynx (length)	26-33	31
Pharynx	27-31 x 21-26	29 x 24
Oesophagus (length)	90-112	107

to anterior fifth of tail, then bifurcates into two lateral branches. Flame cell formula: $2[(3+3+3)+(3+3+3+3)] = 42$.

Life Cycle

Metacercaria (Fig. 2c)

Natural infections by metacercariae were found in the hepatopancreas, mantle cavity, kidney and gonad of *Lymnaea luteola*, *Bellamya dissimilis* and *Indoplanorbis exustus*.

Cysts spherical, measured 137-163 (154) in diameter. Cyst wall double-layered, consisting of an outer, transparent layer, 9.8-12.3 thick and an inner, opaque layer, 2.6-4.1 thick. Metacercaria remains folded inside the cyst cavity. Internal structures like collar spines, suckers, excretory concretions and parts of caeca visible through the cyst wall. Development of the metacercaria was followed in one-day-old white leg-horn chicks.

Adult (Fig. 3a; Table 4)

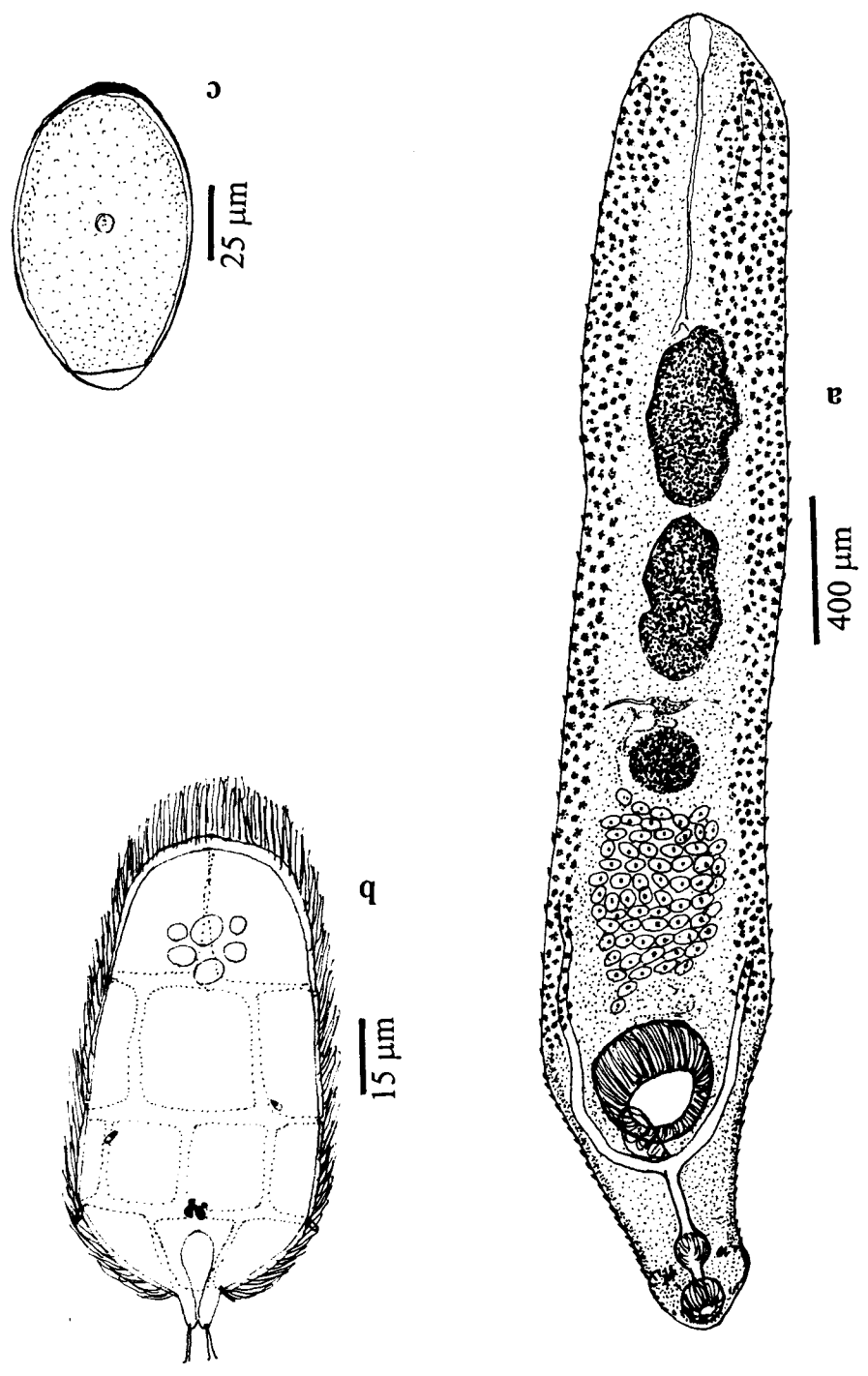
Mature adults were recovered from posterior ileum, caecum and rectum of chicks 10 days post-exposure to metacercariae. Flukes measured 2280-3560 (2612) x 464-543 (520). Body spinose; dorsally the spines confined to pre-acetabular region and arranged in transverse rows. Ventral and lateral spines extended posteriorly to halfway between posterior testis and posterior end of body, often becoming sparse. Collar well-developed, 291-356 (320) wide, with 37 conspicuous spines, measuring 59-92 (77) x 10-13 (11.5) in size. Spine

arrangement similar to that of cercaria. Oral sucker oval, 156-173 (166) x 149-169 (158) in size. Acetabulum measured 358-432 (388) x 342-461 (405). Prepharynx 186-243 (228) long; pharynx muscular 169-192 (183) in diameter; oesophagus 226-341 (293) long and bifurcates anterior to acetabulum; caeca 760-2130 (1766) long, extend to posterior extremity. Testes tandem, ovoid, slightly lobate, in middle of hind body; anterior testis measured 330-610 (518) x 266-586 (498); posterior testis 396-700 (641) x 237-519 (427). Cirrus sac club-shaped, extends postero-dorsally from genital atrium to middle of acetabulum; seminal vesicle coiled; pars prostatica poorly developed; cirrus unspined. Genital pore median, pre-acetabular, followed by genital atrium. Ovary round to ovoid, at anterior half of body, 180-510 (376) in size. Ootype with well developed Mehli's gland complex. Laurer's canal and uterine seminal receptacle present. Uterus intercaecal, pre-testicular, with 7-13 coils containing numerous eggs. Vitellaria follicular, lateral, extending from post-acetabular level to caecal ends. Excretory bladder tubular and coiled, opens out at posterior end of body.

Egg (Fig. 3c)

Eggs ovoid, operculate, yellow-brown, measured 76-106 (93) x 67-78 (71) in size.

Fig. 3. *Echinostoma revolutum* (Frölich, 1802) Dietz, 1909.
a. Mature adult; b. Miracidium; c. Egg.



**Table 4. Measurements of adult of *Echinostoma revolutum*
(Frolich, 1802) Dietz,1909**

Character	Range	Mean
Body	2280-3560 x 464-543	2612 x 520
Collar width	291-356	320
Collar spines	59-92 x 10-13	77 x 11.5
Oral sucker	156-173 x 149-169	166 x 158
Acetabulum	358-432 x 342-461	388 x 405
Prepharynx (length)	186-243	228
Pharynx	169-192	183
Oesophagus (length)	226-341	293
Caeca (length)	760-2130	1766
Testis (Anterior)	330-610 x 266-586	518 x 498
Testis (Posterior)	396-700 x 237-519	641 x 427
Ovary	180-510	376
Egg	76-106 x 67-78	93-71

Miracidium (Fig. 3b)

Miracidia, fixed in 2% silver nitrate, measured 72-88 (78) x 43-70 (60). Apical papilla with 2 pairs of setae. Body covered with ciliated epidermal plates arranged in 6,6,4,2 pattern. Paired lateral processes, eyespots and flame cells present. Germinal cells scattered at the posterior half of miracidium.

Sporocyst (Fig. 4a)

Sporocysts were recovered from heart and kidney of infected snails. Body elongated, colourless, sac-like, measured 344-691 (560) x 92-267 (154).

Redia (Figs. 4b-c)

Mother rediae found in kidney were motile, elongate, pale-yellow, with terminal mouth, conspicuous collar and locomotor organs. Fully developed rediae measured 392-977 (822) x 112-266 (190), contained 3-12 daughter rediae and a few germ balls.

Daughter rediae were found in hepatopancreas, kidney, intestine and gonad. They were motile, elongate, yellow-brown, measured 911-2200 (1650) x 216-490 (378) in size. Fully grown daughter rediae contained developing cercariae and many germ balls.

Natural definite host	:	Could not be recovered
Experimental host	:	White leg-horn chicks
Location in the host	:	Posterior ileum, caecum and rectum

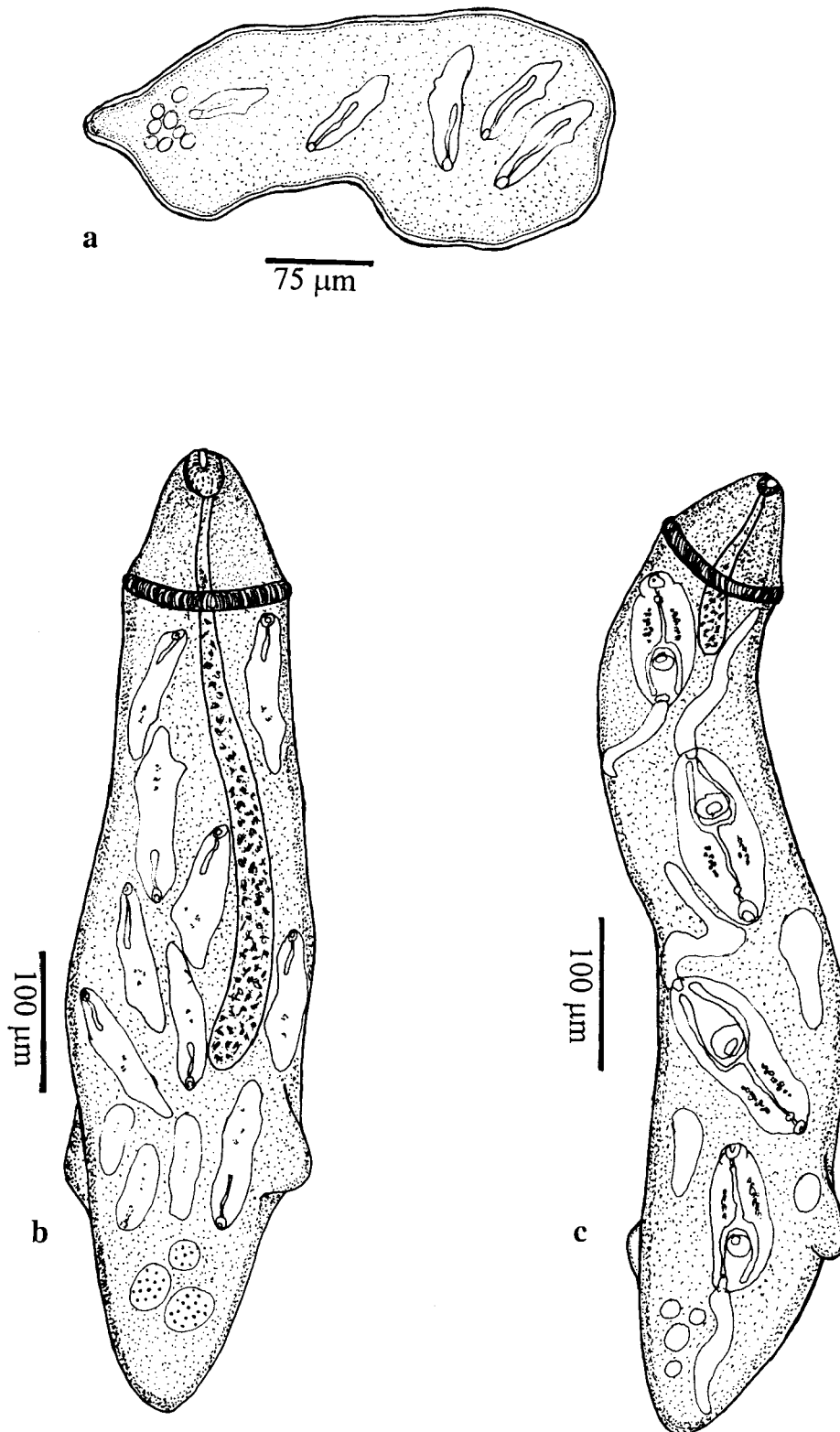


Fig. 4. *Echinostoma revolutum* (Frolich, 1802) Dietz, 1909.
 a. Sporocyst; b. Mother redia with daughter rediae and germ balls;
 c. Daughter redia with cercariae and germ balls.

First intermediate host	:	<i>Lymnaea luteola</i>
Locality	:	Kanhangad in Kasargod district, Payyanur in Kannur district, Chorode, Feroke and Ramanattukara in Kozhikode district and Malampuzha in Palakkad district.
Period of Collection	:	July and August 1996, November 1996 to April 1997, June to October 1997, December 1997 to February 1998 and April to August 1998.
Prevalence	:	2.28% (65 out of 2850 were infected)
Second intermediate host	:	<i>L. luteola</i> , <i>Bellamya dissimilis</i> and <i>Indoplanorbis exustus</i> ,

Remarks

The present day knowledge of *Echinostoma revolutum* (Frölich, 1802) Dietz, 1909 is based mainly on descriptions and illustrations published by several authors. Of all the published descriptions, the most important are by Johnson (1920), Lutz (1924) Beaver (1937), Mendheim (1940, 1943), Supperer (1959), Skryabin (1947, 1956), Bisseru (1953, 1967), Yamaguti (1975), Odening (1964), Patnaik and Ray (1966), Mc Donald (1981), Kosupko (1972), Moravec *et al.* (1974) and Nasinkova (1986). These authors gave conflicting information on the validity, identity, synonymy, morphology, life history, hosts and distribution of adults and larvae described by them as *E. revolutum*. The matter has been resolved by the comparative experimental studies on the life cycle of *E. revolutum* by Kanev (1994), beginning with infected snails from the type-locality, near Erlangen, Germany. Based on the extensive studies he concluded that lymnaeid snails serve as first intermediate host, various pulmonate and

prosobranch snails, mussels, frogs and freshwater turtles as second intermediate hosts and birds as the final host. Further, the cercariae and adults have 37 collar spines and the species occurs only in Europe and Asia.

Based on extensive studies on adult and larval morphology and biology, Kanev (1994) synonymised *E.audyi* Lie and Umathevy, 1965 (from Southeast Asia), *E. paraulum* Dietz, 1909 (from Europe and Asia) and *E. ivaniosi* Mohandas, 1973 (from India) with *E. revolutum*. He preferred to include all the worms with 37 collar spines under the genus *Echinostoma* and treat all those occurring in naturally infected birds in Europe and Asia as belonging to the “*E. revolutum* group”.

Results of the experimental life cycle studies beginning with 37-spined echinostome cercariae from *Lymnaea luteola*, (also the first intermediate host of *E. ivaniosi*) presented here show that the cercaria under study resembles the cercaria of *E. ivaniosi* in the following characters: distribution of body spines, arrangement of collar spines, number of cells in oesophagus and caecum, nature of cystogenous material, number and position of penetration glands, number of flame cells and disposition of setae. Therefore, I am sure that the present cercaria recovered from *L. luteola* in North Kerala and that of *E. ivaniosi* Mohandas, 1973 infecting the same snail species in South Kerala are identical. Besides, the other larval stages and adults of the present worm completely correspond with those reported for *E. ivaniosi* by Mohandas (1973).

A comparative study of the morphology and biology of the present worm (or of *E. ivaniosi*) with descriptions and illustrations of *E. revolutum* presented by Kanev (1994) shows that the present form and *E. ivaniosi* are identical with *E. revolutum*, and the decision of Kanev to treat *E. ivaniosi* as a synonym of *E. revolutum* is valid. I would agree with this synonymity and report the present fluke as *Echinostoma revolutum* (Frölich, 1802) Dietz, 1909.

Echinostoma sp.I n. sp.

Cercaria (Figs. 5a-b; Table 5)

Natural infections by the cercariae were found in the apple snail, *Pila virens* collected from Kanhangad in Kasargod, Cherukunnu, Payangadi and Pinarayi in Kannur, Chorode, Ramanattukara and Kundayithode in Kozhikode and Idimuzhikkal and Nilambur in Malappuram districts of Kerala. Infections were prevalent throughout the year, especially common during the monsoon period (June-September) when 27% of the snails examined were infected.

Behaviour: Cercariae were found emerging from snails in moderate numbers during the morning hours. They showed photopositivity and accumulated in large numbers on the brighter side of the container. They exhibited typical wobble-like motion with constant flexion and extension of tail. The cercariae performed leech-like movements on a glass slide with a thin film of water or at the bottom of the container. They remained alive for about 12 h in well-water.

Description: Body elongate oval, spinose, with 8 pairs of sensory hairs at anterior half; measured 355-490 (412) x 132-290 (212). Collar distinct, 74-119 (87) wide, with 37-spines, 8.5-10.5 (9.8) in length. Spine arrangement characteristic: five corner spines on each ventral lappet, 3 oral and 2 aboral; 6 laterals on each side in single row; 15 dorsals, 8 oral and 7 aboral. Oral sucker roughly oval, measured 44-71 (56) x 41-65 (54). Acetabulum post-equatorial, protrusible, 65-89 (75) in diameter. Tail cylindrical, aspinose, set in a concavity at the posterior end of body; measured 327-550 (482) x 66-93 (70), with 2 dorsal, 2 ventral, 2 ventro-lateral and one ventral finfolds. At anterior half, 12-14 sensory hairs present. Tip of tail finger-like, capable of independent contraction and expansion.

Prepharynx 18-25 (22) long, with a small glandular prepharyngeal body. Pharynx muscular, 31-43 (36) x 17-22 (19) in size. Oesophagus solid, 142-176 (161) long, consisting of 9-10 cells. Intestinal caeca solid, 231-249 (235) long; each caecum composed of 10 cells and an additional cell at the point of bifurcation.

Penetration glands 4 pairs, lobate, along oesophagus; gland ducts open on dorsal lip of oral sucker in 2 pairs. Paraoesophageal gland-cells 16-18 in number, with duct outlets around oral sucker and pharynx. Cystogenous glands numerous, densely distributed throughout body, and filled with rod-shaped contents.

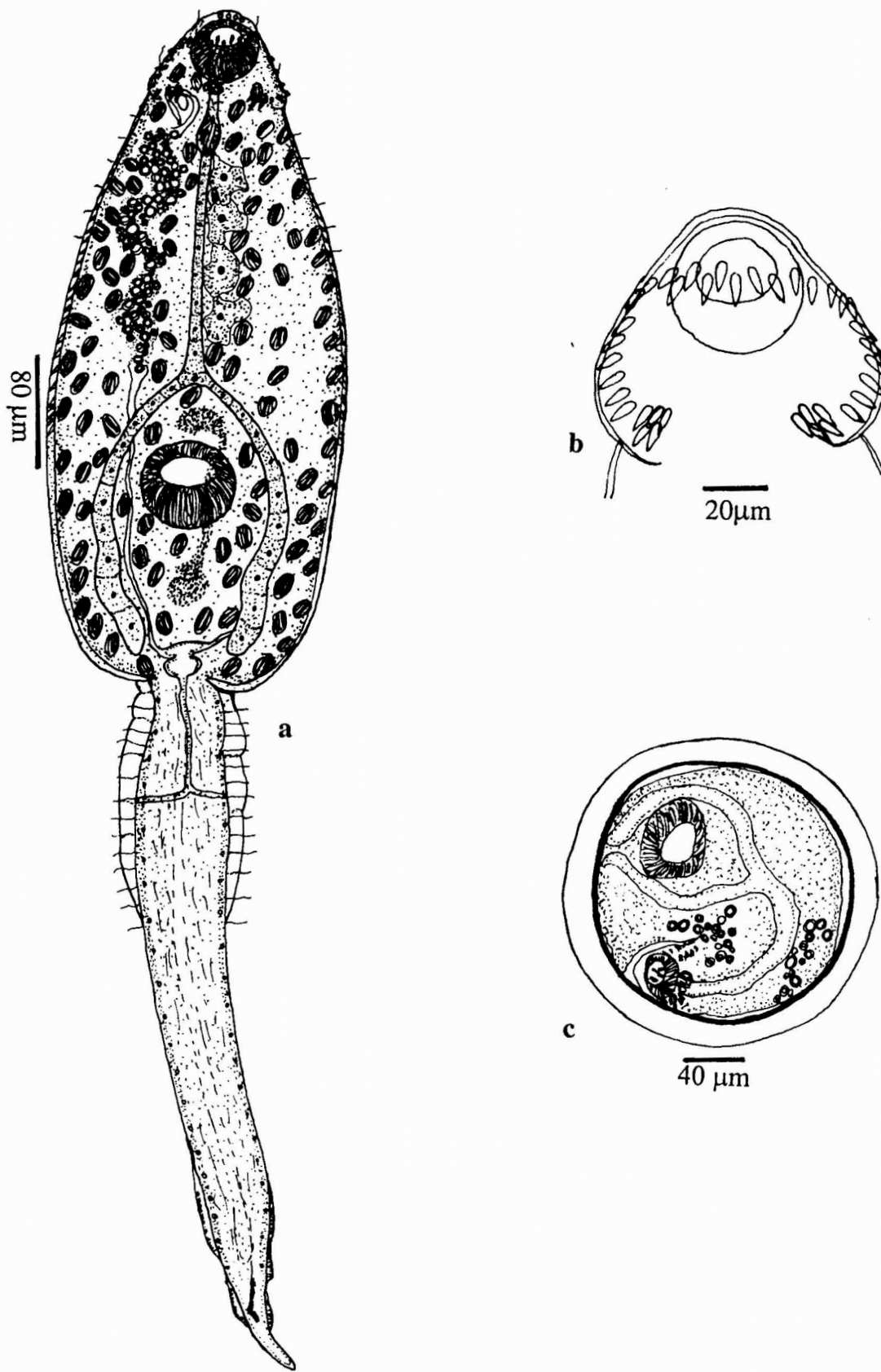


Fig. 5. *Echinostoma* sp. I n. sp.
a. Cercaria; b. Collar with collar spines; c. Encysted metacercaria.

Table 5. Measurements of cercariae of *Echinostoma* sp. I n. sp.

Characters	Range	Mean
Body	355-490 x 132-290	412 x 212
Collar width	74-119	87
Collar spines (length)	8.5-10.5	9.8
Oral sucker	44-71 x 41-65	56 x 54
Acetabulum	65-89	75
Tail	327-550 x 66-93	482 x 70
Prepharynx (length)	18-25	22
Pharynx	31-43 x 17-22	36 x 19
Oesophagus (length)	142-176	161
Caeca (length)	231-249	235

Genital primordia consist of two masses of cells, one at the pre-acetabular region, the other between acetabulum and base of tail and connected by a string of cells passing dorsal to acetabulum. Excretory system stenostomate; excretory bladder bipartite, at posterior end of body; main collecting ducts distended between collar and acetabulum, each containing 60-90 excretory granules. Caudal excretory duct extends to anterior fifth of tail, then bifurcates into two lateral branches. Flame cell formula: $2[(3+3+3) + (3+3+3)] = 36$.

Life Cycle

Metacercaria (Figs. 5c; 6)

The cercariae encysted in the auricle of *Bellamya dissimilis* and hepatopancreas of *Indoplanorbis exustus* and *Lymnaea luteola*. Occasionally, a few metacercariae were found in the kidney and hepatopancreas of the host snail, *P. virens*. They also encysted on gill arches of tadpoles and fishes. Under experimental conditions, metacercariae were obtained from these hosts.

Description: Cyst spherical, 192-204 (198) in diameter. Cyst wall three-layered, 13-15 thick; outer, thin, transparent layer, 0.7-2 thick; inner, translucent layer, 3-4 thick and middle, transparent layer 7-9 thick. The cysts recovered from gill arches of fishes and tadpoles were without the outer layer. Inside the cyst the larva remained curled up. Oral sucker, acetabulum, collar and collar spines, pharynx, oesophagus and caeca visible through cyst wall. Excysted metacercaria measured 427-635 (547) x 177-332 (294).

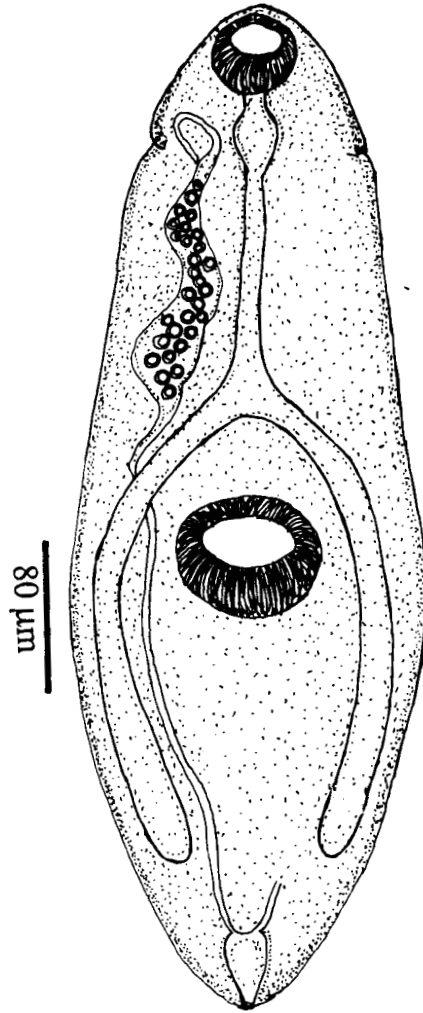


Fig. 6. *Echinostoma* sp. I n. sp.
Excysted metacercaria.

Metacercariae recovered from snails 4 months after exposure to cercariae were viable, and chicks fed with these metacercariae developed adults in the posterior region of ileum and rectum.

Adult (Figs. 7a-b, 8a-b; Table 6)

In laboratory infections, adult worms were found in the posterior half of ileum and rectum of white leg-horn chicks and albino-rats. Development of the flukes was followed in chicks.

The 3-day-old flukes long, slender maximally wide at acetabular region, measured 966-1540 (1214) x 290-385 (346), and occupied the middle region of ileum. The suckers increased in size; acetabulum moved from the equator to a more anterior position. Primordia of testes and ovary visible. Differentiating cirrus sac traceable between caecal bifurcation and acetabulum.

The 6-day-old specimens 3070-3840 (3327) long 392-629 (520) wide, with narrow pre-acetabular and broad post-acetabular regions. Cirrus sac became prominent, but its component parts not distinguishable. Ovary and testes increased in size; uterus became complexly coiled, without any eggs. Vitelline follicles developed in lateral fields below the level of acetabulum; the main collecting ducts were not visible.

The flukes recovered on the 9th day were fully mature, most of them occurring in posterior ileum and rectum. Body slender, elongate, 6120-7266 (6860) x 950-1310 (1196) in size, attaining maximum width at acetabular region.

Pre-acetabular body slender, tapering; post-acetabular body broad, uniformly wide. Body spines posterior to collar differing in distribution on dorsal, ventral and lateral surfaces. Dorsal spines scale-like, arranged in transverse rows and confined to pre-acetabular region; ventral and lateral spines slender, long, extend posteriorly to half way between posterior testis and posterior extremity of body, often becoming sparse.

Collar well-developed, 520-565 (534) wide, with 37 collar spines, measuring 44-59 (52) x 9-13 (12). Spine arrangement similar to that in cercaria. Latero-aboral of corner group the largest (59 long) of all collar spines and inner-oral of corner group and latero-oral of dorsal spines the smallest (44 long). Oral sucker subterminal, roughly round, 176-223 (209) in diameter. Acetabulum in anterior third of body, 451-566 (493) x 476-548 (510) in size.

Prepharynx 62-79 (67) long; pharynx muscular, oval, measured 161-190 (170) x 120-147 (138). Oesophagus 402-510 (465) long; bifurcating in front of acetabulum; caeca almost reaching posterior end of body.

Testes tandem, intercaecal, spherical or ovoid, smooth, in posterior half of body. Anterior testis 420-516 (497) x 481-627 (458); posterior testis 506-648 (592) x 428-579 (539). Cirrus sac ovoid, 231-332 (249) long, 296-393 (373) wide, extending to mid-region of acetabulum, containing coiled seminal vesicle, well developed pars prostatica and unspined cirrus. Genital pore pre-acetabular, followed by genital atrium.

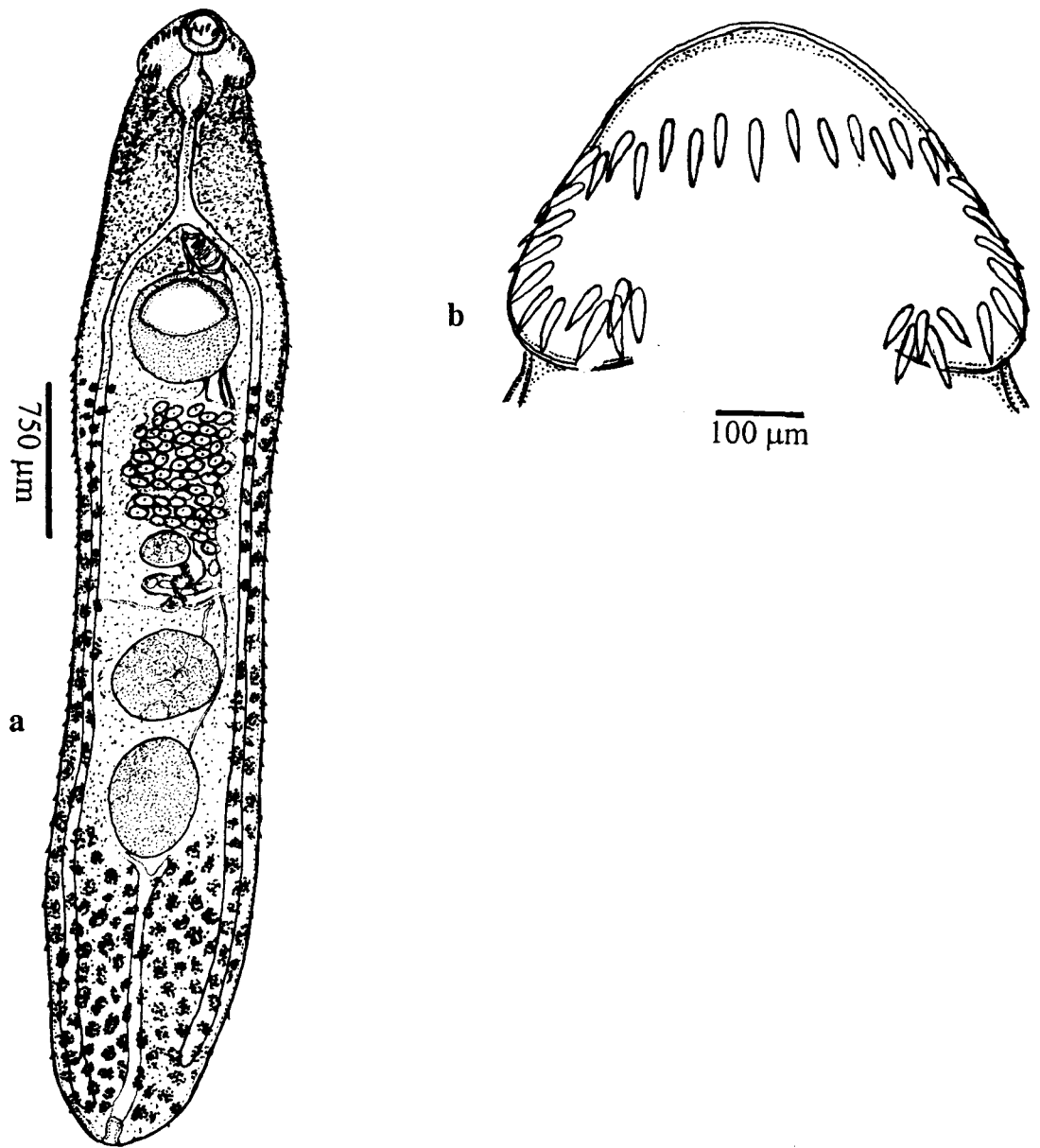


Fig. 8. *Echinostoma* sp. I n. sp.
a. Mature adult; b. Collar with collar spines.

Ovary pre-testicular, near equator, elliptical, 179-202 (194) x 230-271 (242) in size. Mehli's gland complex between ovary and anterior testis with dilated ovicapt and ciliated oviduct. Laurer's canal opening medially on dorsal surface. Uterine seminal receptacle filled with masses of sperms. Small vitelline reservoir present. Uterus intercaecal, pre-testicular, with 8-10 coils containing 16-80 eggs and connected to genital atrium through metraterm. Vitellaria follicular, lateral, overlapping caeca, extending from just behind acetabulum to posterior end of body, rarely confluent in post-testicular region.

Excretory bladder with small branches; stem more or less coiled before opening at posterior extremity.

Egg (Figs. 9a-b)

Eggs usually appear in faeces of chicks and rats 9 days after infection by metacercariae. Eggs ovoid, yellow-brown, with inconspicuous operculum at narrow end and shell thickening with striated appearance at opposite end; measured 106-128 (118) x 63-77 (71). Eggs kept in well-water at room temperature (27° - 28°C) began to hatch in 14 days. Exposure to light stimulated hatching.

Miracidium (Figs. 9c-d)

Newly hatched miracidia swim rapidly in water, but later show creeping movements on the bottom of the container.

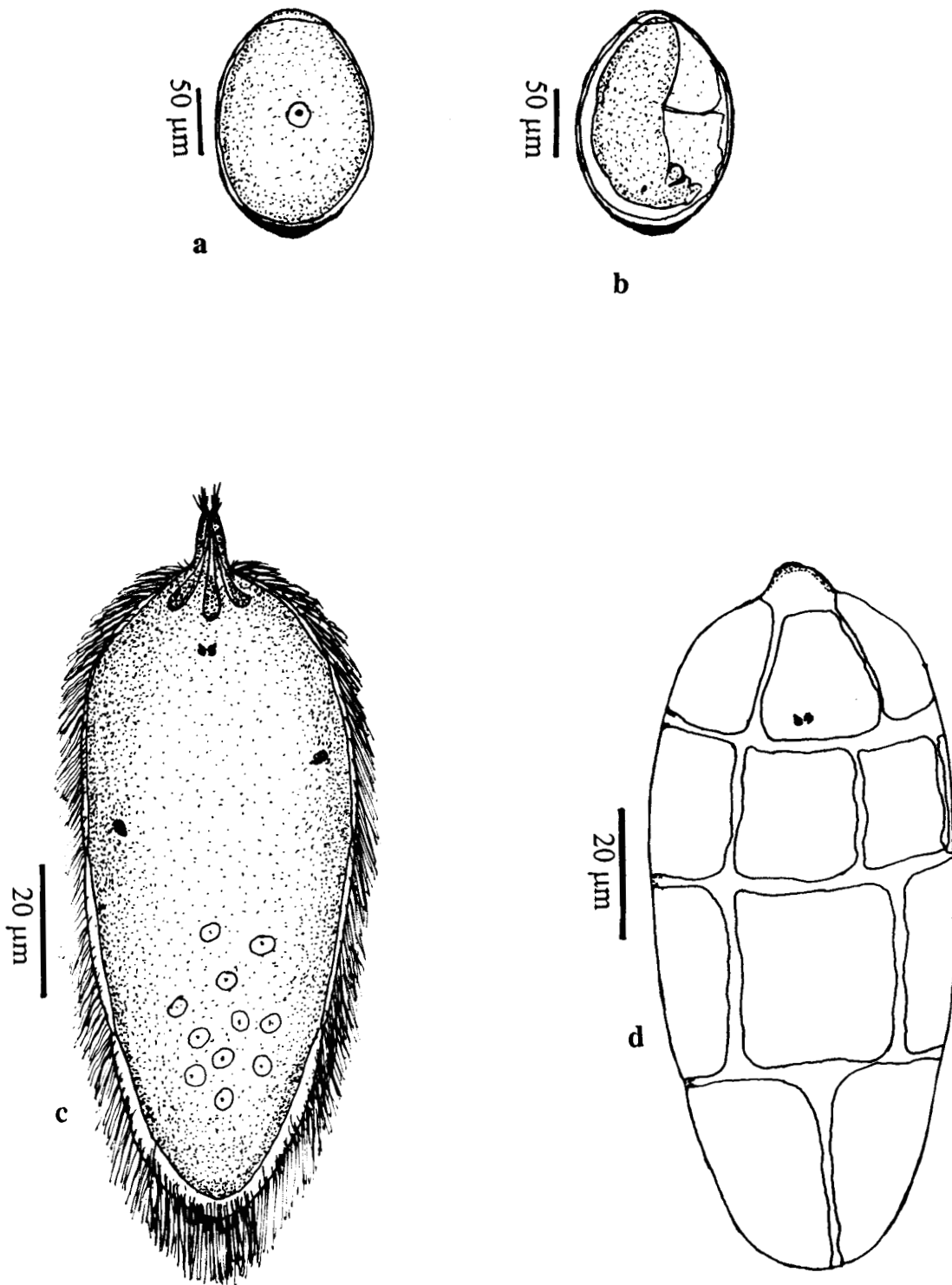


Fig. 9. *Echinostoma* sp. I n. sp.
 a. Egg; b. Egg with miracidium; c. Miracidium; d. Epidermal plates of miracidium.

Table 6. Measurements of adults of *Echinostoma* sp. I n. sp.

Character	Range	Mean
Body	6120-7266 x 950-1310	6860 x 1196
Collar width	520-565	534
Collar spines	44-59 x 9-13	52 x 12
Oral sucker	176-223	209
Acetabulum	451-566 x 476-548	493 x 510
Prepharynx (length)	62-79	67
Pharynx	161-190 x 120-147	170 x 138
Oesophagus (length)	402-510	465
Anterior testis	420-516 x 481-627	497 x 458
Posterior testis	506-648 x 428-579	592 x 539
Cirrus sac	231-332 x 296-393	249 x 373
Ovary	179-202 x 230-271	194 x 242
Egg	106-128 x 63-77	118 x 71

Miracidia fixed in hot 2% silver nitrate measured, 106-117 (113) x 39-45 (43). Body covered with 4 rows of ciliated epidermal plates consisting respectively 6, 6, 4 and 2 plates from anterior to posterior end. Apical papilla with 2 bunches of setae, measured 13 long and 5 wide. Body cilia 12-16 long. Lateral process absent. Primitive gut filled with refractile granules open at tip of apical papilla. Penetration gland cells 2, filled with refractile granular contents. Eyespots anterior, median, consist of 2 pairs of dark-brown pigmented bodies lying side by side. Flame cells 2; left one ventral and posterior, right one dorsal and anterior with duct openings situated between third and fourth rows of epidermal plates. A few germ balls occupy the middle and posterior parts of body.

Sporocyst (Fig. 10a)

Sporocysts were recovered from the pericardial cavity of snails. Fully developed sporocysts colourless, elongated sacs, 110-260 (178) long, 67-130 (89) wide, with 5-10 rediae and a few germ balls.

Redia (Figs. 10b-c)

Mother rediae were found in pericardium and kidney of snails. Fully developed mother rediae measured 320-996 (690) x 132-235 (172). Collar 67-163 (134) wide, 157-231 from anterior end. Locomotor processes 246-396 (311) from posterior end. Mouth terminal; pharynx globular, 47-68 (59) in diameter; gut 314-722 (488) long, filled with dark brown contents and reaching the level of

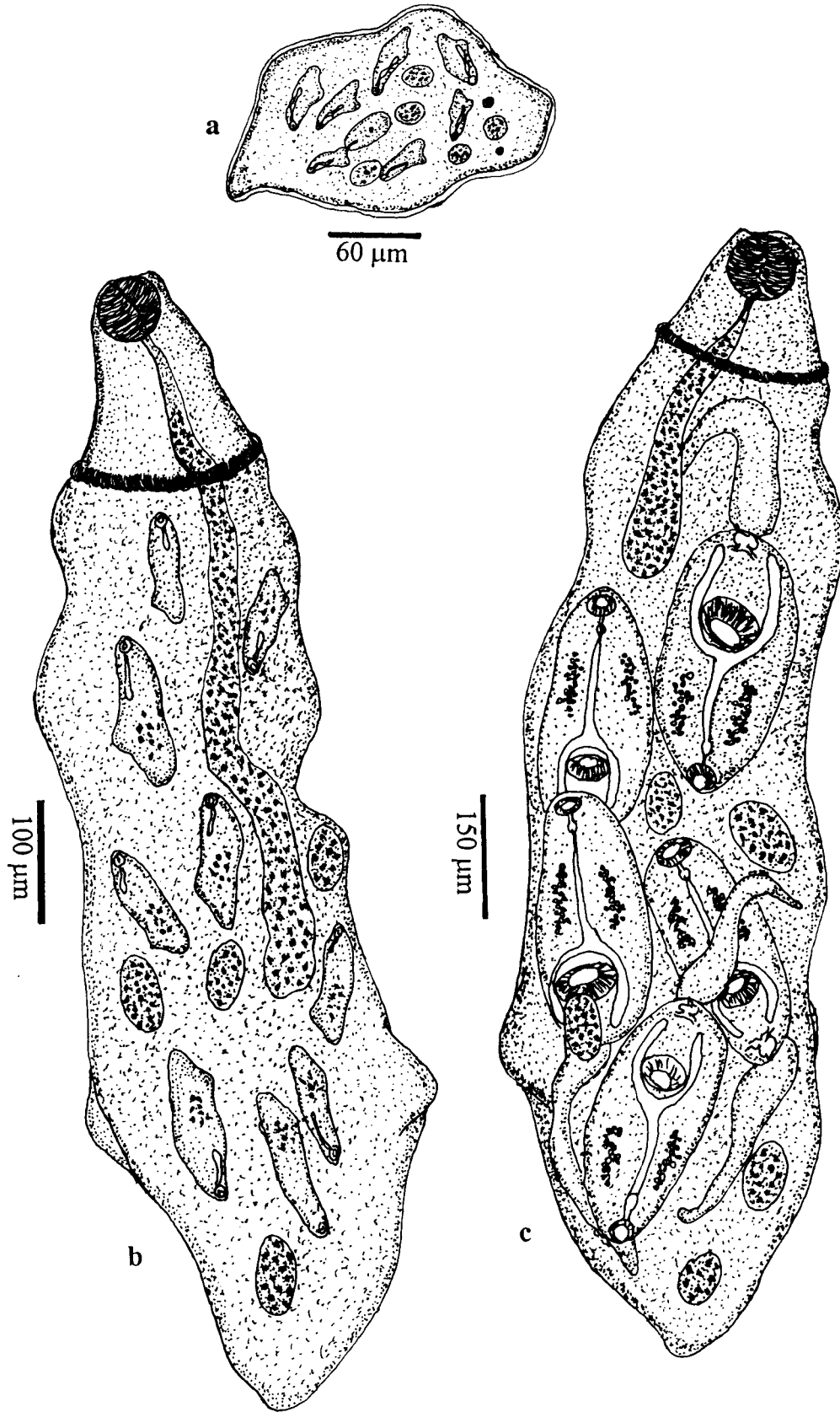


Fig. 10. *Echinostoma* sp. I n. sp.

a. Sporocyst; b. Mother redia with daughter rediae and germ balls;
c. Daughter redia with cercariae and germ balls.

locomotor processes. Rediae contained 6-10 daughter rediae and some germ balls at different stages of development. Birth pore not discernible.

Daughter rediae remained in hepatopancreas, although a few migrated to kidney and gonads. Fully grown daughter rediae elongated, motile, yellow-brown, 460-1950 (1226) x 186-392 (284) in size; contain 4-6 cercariae and many germ balls. Mouth terminal; pharynx globular, 60-95 (78) in diameter; collar conspicuous, 160-277 (212) from anterior end, 126-298 (204) wide. Birth pore dorsal and immediately posterior to collar. Gut saccular 236-418 (315) long, filled with dark brown granules.

Cercariae started emerging from snails 42 days after exposure to miracidia.

Natural definitive host	:	Unknown
Experimental hosts	:	Albino-rats; White leg-horn chicks
Location in the host	:	Posterior region of ileum and rectum
First intermediate host	:	<i>Pila virens</i>
Locality	:	Kanhangad in Kasargod district, Cherukunnu, Payangadi and Pinarayi in Kannur district, Chorode, Ramanattukara and Kundaithode in Kozhikode district and Idimuzhikkal and Nilambur in Malappuram district
Period of Collection	:	June 1996 to October 1997, January 1998, October to December 1998, April 1999.
Prevalence	:	5.87% (131 out of 2230 were infected)

- Second intermediate hosts : *Bellamyia dissimilis*, *Indoplanorbis exustus*, *Lymnaea luteola*, *P. virens* and fishes and tadpoles
- Holotype : To be deposited in the parasite collections, Parasitology Laboratory, Department of Zoology, University of Calicut, Kerala (India).

Remarks

The adults obtained experimentally from albino-rats and white leg-horn chicks are identified as belonging to the genus *Echinostoma* Dietz, 1909 on account of the well-developed collar with 37 spines arranged in double dorsally uninterrupted rows, maximally wide acetabular zone, location of cirrus sac in antero-lateral position of acetabulum, coiled seminal vesicle, unspined cirrus and highly coiled uterus with numerous eggs. Fried *et al.* (1998) based on comparative observations on cercariae of *Echinoparyphium* sp. and *Echinostoma trivolvis* reported that the cercariae of *Echinoparyphium* is characterized by the presence of a conical tail without fin folds while that of *Echinostoma* has a finger-like process at the tip of tail and fin folds on the tail. The cercaria of the present fluke has a tail identical with that of the cercariae of the genus *Echinostoma*, and therefore, the fluke under discussion undoubtedly belongs to the genus *Echinostoma* of the family Echinostomatidae Poche, 1926.

The number and arrangement of collar spines can be taken as one of the important distinguishing characters of echinostomes. We know that creation of

new species based on adult characters alone in echinostomes will often be misleading and confusing. Therefore, Mohandas (1973) and Kanev (1994) suggested to use cercarial characters as a major criterion to distinguish closely related species. Hence *Echinostoma* sp. n. sp. is compared with other 37 spined echinostomes, the life cycles of which have been established. As far as is known, more than 16 valid species of *Echinostoma* possessing 37 collar spines have reported all over the world. Of these, *E. echinatum* (Zeder, 1803) Kanev, 1994, *E. trivolvis* Cort, 1914, *E. cinetorchis* Ando and Ozaki, 1923, *E. caproni* Richard, 1964 and *E. rodriguessi* Hsu, Lie and Basch, 1968 have been found to develop both in birds and mammals. The present fluke developed experimentally in white leg-horn chicks and albino-rats and therefore, deserves comparison with the above 5 species.

The present *Echinostoma* species differs from *E. echinatum* in the location of cirrus sac and shape of cirrus. Besides, its cercaria is different from that of *E. echinatum* in body spination, number of penetration glands, paraoesophageal gland-cells, cells in the oesophagus and caecum and flame cells and in having a different snail host.

E. trivolvis differs from the present one in the position of cirrus sac, shape of testes and in structural details of its cercaria. The difference in number of paraoesophageal gland-cells and cells in the oesophagus and caecum make the cercaria of *E. trivolvis* distinct from the present cercaria. Further, the hosts utilized by the cercariae of the two species are different.

In the arrangement of collar spines, oblique position of testes, and position of cirrus sac, *E. cinetorchis* differs from the present species. Cercariae of both these species differ in the number of penetration glands, oesophageal and caecal cells, in the nature of cystogenous material and in morphometry. Moreover, the cercaria of *E. cinetorchis* develops in *Hippeutis cantori* whereas that of the present form in *Pila virens*. Though *E. caproni* and *E. rodriguessi* have spine arrangement similar to that of the present species, the size of the collar spines is different. The nature of testes, position and size of cirrus sac and body measurements are also different. In the cercaria of *E. caproni* there are 8 penetration glands, 18 pairs of flame cells, 7 cells each in oesophagus and caecum and in the cercaria of *E. rodriguessi*, the oesophagus and caecum consists of 7 cells, penetration glands are 6 in number and there are 21 pairs of flame cells.

The present cercaria is compared with other 37 spined echinostome cercariae, the adults of which are not known. Among these, the present form has a tail identical with that of *Cercaria unnaoensis* III Pandey and Lal, 1982 and cercaria of *Echinostoma* sp. II n. sp (present report). But it stands distinct from the others in one or more of the following characters: distribution of body spines, number of oesophageal and caecal cells, nature of cystogenous material, number and position of penetration glands, number of flame cells, number and

disposition of setae, presence or absence of paraoesophageal gland-cells. Again it differs in the snail hosts utilized.

The present cercariae needs comparison with *Cercaria andhraensis* Ganapati and Rao, 1969, the only species of echinostome cercaria known from a *Pila* species in India till date. Ganapati and Rao (1969) reported it from *P. globosa* in Waltair. However the presence of 33 collar spines and absence of tail fin folds make it distinctly different from the present form which has 37 collar spines and 7 finfolds on tail. Besides, there are differences in several other characters and in morphometry.

During the course of the present investigation, two other echinostome cercariae with 37 collar spines have been recovered, one from *Bellamya dissimilis* and the other from *Lymnaea luteola*. Life cycle studies revealed that the cercaria from *B. dissimilis* developed into a new species of *Echinostoma* and that from *L. luteola* developed into *E. revolutum*. Comparison of characters (Table. 7) of the 2 species of cercariae with the present form revealed that they are different in the number and arrangement of setae on body and tail, shape and position of penetration glands, number of cells in the oesophagus and caecum, and in morphometry. Life cycle studies made on the cercaria released by *B. dissimilis* revealed that it developed into a new species of *Echinostoma*. The cercaria from *Lymnaea luteola* developed into adults of *E. revolutum* (reported by Mohandas in 1973 as *E. ivniosi*). In order to further confirm the identity of

Table 7. Comparative characters and measurements of cercariae of *Echinostoma revolutum* (Frölich, 1802) Dietz, 1909, *Echinostoma* sp. I n. sp. and *Echinostoma* sp. II n. sp

Characters	Cercaria of <i>Echinostoma revolutum</i> (present report)	Cercaria of <i>Echinostoma</i> sp.I n.sp.	Cercaria of <i>Echinostoma</i> sp.II n.sp.
Body size	312-402 x 150-176	355-490 x 132-290	431-601 x 227-331
Collar spines	37	37	37
Body spination	Spinose	Spinose	Spinose
Body Setae	8-10 Nos.	8 pairs	8 pairs
Tail size	426-478 x 61-86	327-550 x 66-93	354-420 x 73-85
Tail finfolds	7 pairs	7 pairs	7 pairs
Penetration glands	4 pairs	4 pairs	3 pairs
Oesophageal cells	7	9-10 cells	10 cells
Caecal cells	7+1+7	10+1+10	14+1+14
Cystogeneous gland content	Ovoid	Rod-shaped	Round or oval
Flame cells	21 pairs	18 pairs	18 pairs
Snail host	<i>Lymnaea luteola</i>	<i>Pila virens</i>	<i>Bellamya dissimilis</i>

the 3 flukes and to show the type of specificity exhibited by them toward their first intermediate hosts, the miracidia of these flukes were exposed to laboratory-bred clean *P. Virens*, *B. dissimilis* and *L. luteola*. The miracidia developed into cercariae in their natural hosts only showing that they are species specific. Thus the 3 flukes with 37 collar spines recovered during the present study proved to be distinctly different. In view of these reasons which differentiate the present echinostome from the closely related forms, this species is considered new to science and is reported here as *Echinostoma* sp. I n. sp. Attempts made to recover the adults from natural hosts were unsuccessful.

Three *P. virens* exhibited dual infection by cercaria of *Echinostoma* sp. I n. sp. and *Cercaria pilai* II Janardanan and Shiny, 1989 and one specimen exhibited concurrent infection by 3 species, *Echinostoma* sp. I n. sp., *C. pilai* I Janardanan and Shiny, 1989 and *C. pilai* II Janardanan and Shiny, 1989.

***Echinostoma* sp. II n. sp.**

Cercaria (Figs. 11a-b; Table 8)

This cercaria having 37 collar spines occurred in the viviparid snails, *Bellamya dissimilis* throughout the year, but were prevalent during June-September when 11.3 % of the snails examined were found infected. The infected snails were collected from Payyanur, Cherukunnu and Thazhechovva in Kannur and Kizhisseri in Malappuram districts of Kerala.

Behaviour: Cercariae were released by snails throughout day-time. They were found swimming actively for about 6 hours, then sank to the bottom and died several hours later. Cercariae were negatively phototactic, and performed leech-like movements at the bottom of the container.

Description: Body elongate, spinose, measured 431-601 (527) x 227-331 (283), with 8 pairs of sensory hairs in anterior half. Body spines 3-4.5 long in pre-acetabular region and 1.8 to 2.7 in post-acetabular region. Collar distinct, 119-146 (132) wide, armed with 37 spines, 11-13 (12.1) long. Spine arrangement characteristic: five corner spines on each ventral lappet, 3 oral and 2 aboral; 6 laterals on each side in single row; 15 dorsals, 8 oral and 7 aboral. Oral sucker round to oval, measured 62-69 (65) x 69-73 (71). Acetabulum post-equatorial, protrusible, 73-84 (79) in diameter. Tail cylindrical, aspinose, ending in a finger-like projection, capable of independent movement; 354-420 (382) long, 73-85 (82) wide, with 7 finfolds, 2 dorsal, 2 ventral, 2 ventro-lateral and a small ventral just anterior to the finger-like projection. Ten pairs of sensory hairs at anterior half of tail.

Prepharynx 23-35 (28) long; pharynx ovoid, muscular, 31-39 (34) x 39-42 (41) in size. Oesophagus solid, 193-220 (199) long, consisting of 10 cells filled with coarse granular contents. Intestinal caeca solid, 163-293 (236) long, extending to posterior end of body, each caecum with 14 cells and an additional cell at bifurcation.

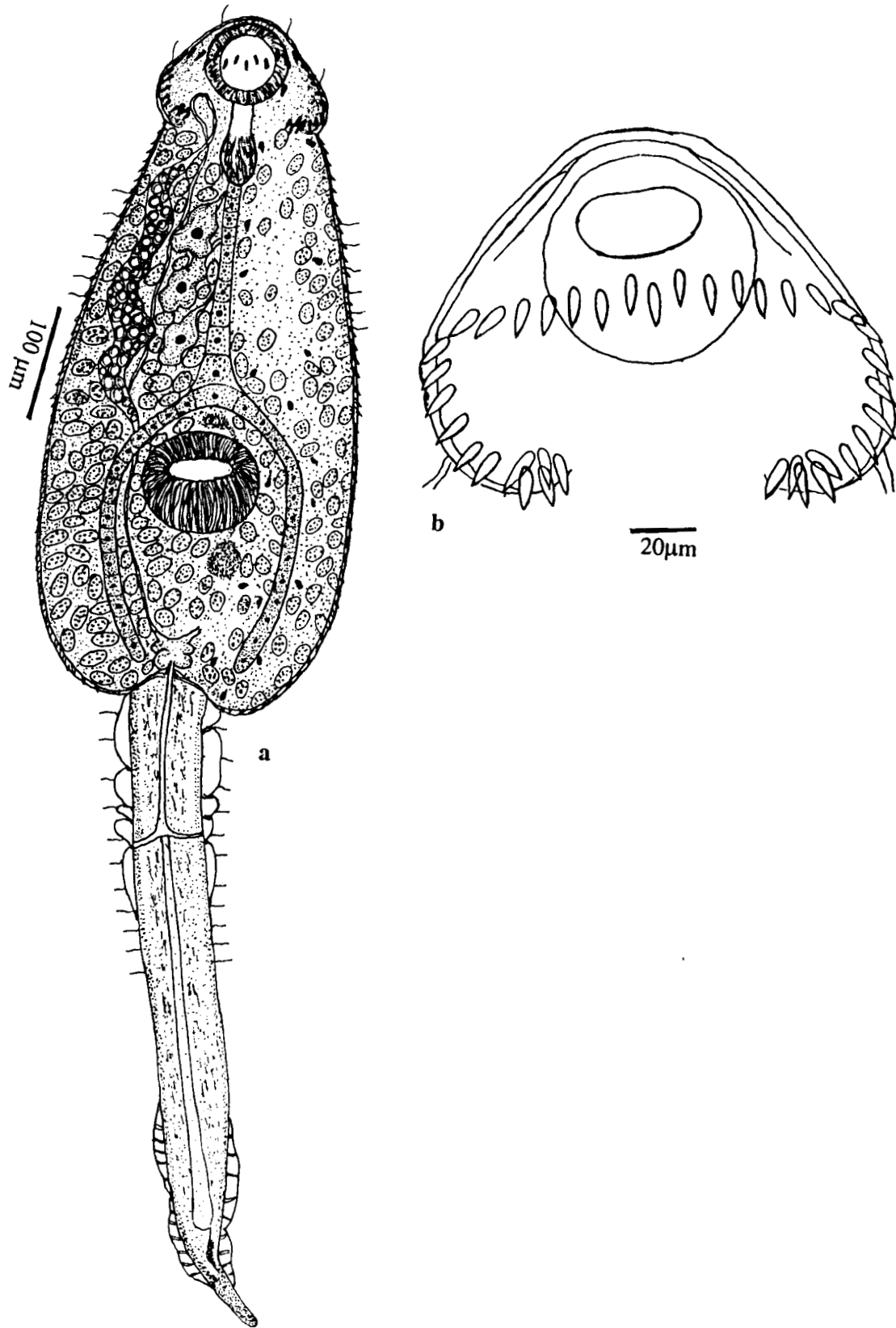


Fig. 11. *Echinostoma* sp. II n. sp.
a. Cercaria; b. Collar with Collar spines.

Penetration glands 3 pairs, located along oesophagus; each gland lobate, with round nucleus and finely granular contents. Paraoesophageal gland-cells at pharyngeal region with 12-14 outlets opening at oral sucker and 6 at pharyngeal zone. Cystogenous glands abundant, distributed throughout body, below the level of prepharynx; filled with round or oval contents.

Genital primordia consist of two cell masses, one anterior to and the other posterior to acetabulum, both connected by a string of cells passing dorsally to acetabulum. The excretory system stenostomate; excretory bladder bipartite, at posterior end of body; main collecting ducts distended between pharyngeal region and acetabulum, each containing 30-60 excretory granules. The caudal excretory duct extends one fourth of tail-length before bifurcating into two lateral branches. Flame cell formula: $2[(3+3+3)+(3+3+3)] = 36$.

Life Cycle

Metacercaria (Figs. 12a-b)

The cercariae encysted in the same snail host which liberated them or in other snails of the locality. Cysts were recovered from the pericardial cavity, kidney and muscles of *Bellamya dissimilis*, *Lymnaea luteola*, *Pila virens*, *Indoplanorbis exustus* and *Thiara tuberculata* and tadpoles of *Bufo bufo*. Within 5 days metacercariae became infective.

Table 8. Measurements of cercariae of *Echinostoma* sp. II n. sp.

Characters	Range	Mean
Body	431-601 x 227-331	527 x 283
Collar width	119-146	132
Collar spines (length)	11-13	12.1
Oral sucker	62-69 x 69-73	65 x 71
Acetabulum	73-84	79
Tail	354-420 x 73-85	382 x 82
Prepharynx (length)	23-35	28
Pharynx	31-39 x 39-42	34 x 41
Oesophagus (length)	193-220	199
Caeca (length)	163-293	236

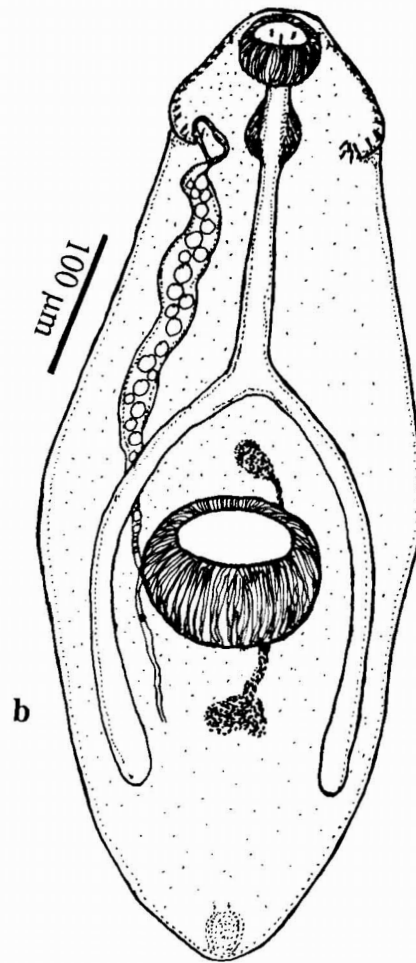
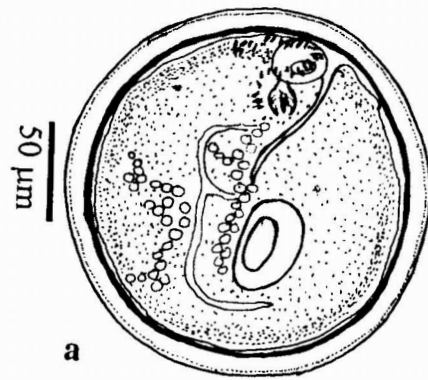


Fig.12. *Echinostoma* sp. II n. sp.
a. Encysted metacercaria; b. Excysted metacercaria

Metacercarial cysts spherical, 185-191(188) in diameter. Cyst wall double-layered with an outer, transparent, delicate, easily detachable layer of 5-6 thickness and an inner, translucent layer of 2-3 thickness. Inside the cyst the larva remained curled up.

Metacercariae exposed to digestive juice of chicks excysted in 3-4 hours. Excysted metacercariae measured 551-648 (592) x 216-326 (285).

Adult (Figs. 13a-b, 14a-b; Table 9)

Clean chicks of whitebreasted waterhen and one-month-old albino-rats, force-fed with infective metacercariae, yielded mature adults in 8 days. Development of the flukes was followed in experimentally infected chicks.

The one-day-old flukes measured 607 x 299, and were almost identical to excysted metacercariae.

The four-day-old specimens measured 766-1302 (930) x 276-418 (355). Differential growth of anterior and posterior halves of the body shifted the acetabulum from its median position to a more anterior region. The suckers increased in size; acetabulum larger than oral sucker. Primordia of cirrus sac, ovary and testes appeared.

Flukes recovered on the 8th day were fully mature, measured 4940-6150 (5210) x 942-1526 (1220); uterine coils were filled with numerous eggs. Body elongate with pointed anterior and broadly round posterior ends, attaining

maximum width at the junction between first and second thirds of body. Body spines posterior to collar, differing in distribution on dorsal, ventral and lateral surfaces; dorsally, the scale-like spines in transverse rows confined to a region anterior to acetabulum, with density of distribution usually decreasing from anterior to posterior; ventrally and laterally, spines extend posteriorly to half way between posterior testis and posterior extremity of body, often becoming sparse.

Collar well developed, 418-568 (496) wide, with 37 collar spines, measuring 48-61 (57) x 13-19 (16). Spine arrangement similar to that in cercaria. Latero-aboral of corner spines the largest (61 long) of all collar spines and inner-oral of corner spines and latero-oral of dorsals the smallest (48 long). Oral sucker subterminal, oval, 156-214 (163) x 172-234 (212) in size. Acetabulum in anterior third of body, slightly broader than long, measured 406-492 (446) x 428-504 (488).

Prepharynx 72-79 (75) long; pharynx muscular, ovoid, measured 122-169 (157) x 118-165 (152). Oesophagus 298-462 (398) long, bifurcating in front of acetabulum; caeca extending almost to posterior end of body, 2200-4870 (3560) long.

Testes tandem, intercaecal, ovoid, irregular in outline, in posterior half of body. Anterior testis 240-322 (310) x 334-580 (508); posterior testis 295-424 (381) x 350-610 (521). Cirrus sac median, ovoid, 202-368 (314) long, 161-216

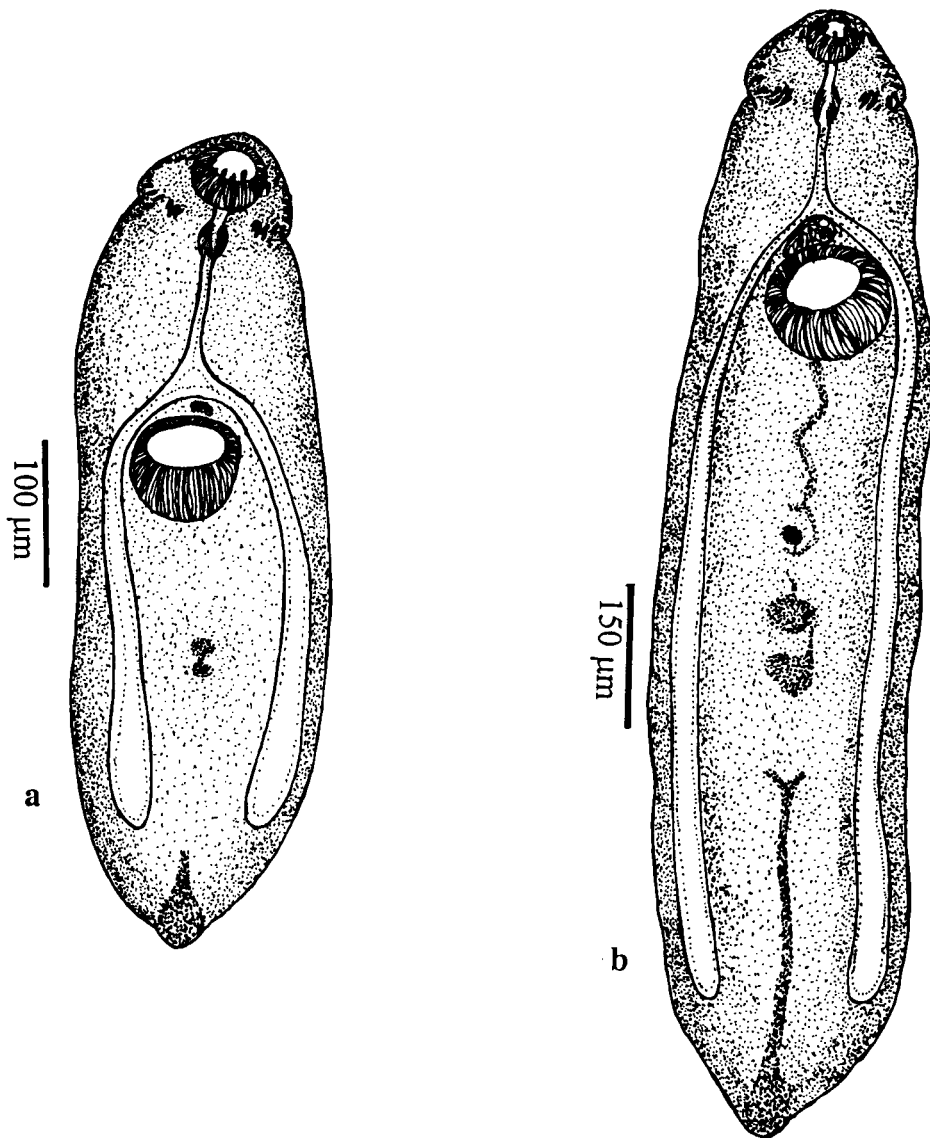


Fig. 13. *Echinostoma* sp. II n. sp.
a. One-day-old adult; b. 4-day-old adult.

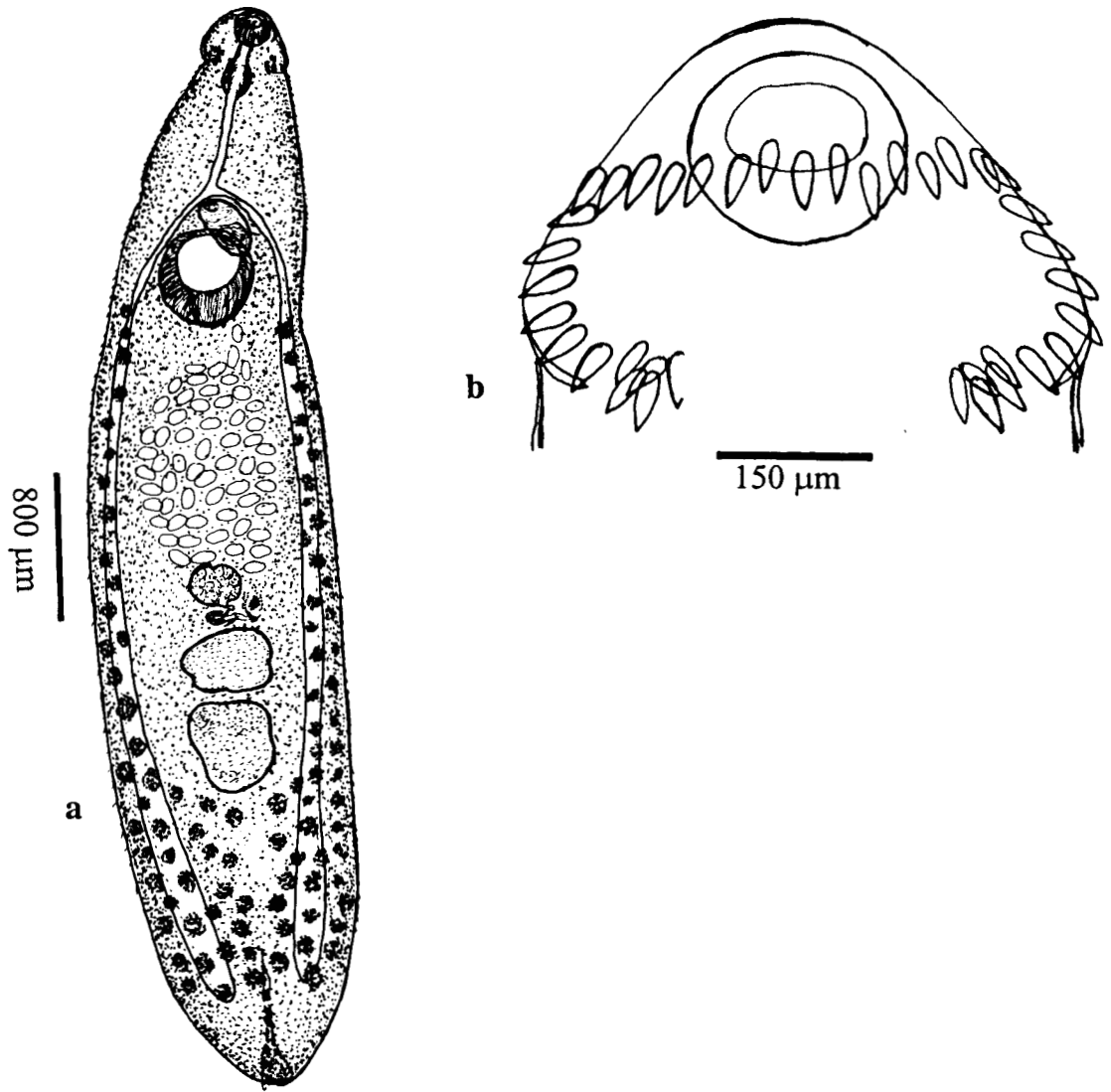


Fig. 14. *Echinostoma* sp. II n. sp.
a. Mature adult; b. Collar and collar spines

(189) wide, between caecal bifurcation and acetabulum and containing bipartite seminal vesicle, feebly developed pars prostatica and unspined cirrus.

Ovary pre-testicular, near equator, oval to elongate, measured 149-230 (184) x 224-327 (276). Mehli's gland complex poorly developed. Laurer's canal opening medially on dorsal surface. Uterine seminal receptacle filled with many sperms. Vitelline reservoir small, median. Uterus intercaecal, pre-testicular with 10-12 coils containing 40 or more eggs, and connected to genital atrium through metraterm. Vitellaria follicular, lateral, extend from mid-level of acetabulum to near posterior end of body, do not confluent posterior to testes.

Excretory bladder with small branches; stem coiled before opening at posterior tip of worm.

Egg (Fig. 15a)

Eggs ovoid, yellow-brown, with conspicuous operculum at narrow anterior end and shell thickening with striated appearance at posterior end; measured 110-130 (121) x 72-89 (80). Most of the eggs hatch on the 12th day at room temperature (26°-28°C). Hatching usually occurs in the morning.

Miracidium (Figs. 15b-c)

Newly hatched miracidia swim rapidly in water for 4-6 h, then settle to the bottom of the container and show creeping movements.

Table 9. Measurements of adults of *Echinostoma* sp. II n.sp.

Characters	Range	Mean
Body	4940-6150 x 942-1526	5210 x 1220
Collar width	418-568	496
Collar spines	48-61 x 13-19	57 x 16
Oral sucker	156-214 x 172-234	163 x 212
Acetabulum	406-492 x 428-504	446 x 488
Prepharynx (length)	72-79	75
Pharynx	122-169 x 118-165	157 x 152
Oesophagus (length)	298-462	398
Caeca (length)	2200-4870	3560
Anterior testis	240-322 x 334-580	310 x 508
Posterior testis	295-424 x 350-610	381 x 521
Cirrus sac	202-368 x 161-216	314 x 189
Ovary	149-230 x 224-327	184 x 276
Egg	110-130 x 72-89	121 x 80

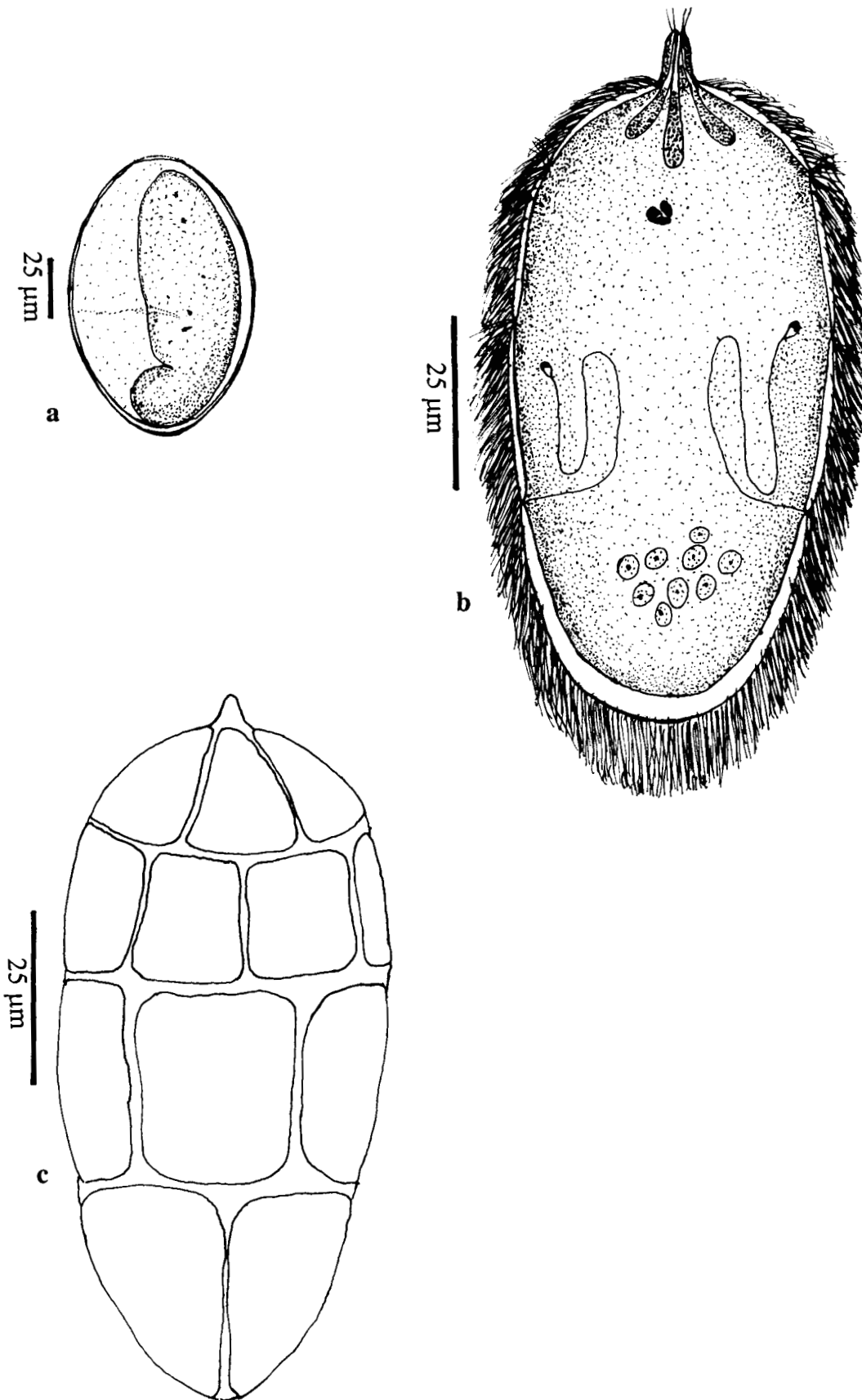


Fig. 15. *Echinostoma* sp. II n. sp.
a. Egg with miracidium; b. Miracidium; c. Epidermal plates of miracidium.

Miracidia measured 102-112 (106) x 53-69 (64). Body covered with 4 rows of ciliated epidermal plates consisting respectively 6,6,4 and 2 plates from anterior to posterior end. Refractile apical papilla with 2 pairs of long setae measuring 12 x 5 in size. Body cilia 12-14 long. Lateral processes 2 pairs, one pair each at the junction of first and second rows, and second and third rows of epidermal plates. Primitive gut filled with refractile granules open at tip of apical papilla. Penetration gland cells 2, filled with granular contents. Eyespots consist of 2 pairs of dark-brown pigmented bodies lying side by side. Flame cells 2; left one ventral and posterior; right one dorsal and anterior. Two excretory pores open between third and fourth rows of epidermal plates. Several germinal cells occupy the posterior half of body.

Sporocyst (Fig. 16a)

Sporocysts usually found in head and surrounding muscles of snails. Mature sporocysts colourless, elongated sacs, 155 x 89 in size, containing 2-5 rediae and a few germ balls.

Redia (Figs. 16b-c)

Mother rediae were recovered from heart and kidney of snails. Fully grown mother rediae measured 315-1802 (1124) x 85-376 (227), possess well-developed collar and locomotor processes, and contain 10-16 daughter rediae and some germ balls at different stages of development. Birth pore not discernible.

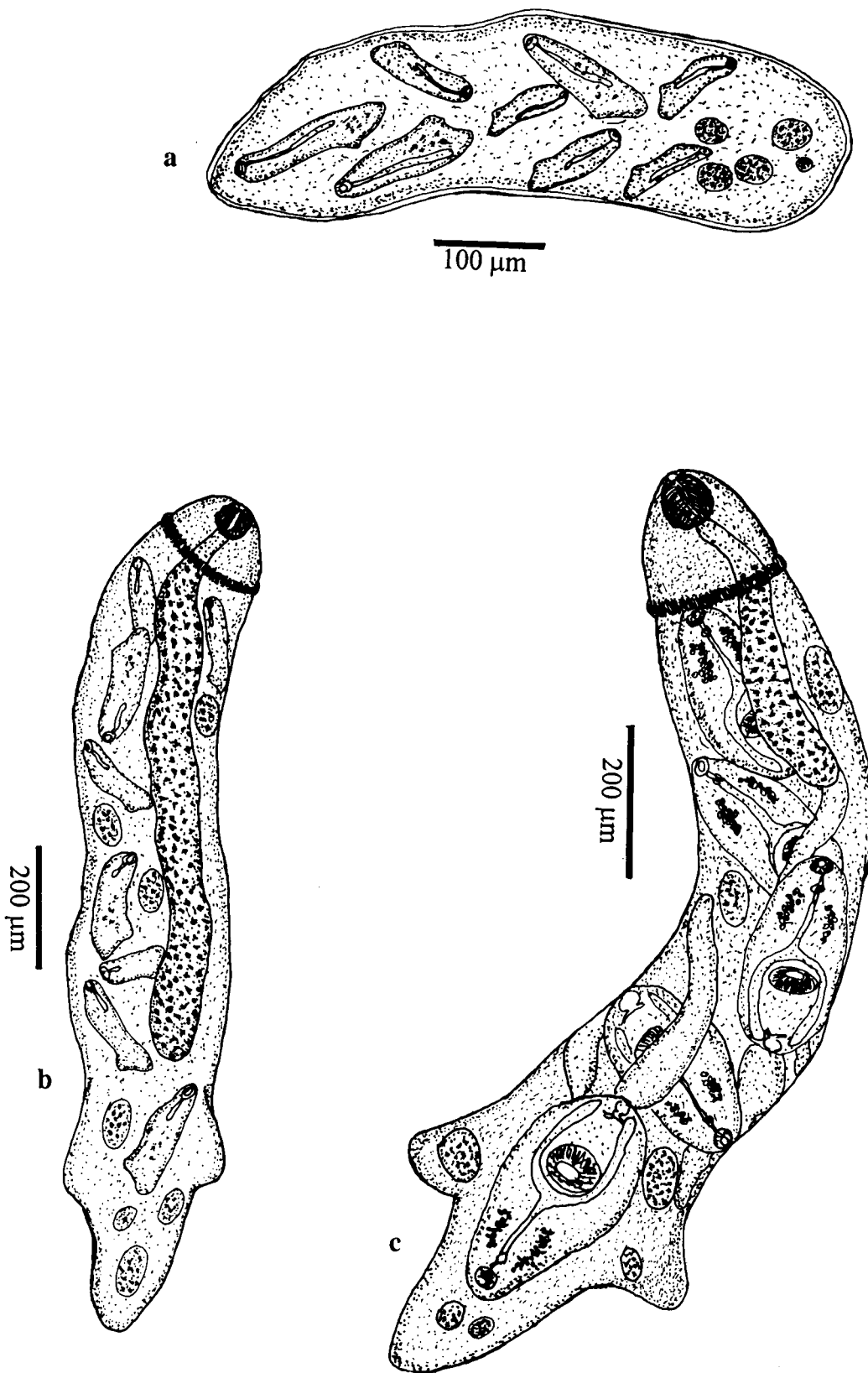


Fig. 16. *Echinostoma* sp. II n. sp.

a. Sporocyst; b. Mother redia with daughter rediae and germ balls;
c. Daughter redia with Cercariae and germ balls.

Daughter rediae were found in hepatopancreas. Fully developed daughter rediae elongate, motile, yellow-brown, 400-2387 (1802) x 100-416 (286) in size; contain 4-6 cercariae and many germ balls. Collar conspicuous, 90-296 (171) wide, 50-215 from anterior end. Mouth terminal; pharynx globular, 32-61 (47) in diameter. Gut saccular, 120-560 (394) long, filled with dark contents. Locomotor process paired, 106-419 from posterior end.

Natural definitive host	: Whitebreasted waterhen, <i>Amaurornis phoenicurus</i>
Location in the host	: Ileum
Locality	: Kizhisseri
Prevalence	: 25 % (One out of four were infected)
Experimental host	: Albino-rats and whitebreasted waterhen, <i>A. phoenicurus</i>
Location in host	: Ileum and rectum
First intermediate host	<i>Bellamya dissimilis</i>
Locality	: Payyanur, Cherukunnu and Thazhechovva in Kannur district and Kizhisseri in Malappuram district.
Period of collection	: July to September 1997, December 1997, June 1998, February 1999.
Prevalence	: 9.22 % (353 out of 3827 were infected).
Second intermediate host	: <i>B. dissimilis</i> , <i>Lymnaea luteola</i> , <i>Pila virens</i> , <i>Indoplanorbis exustus</i> and <i>Thiara tuberculata</i> and tadpoles of <i>Bufo bufo</i>
Holotype	: To be deposited in the parasite collections, Parasitology Laboratory, Department of Zoology, University of Calicut, Kerala (India).

Remarks

The present fluke recovered from naturally and experimentally infected *Amaurornis phoenicurus* and from experimental infections of albino-rats has a well-developed collar with 37 spines arranged in double uninterrupted rows, maximally wide acetabular zone, antero-laterally located cirrus sac, coiled seminal vesicle, unspined cirrus and highly coiled uterus with numerous eggs. These characters are significant enough for inclusion of worm under the genus *Echinostoma* Dietz, 1909 of the family Echinostomatidae Poche, 1926.

The present species of *Echinostoma* developed both in birds and mammals, and therefore, needs comparison with those species which have been reported from these hosts. The species are *E. echinatum* (Zeder, 1803) Kanev, 1994, *E. trivolvis* Cort, 1914, *E. cinetorchis* Ando and Ozaki, 1923, *E. caproni* Richard, 1964, *E. rodriguesi* Hsu, Lie and Basch, 1968 and *Echinostoma* sp. I n. sp. (Present report). From a comparison of characters, it is evident that *E. echinatum*, differs from the present form in the position of cirrus sac, in the shape of cirrus and in morphometry. In addition, the cercaria of *E. echinatum* differs from that of the present form in the number of paraoesophageal gland-cells, penetration glands, flame cells and oesophageal and caecal cells.

E. trivolvis superficially resembles the present species, but differs in the position and size of cirrus sac and testes and in body measurements. Moreover,

cercariae of the two species differ in body spination, number of paraoesophageal gland-cells and caecal cells.

E. cinetorchis is distinct from the present form in its collar spine arrangement and oblique position of testes. Differences are also found in the cercariae of the two species. In the number of penetration glands, number and arrangement of finfolds, number and distribution of paraoesophageal gland-cells, and in morphometry they are different.

E. caproni differs from the present fluke in shape and size of testes. Further, the cercaria of *E. caproni* is distinct in the number of penetration glands, body setae, nature of cystogeneus material and number of oesophageal and caecal cells.

E. rodriguessi has elongate, antero-lateral cirrus sac, smooth testes and anteriorly extended vitellaria making it different from the present fluke. Besides, the cercaria of *E. rodriguessi* different from the present cercaria in flame cell formula, number of oesophageal and caecal cells and in morphometry.

Echinostoma sp. I n. sp. is distinct from the present fluke in shape and size of testes, position of cirrus sac, distribution of vitelline follicles and morphometry. The cercaria of *Echinostoma* sp. I n. sp. has 4 pairs of penetration glands, 10 cells in each caecum and rod-shaped cystogenous material. In addition, all the six species are found to develop in different snail hosts.

The present cercaria needs comparison with other cercariae, the adults of which are not known. It comes close to *Cercaria spinosa* Pandey and Singh, 1984 infecting *Bellamya bengalensis* from Uttar Pradesh in most of its characters except in the number and arrangement of tail finfolds on tail. Comparative studies on the tail finfolds of 37-collar-spined cercariae (Kanev, 1994, Kanev *et al.*, 1995 and present study) showed that the finfolds are delicate and difficult to see if not fixed properly. Therefore, the cercaria under discussion cannot be treated as one different from *C. spinosa* based on the number and arrangement of finfolds alone. In all probability these two are identical. Since the present fluke cannot be identified with any known species of *Echinostoma*, it is considered as new and reported here as *Echinostoma* sp. n. sp.

***Echinostoma malayanum* Leiper, 1911**

Cercaria (Figs. 17a-b; Table 10)

Infections by this cercaria were found in the planorbid snails, *Indoplanorbis exustus*, collected from Kanhangad in Kasargod, Payyanur and Melechovva in Kannur, Feroke in Kozhikode and Idimuzhikkal in Malappuram districts of Kerala. Sixty-one out of 835 snails (7.31%) collected were infected. The infection occurred throughout the year.

Behaviour: The cercariae emerged from snails chiefly in the first four hours after sunrise. They were active swimmers, and are not phototactic. They remain alive for about 12 h in well-water.

Description: Body oval to elliptical, aspinose, with 6-10 pairs of sensory hairs at anterior half; measured 213-309 (267) x 90-167 (135) in size. Collar distinct 65-89 (73) wide, with 43 or 45 spines, 8-10.5 (9.6) in length. Spine arrangement characteristic: five corner spines on each ventral lappet, 3 oral and 2 aboral; 11 or 12 lateral spines in single row on each side and 11 dorsals, 6 oral and 5 aboral. Oral sucker oval, measured 40-47 (43) x 36-42 (39) in size. Acetabulum post-equatorial, protrusible, circular, measured 57-72 (63) in diameter. Tail cylindrical, 356-458 (411) long, 47-70 (62) wide, with 7 finfolds, 2 dorsal, 2 ventral, 2 ventro-lateral and a small ventral one near tip of tail. The end of tail modified into a slender, finger-like process capable of independent movement.

Prepharynx 19-23 (21) long, with a prepharyngeal body. Pharynx oval, measured 19-27 (24) x 16-24 (21). Oesophagus solid 47-69 (61) long, with 6 cells; caeca narrow, solid, 130-152 (143) reaching nearly to posterior end of body; each caecum consists of 6 cells and an additional cell at the point of bifurcation.

Penetration glands 3 pairs, along oesophagus; gland cells open through 3 pairs of outlets on the dorsal lip of oral sucker. Cystogenous glands numerous, densely distributed throughout body and filled with rod-shaped contents.

Genital primordia consist of two masses of cells, one at anterior margin of acetabulum, the other between acetabulum and base of tail, and connected by a string of cells passing dorsal to acetabulum. Excretory system stenostomate type;

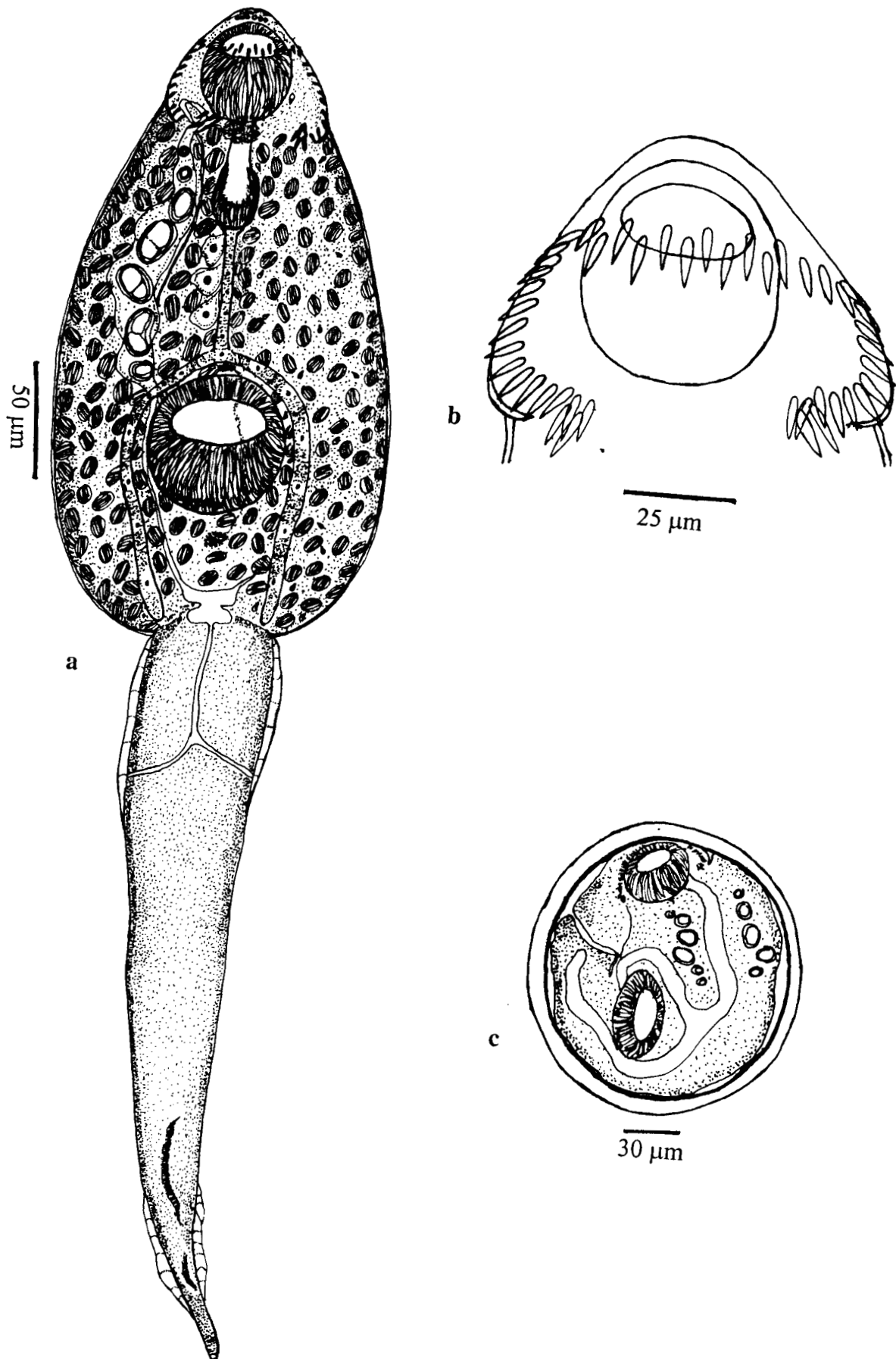


Fig. 17. *Echinostoma malayanum* Leiper, 1911
a. Cercaria; b. Collar with collar spines; c. Encysted metacercaria.

excretory bladder bipartite, at posterior end of body; main collecting ducts distended between collar region and acetabulum, each containing 4-7 excretory concretions, with the largest one (29 x 18) lying in the middle and the smaller ones (7 x 6) on either side. Flame cell formula: $[(3+3+3) + (3+3+3)] = 36$.

Life cycle

Metacercaria (Fig. 17c)

Natural infections by the metacercariae were found in hepatopancreas, kidney, gonad and muscles of *Indoplanorbis exustus*, *Lymnaea luteola* and *Bellamya dissmilis*. Occasionally they were also found encysting in *Pila virens*, *Paludomous tanschauricus*, *Thiara tuberculata* and on gill arches of fishes and tadpoles. Cysts ovoid, 135-170 (162) x 123-138 (131) in size. Cyst wall double-layered, consisting of an outer, transparent layer, 6.5-8 thick and an inner, opaque layer, 1-2 thick. Inside the cyst the larva remained curled up.

Adult (Fig. 18a; Table 11)

During the course of present study, the life cycle of *Echinostoma malayanum* Leiper, 1911 has been elucidated in the Laboratory. Metacercariae, force-fed to one-month-old albino rats developed into mature adults in 10 days. Characters of the adult worms agreed fully with those given by Mohandas (1971) for *E. malayanum*. A brief description of the adult is given here.

Table 10. Measurements of cercariae of *Echinostoma malayanum* Leiper, 1911.

Characters	Range	Mean
Body	213-309 X 90-167	267 X 135
Collar width	65-89	73
Collar spines (length)	8-10.5	9.6
Oral sucker	40-47 x 36-42	43 x 39
Acetabulum	57-72	63
Tail	356-458 x 47-70	411 x 62
Prepharynx (length)	19-23	21
Pharynx	19-27 x 16-24	24 x 21
Oesophagus (length)	47-69	61
Caeca (length)	130-152	143

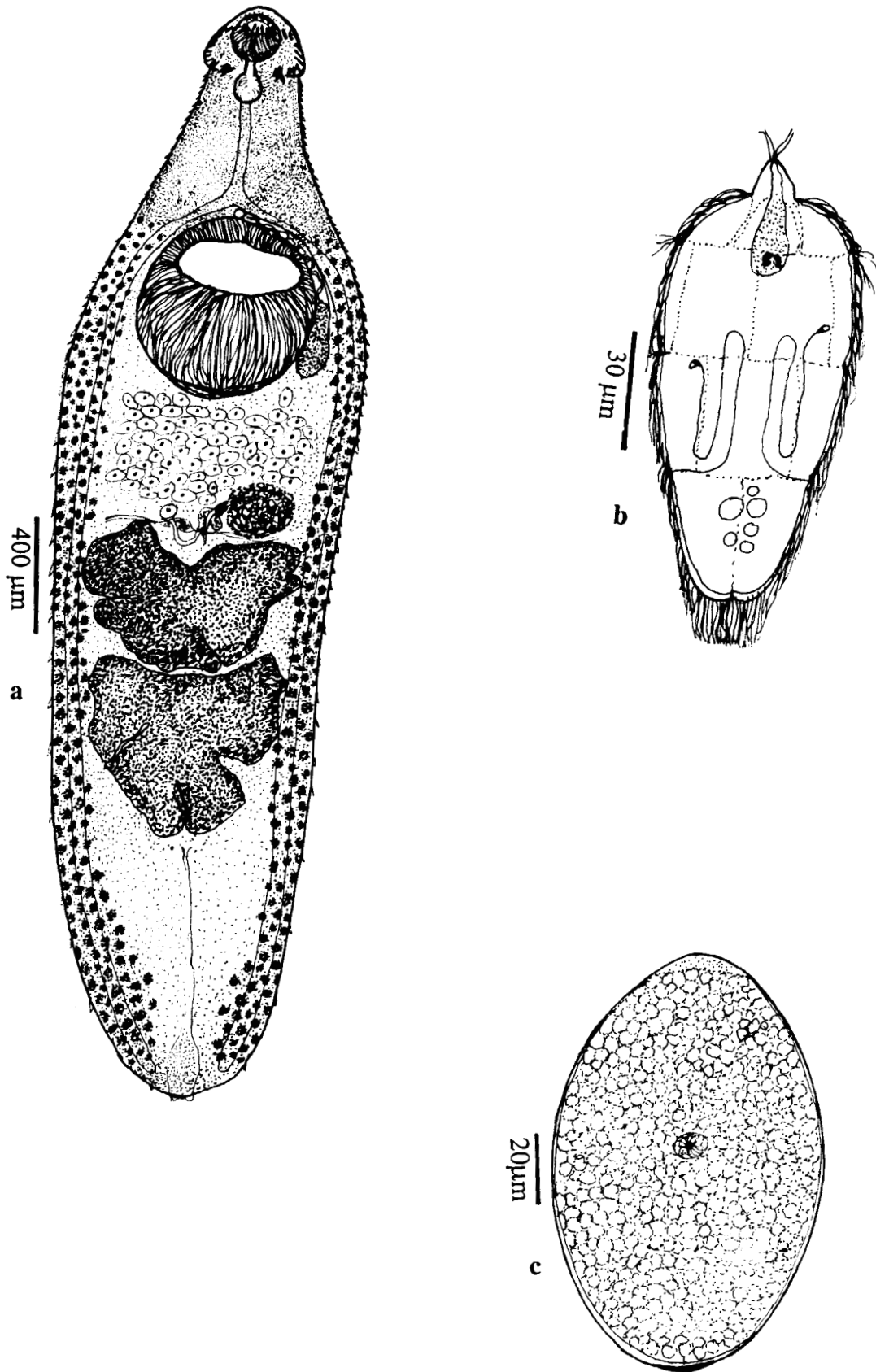


Fig. 18. *Echinostoma malayanum* Leiper, 1911
a. Mature adult; b. Miracidium; c. Egg.

Description: Body elongate, spinose, with pointed anterior and broadly rounded posterior ends; measured 3900-8150 (6350) x 890-2010 (1676). Collar 360-615 (520) wide, with 43 or 45 spines arranged as in cercariae. Oral sucker ovoid, measured 150-189 (176) x 180-239 (209). Acetabulum in anterior quarter of body, slightly broader than long, measured 682-1035 (806) x 755-1060 (960) in size. Prepharynx 26-40 (37) long; pharynx globular, 80-112 (97) in diameter; oesophagus 190-410 (285) long. Caeca reach near the posterior end.

Testes tandem, in middle of hind body, lobate; anterior testis measured 618-827 (721) x 702-983 (875); posterior testis 816-1200 (1056) x 796-1140 (967). Cirrus sac oval to elongate; cirrus unspined. Seminal vesicle bipartite, swollen at the base. Ovary ovoid, 292-486 (376) x 174-409 (298) in size. Laurer's canal and uterine seminal receptacle present. Uterus consists of 4-7 coils with many eggs. Genital pore medium, pre-acetabular. Vitellaria follicular, lateral, extending from acetabular region to near caecal ends.

Egg (Fig.18c)

Eggs ovoid, yellow-brown, operculate, measured 123-140 (134) x 60-76 (66).

Miracidium (Fig. 18b)

Miracidia, fixed in 2% silver nitrate measured 76-109 (91) x 37-48 (42). Body covered with 4 rows of ciliated epidermal plates, arranged in 6+6+4+2 pattern; cilia 10-14 long. Paired lateral processes, eyespots, penetration glands and flame cells are similar to that described by Mohandas (1971).

Table 11. Measurements of adults of *Echinostoma malayanum* Leiper, 1911

Character	Range	Mean
Body	3900-8150 x 890-2010	6350 x 1676
Collar width	360-615	520
Collar spines	43-64 x 10-17	56 x 14
Oral sucker	150-189 x 180-239	176 x 209
Acetabulum	682-1035 x 755-1060	806 x 960
Prepharynx (length)	26-40	37
Pharynx	80-112	97
Oesophagus (length)	190-410	285
Anterior testis	618-827 x 702-983	721 x 875
Posterior testis	816-1200 x 796-1140	1056 x 967
Ovary	292-486 x 174-409	376 x 298
Egg	123-140 x 60-76	134 x 66

Sporocyst (Fig. 19a)

Sporocysts found in pericardial cavity of *Indoplanorbis exustus* were colourless, sac-like, measured 162-298 (196) x 85-130 (197), and contained a few rediae and germ balls at different stages of development.

Redia (Figs. 19b-c)

Mother rediae, recovered from heart and kidney of the snails, were motile, colourless with conspicuous collar and locomotor organs; measured 640-872 (785) x 173-260 (220) in size. Fully grown mother rediae filled with different stages of developing daughter rediae.

Daughter rediae measured 1873-2560 (2350) x 218-474 (390) in size. Fully formed daughter rediae contained 4-16 cercariae and a few germ balls.

Natural definitive host	: Could not be recovered
Experimental host	: Albino-rats
Location in host	: Duodenum and anterior ileum
First intermediate host	: <i>Indoplanorbis exustus</i>
Locality	: Kanhangad in Kasargod district, Payyanur and Melechowva in Kannur district, Feroke in Kozhikode district and Idimuzhikkal in Malappuram district.
Period of collection	: December 1996 to June 1997, August to October 1997, February and March 1998, October and November 1998 and February 1999

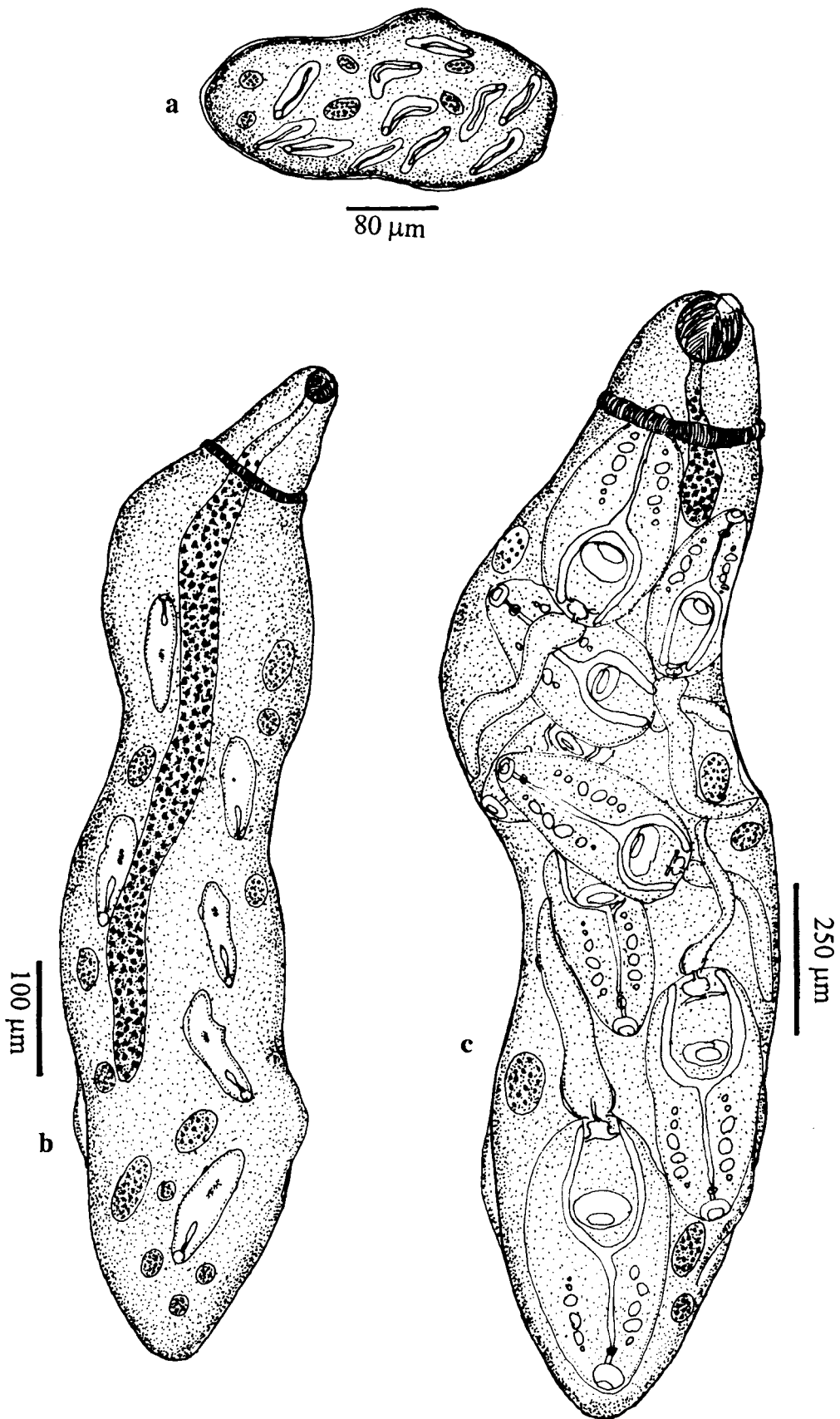


Fig. 19. *Echinostoma malayanum* Leiper, 1911
a. Sporocyst; b. Mother redia with daughter rediae and germ balls;
c. Daughter redia with cercariae and germ balls.

- Prevalence : 1.5 % (61 out of 4056 were infected)
- Second Intermediate hosts : *I. exustus*, *Lymnaea luteola*, *Bellamya dissimilis*, *Pila virens*, *Paludomous tanschauricus*, *Thiara tuberculata* and fishes and tadpoles.
- Paratype : To be deposited in the parasite collections, Parasitology Laboratory, Department of Zoology, University of Calicut, Kerala (India)

Remarks

Echinostoma malayanum was first described by Leiper (1911) who obtained the worm from an autopsy of an Indian labourer in Malay states. Lie (1963a) found an echinostome metacercaria in *L. rufiginosa* and *I. exustus* in the neighbourhood of Kuala Lumpur and developed it into adult in white rats and other mammals. The adult turned out to be that of *E. malayanum*. Then he established the life cycle of this species in laboratory. This formed the first report on the life cycle of *E. malayanum*.

Lane (1915) erected the genus *Artyfechinostomum* to accommodate an echinostome fluke recovered from an Assamese girl and named it *Artyfechinostomum sufratyfex*. The description of this species was based on macerated specimen. Eversince the creation of the genus *Artyfechinostomum*, it attracted controversy over its validity. Of equal importance is the validity of the species *A. sufratyfex* for which the genus was erected. As *A. sufratyfex* bears similarly with *Echinostoma malayanum*, many workers considered them

identical but under different combinations. Reddy and Verma (1950) and Ramanujachari and Alwar (1953) preferred to treat it under *Paryphostomum*, while Rai and Ahluwalia (1958) and Jain (1960) under *Artyfechinostomum*. Leiper (1915), Lie (1963b) and Mukherjee and Ghosh (1968) treated *A. sufratyfex* as a synonym of *E. malayanum*. In order to prove the synonymity of *A. sufratyfex* with *E. malayanum*, Mohandas (1971) made studies on the life cycle study of *A. sufratyfex* and concluded that these two are synonyms.

During the present study, I could recover a 43-45 spined echinosome cercaria from *I. exustus*. Experimentally, this cercaria developed into metacercaria in snails, *Bellamya dissimilis*, *I. Exustus*, *L. luteola*, *P. virens*, *P. tanschauricus*, and *T. tuberculata*, fishes and tadpoles. The metacercariae when fed to albino rats developed into *E. malayanum*.

The complete life cycle from egg to egg-producing adults has been established in the laboratory. All the stages developed during the present investigation are identical with those recorded by Lie (1963a) and Mohandas (1971) except for slight variation in morphology. These variations can be attributed to the different environment provided by different hosts. Therefore, the present worm is identified and reported here as *Echinostoma malayanum* Leiper, 1911.

Mixed infections by different species of cercariae were observed in 22 specimens of *Indoplanorbis exustus*. In 3 cases concurrent infections were by

cercariae of *E. malayanum* and *Cercaria* sp. II Malabar n. sp., in 11 instances by cercaria of *E. malayanum* and that of *Cotylophoron cotylophoron* and in 7 cases with unidentified furcocercous cercaria. Triple infection by cercariae of *E. malayanum*, that of *C. cotylophoron* and an unidentified furcocercous cercaria was found in one specimen.

***Echinostoma* sp. III n. sp.**

Cercaria (Figs. 20a-b; Table 12)

The lymnaeid snails, *Lymnaea luteola* were found infected by echinostome cercariae with 43 collar spines. Infections were found in snails collected from Trikaripur in Kasargod, and Cherukunnu and Pinarayi in Kannur districts of Kerala. Of 2850 snails examined 37 were infected, the prevalence of infection being 1.3%.

Behaviour: Cercariae emerged from snails during morning hours and were found swimming actively, curving the body ventrally. They remained alive for about 24 h at room temperature, and were positively phototactic.

Description: Body oval to elliptical, covered with minute cuticular spines up to the level of acetabulum; measured 330-408 (346) long, 193-316 (241) wide, with maximum width at mid-body. Collar well developed, 80-102 (91) wide, with 43 conspicuous spines, 9-12 (10.8) in length. Spine arrangement characteristic: five corner spines on each ventral lappet, 3 oral and 2 aboral; 7 laterals on each side in single row; 19 dorsals, 10 oral and 9 aboral. Oral sucker circular, 51-62 (58)

in diameter. Acetabulum post-equatorial, protrusible, oval, 57-65 (62) x 65-79 (69) in size. Tail 339-377 (347) long, 54-63 (57) wide, with 7 finfolds, 2 dorsal, 2 ventral, 2 ventro-lateral and a small ventral one near tip of tail. Tail tip finger-like, capable of independent contraction and expansion.

Prepharynx 26-33 (31) long, with a prepharyngeal body. Pharynx globular, 29-34 (31) in diameter. Oesophagus 80-97 (88) long, consisting of 9-10 cells. Caeca narrow, solid, 85-103 (97) extending to the level of excretory bladder; each caecum consisting of 10 cells with an additional cell at bifurcation.

Penetration glands 5 pairs, lobate, along oesophagus, opening through 2 pairs of gland outlets on the dorsal lip of oral sucker. Para-oesophageal gland cells present, with 10-12 outlets around oral sucker and 6 in pharyngeal region. Cystogenous glands numerous, densely distributed in lateral fields, extending from level of pharynx to posterior end of body and containing rod-shaped contents.

Genital primordia consist of two masses of cells, one at anterior margin of acetabulum, the other between acetabulum and base of tail, and both connected by a string of cells passing dorsal to acetabulum. Excretory system stenostome type; excretory bladder bipartite, at posterior end of body; main collecting ducts distended between collar and acetabulum, each containing 7-12 excretory concretions, 7-19 x 6-16 in size; caudal excretory duct extending one-fourth of tail length before bifurcating into two lateral branches. Flame cell formula: $2[(3+3) + (3+3+3)] = 30$.

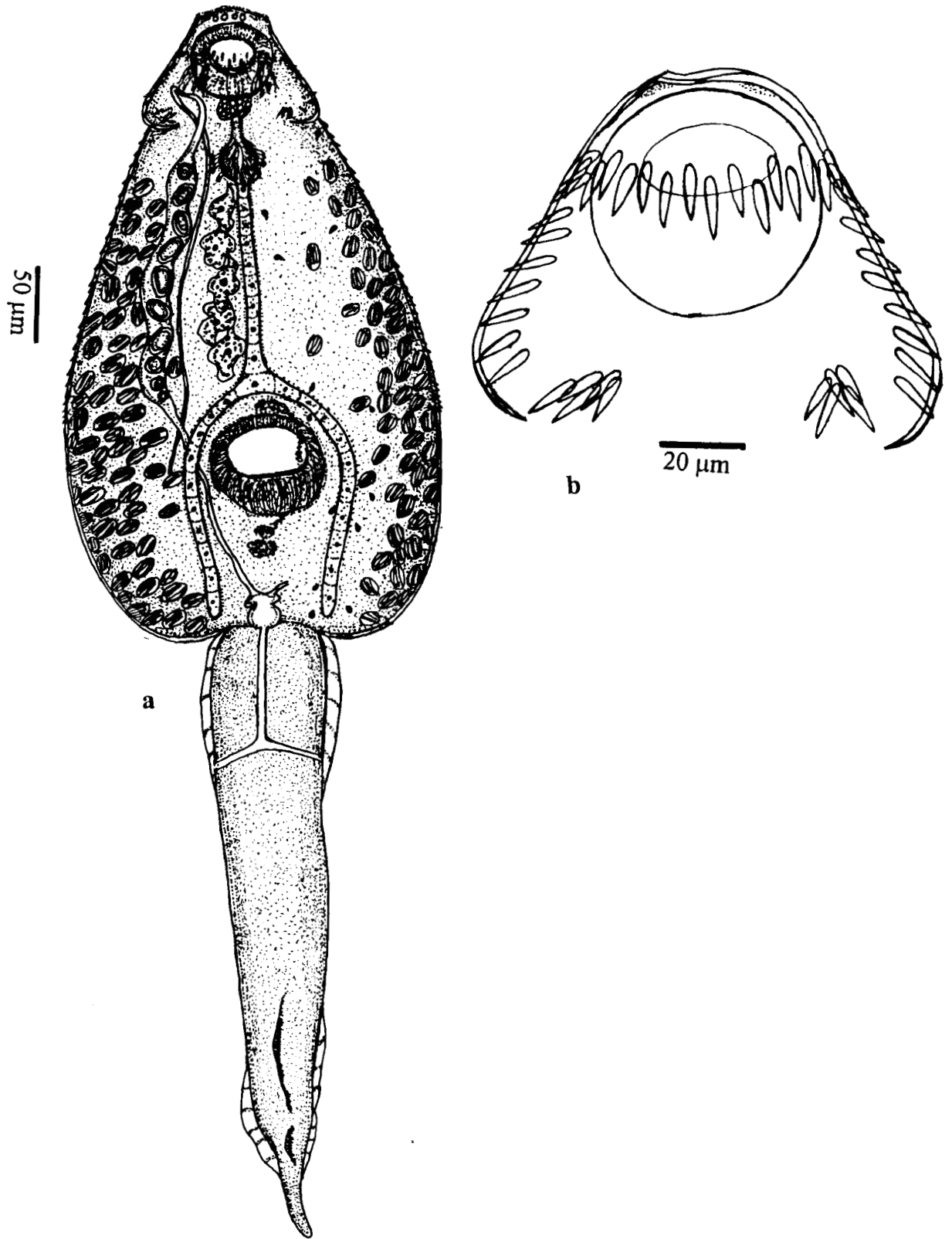


Fig. 20. *Echinostoma* sp. III n. sp.
a. Cercaria; b. Collar with collar spines.

Table 12. Measurements of cercariae of *Echinostoma* sp. III n. sp.

Characters	Range	Mean
Body	330-408 x 193-316	346 x 241
Collar width	86-102	91
Collar spines (length)	9-12	10.8
Oral sucker	51-62	58
Acetabulum	57-65 x 65-79	62 x 69
Tail	339-377 x 54-63	347 x 57
Prepharynx (length)	26-33	31
Pharynx	29-34	31
Oesophagus (length)	80-97	88
Caeca	85-103	97

Life Cycle

Metacercaria (Figs. 21a-b)

The cercariae encysted in the hepatopancreas, intestinal wall, kidney and heart of *Lymnaea luteola*, *Indoplanorbis exustus* and *Bellamya dissimilis*, and in the hepatopancreas of *Paludomous tanschauricus*. They also encysted on gill arches of tadpoles and fishes. Under experimental conditions, metacercariae were obtained from these hosts.

Cysts oval, measured 167-176 (171) x 155-164 (159). Cyst wall three-layered, 14 to 16 thick; outer layer transparent, 1.5 thick; inner layer translucent, 3 thick and middle layer transparent, 9-11 thick. Inside the cyst the larva remained curled up. Oral sucker, acetabulum, collar and parts of caeca visible through cyst wall. Excysted metacercaria measured 427-635 (547) x 277-332 (294).

The metacercariae recovered after 2 months of exposure to cercariae were infective, and chicks and albino rats fed with these metacercariae developed infection by adults.

Adults (Figs. 22a-b, 23a-b; Table 13)

In experimentally infected white leg-horn chicks and albino rats, adult worms were found to develop in the posterior region of ileum. Development of the flukes was followed in experimentally infected albino-rats.

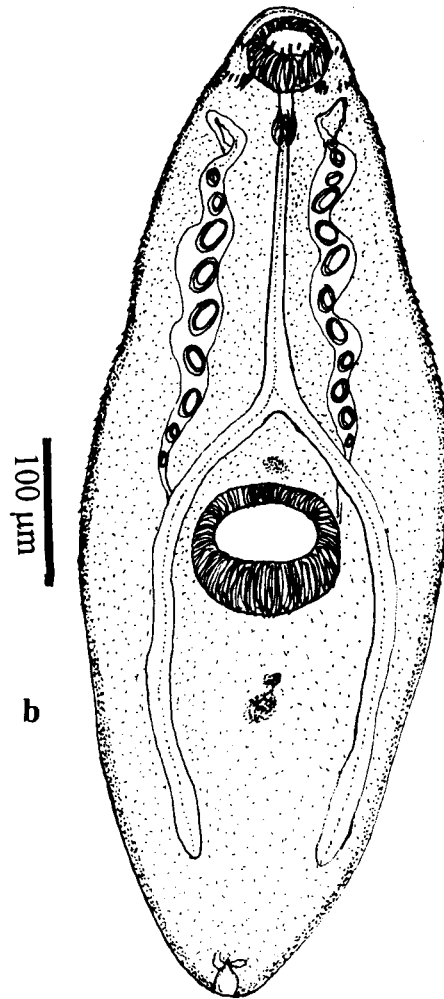
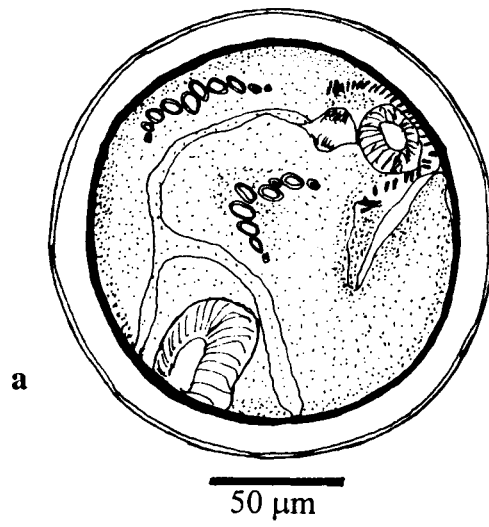


Fig. 21. *Echinostoma* sp. III n. sp.
a. Encysted metacercaria; b. Excysted metacercaria

The 3-day-old flukes measured 816-1025 (923) x 227-386 (311). The suckers increased in size; acetabulum moved to a more anterior position. Primordia of testes and ovary visible. Differentiating cirrus sac traceable between caecal bifurcation and acetabulum; its component parts not distinguishable.

The 8-day-old specimens measured 4715-5975 (4932) long, 976-1279 (1160) wide. Testes and ovary increased in size. Follicular vitellaria became visible in the extracaecal field, below the level of acetabulum. Cirrus and seminal vesicle differentiated in cirrus sac. Uterus became complexly coiled, without any eggs.

The flukes recovered on the 12th day were fully mature, measured 6271-7519 (6798) x 1544-1732 (1612), with pointed anterior and broadly round posterior ends. Body spines scale-like, extending from collar to anterior fifth on dorsal surface, to equatorial level on lateral surface, and almost all over ventral surface. Collar well-developed, 432-486 (453) wide, with 43 conspicuous spines. Spine arrangement similar to that in cercaria. Latero-aboral of corner group the largest (64 long) of all spines, and dorso-oral the smallest (44 long). Oral sucker subterminal, oval, 218-234 (222) x 193-211 (205) in size. Acetabulum in anterior fifth of body, protrusible, 577-647 (601) x 531-608 (585).

Prepharynx 179-206 (198) long; pharynx muscular, oval, measured, 156-171 (166) x 172-187 (180). Oesophagus 296-328 (312) long; caeca extending almost to posterior end of body. Testes tandem, intercaecal, in posterior half of

body. Anterior testis 764-811 (790) x 959-983 (961); posterior testis 920-952 (937) x 796-842 (830). Cirrus sac 640-702 (665) long, 187-203 (199) wide, extending postero-dorsally from genital atrium to half way of acetabulum, containing coiled, bipartite seminal vesicle, well developed pars prostatica and unspined cirrus.

Ovary pre-testicular, near equator, oval to elongate, 327-359 (338) x 359-374 (363). Ootype large, conspicuous, with well developed Mehli's gland complex. Laurer's canal median, opened dorsally. Seminal receptacle present; vitelline reservoir small, median. Uterus intercaecal, pre-testicular, with 8-14 coils containing more than hundred eggs, opening into genital atrium through metraterm. Vitellaria follicular, lateral, extending from mid-level of acetabulum to near posterior ends of caeca, confluent posterior to testes.

Excretory system consists of excretory bladder with small branches; stem more or less coiled before opening at posterior extremity.

Egg (Fig. 23c)

Eggs appear in faeces on 12th day of infection. Eggs uncleaved, ovoid, yellow-brown, measured 97-108 (104) x 53-66 (59). Operculum at one end, 27-31 wide, and a shell thickening with striated appearance at the opposite broad end.

Eggs kept in well-water at room temperature (26°-28°c) begin to hatch in 9 days. Exposure to light induces hatching, which usually occurs in the morning hours.

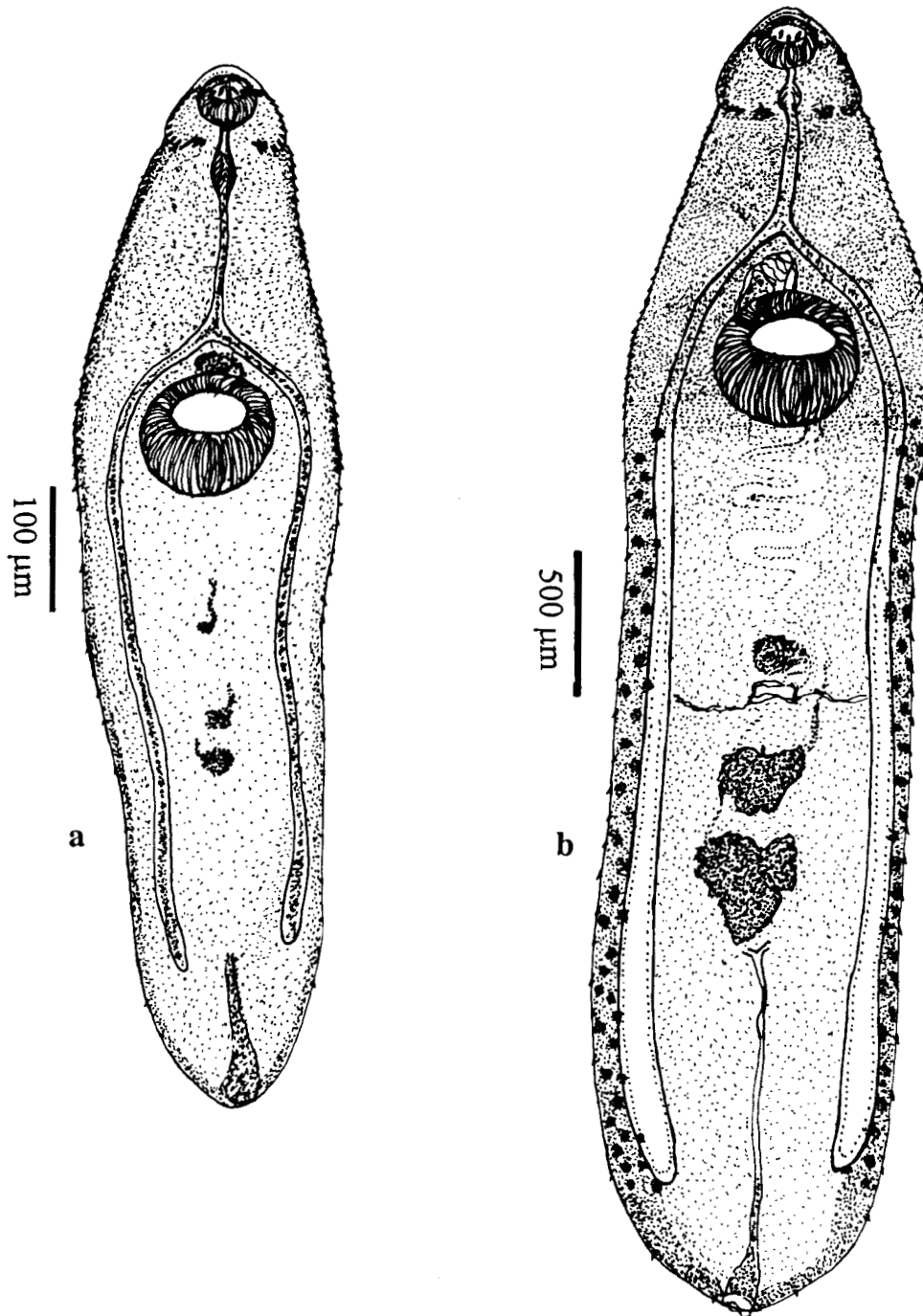


Fig. 22. *Echinostoma* sp. III n. sp.
a. 3-day-old adult; b. 8-day-old adult.

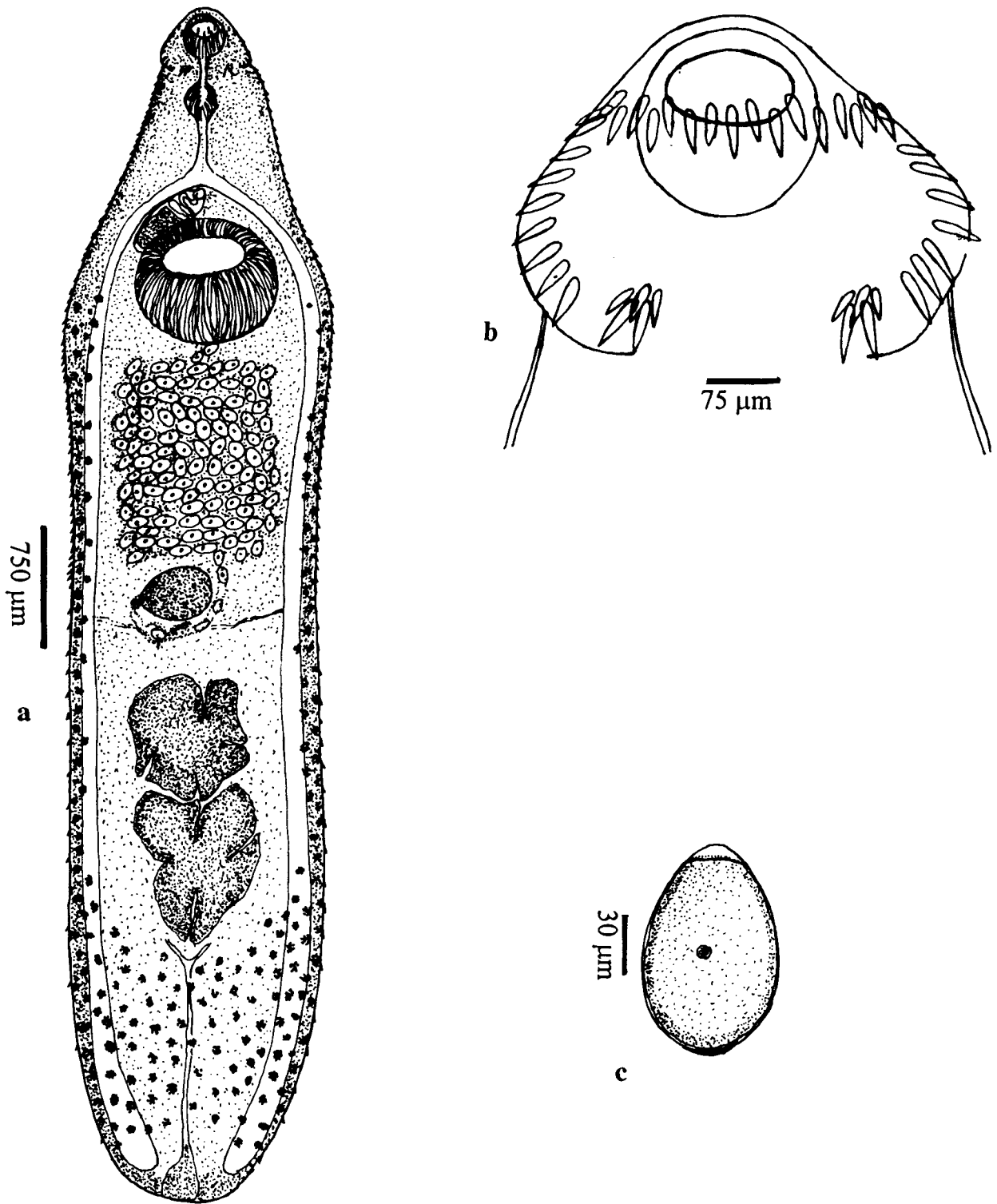


Fig. 23. *Echinostoma* sp. III n. sp.
a. Mature adult; b. Collar with collar spines; c. Egg.

Table 13. Measurements of adults of *Echinostoma* sp. III n.sp.

Character	Range	Mean
Body	6271-7519 x 1544-1732	6798 x 1612
Collar width	432-486	453
Collar spines (length)	44-64	-
Oral sucker	218-234 x 193-211	222 x 205
Acetabulum	577-647 x 531-608	601 x 585
Prepharynx (length)	179-206	198
Pharynx	156-171 x 172-187	166-180
Oesophagus (length)	296-328	312
Anterior testis	764-811 x 959-983	790 x 961
Posterior testis	920-952 x 796-842	937 x 830
Cirrus sac	640-702 x 187-203	665 x 199
Ovary	327-359 x 359-374	338 x 363
Egg	97-108 x 53-66	104 x 59

Miracidium (Figs. 24a-b)

Newly hatched miracidia swim rapidly in water, but later show creeping movements on the bottom of the container and live for about 24 hours.

Miracidium fixed in hot 2% silver nitrate solution measured, 79-87 (83) x 51-58 (54). Body covered with 4 rows of ciliated epidermal plates consisting of 6, 6, 4 and 2 plates from anterior to posterior end. Apical papilla with 2 pairs of setae, 9-10 long. Body cilia 11-15 long. Lateral process 2, found between the first and second row of epidermal plates. Primitive gut filled with refractile granules opens at the tip of apical papilla. Penetration gland 2, filled with granular contents. Eyespots median, anterior, consist of 2 pairs of dark-brown pigmented bodies lying side by side. Flame cells 2, left one ventral and posterior; right one dorsal and anterior. Two excretory pores open between third and fourth row of epidermal plates. Several germinal cells lying scattered in the posterior half of the body.

Sporocyst (Fig. 25a)

Sporocysts were recovered from heart and head regions of snail host. They are colourless, elongated sacs, 375-430 (418) x 189 -316 (261) in size, with 6-12 rediae and a few germ balls.

Redia (Figs. 25b-c)

Mother rediae were found in heart, muscles and hepatopancreas of snails. Mother rediae are elongate, light yellow and measured 528-965 (619) x 298-405 (371). Collar prominent, 210-322 (276) wide, 226-419 from anterior end;

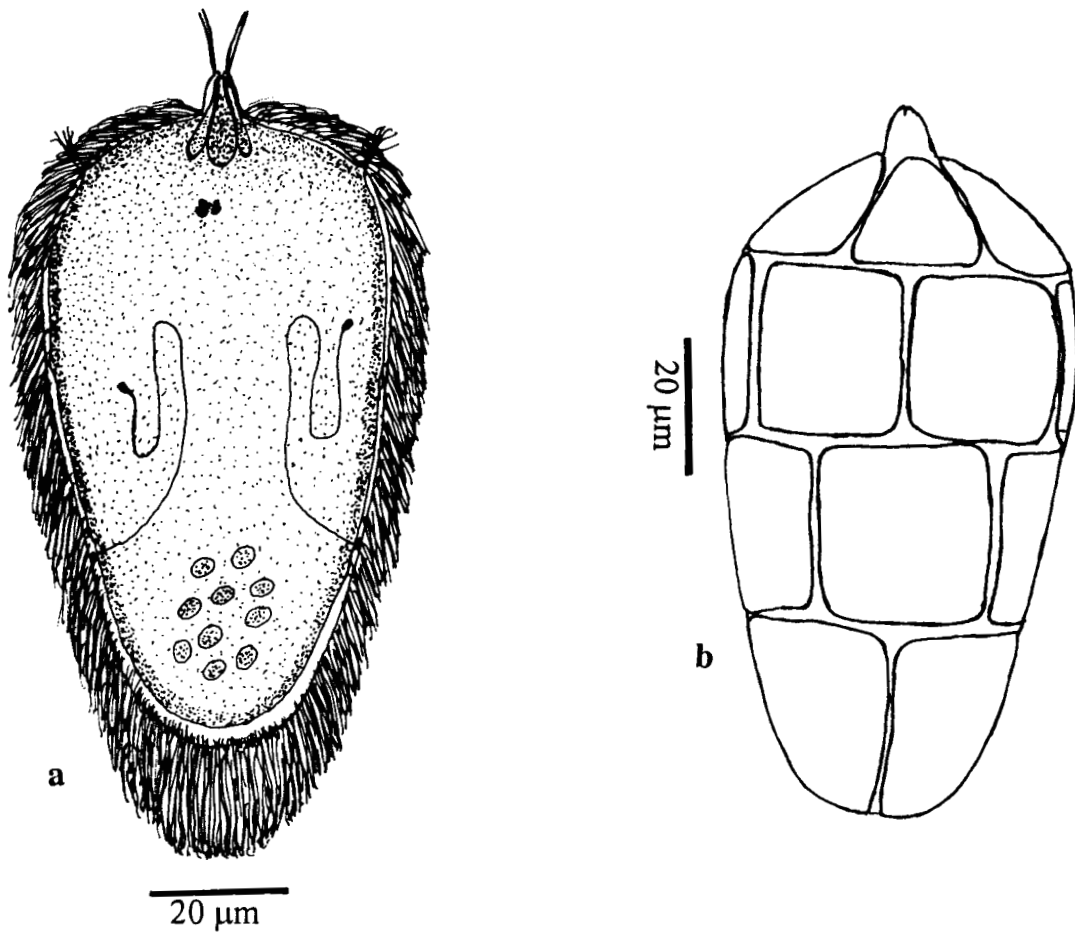


Fig. 24. *Echinostoma* sp. III n. sp.
a. Miracidium; b. Epidermal plates of miracidium.

locomotor processes 173-378 from posterior end. Mouth terminal; pharynx large, globular, 120-192 (166) in diameter; gut 330-726 (433) long, filled with dark-brown contents, and reaching the level of locomotor processes. Fully grown mother redia contained 16-20 daughter rediae and some germ balls at different stages of development. Birth pore not discernible.

Daughter rediae were found in hepatopancreas of snails. Body elongate, motile, yellow-brown, 708-1950 (1690) x 137-496 (328); contain 6-10 cercariae and many germ balls. Birth pore dorsal and immediately posterior to collar.

Natural definitive host	:	Unknown
Experimental hosts	:	Albino-rats and white leg-horn chicks
Location	:	Posterior region of ileum
First intermediate host	:	<i>Lymnaea luteola</i>
Locality	:	Trikaripar in Kasargod district and Cherukunnu and Pinarayi in Kannur district.
Period of Collection	:	October and December 1997, February to April 1998, July 1998.
Prevalence	:	1.3 % (37 out of 2850 were infected)
Second intermediate host	:	<i>L. luteola</i> , <i>Indoplanorbis exustus</i> , <i>Bellamya dissimilis</i> , <i>Paludomous tanschauricus</i> and fishes and tadpoles.
Holotype	:	To be deposited in the parasite collections, Parasitology Laboratory, Department of Zoology, University of Calicut, Kerala (India).

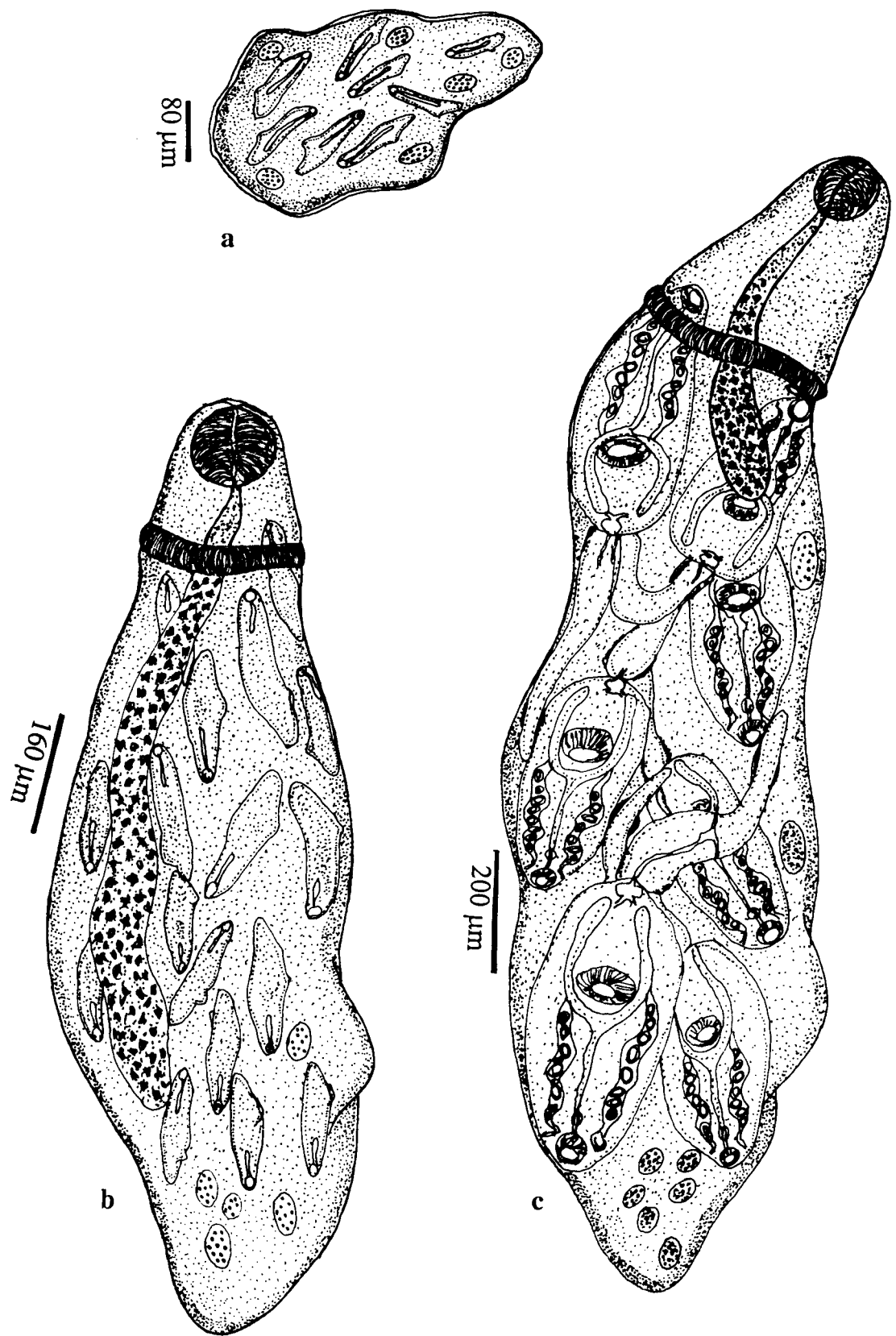


Fig. 25. *Echinostoma* sp. III n. sp.

a. Sporocyst; b. Mother redia with daughter rediae and germ balls;
 c. Daughter redia with cercariae and germ balls

Remarks

The adults obtained experimentally from albino-rats and white leg-horn chicks are characterised by the presence of well-developed collar with 43 spines arranged in double uninterrupted rows, maximally wide acetabular zone, pre-acetabular cirrus sac, coiled seminal vesicle and highly coiled uterus with numerous eggs. Moreover, the cercaria of the present fluke has a finger-like process at the tip of tail and finfolds on the tail. Based on these characters, the present form is included under the genus *Echinostoma* (Frölich, 1802) Dietz, 1909 of the family Echinostomatidae Poche, 1926.

A comparison of characters of all known species of *Echinostoma* with that of present fluke revealed that it is distinctly different from them in one or more characters. However, it deserves comparison with *E. malayanum* Leiper, 1911, the only species of *Echinostoma* having 43 collar spines reported from India. From the descriptions of adults and life cycle stages of *E. malayanum* given by Lie (1963a) and Mohandas (1971) and in this thesis, it is apparent that the present form is different in the arrangement of collar spines, position and size of cirrus sac, nature of testes and in having a seminal receptacle and highly coiled cirrus. Besides, *E. malayanum* is found to develop only in mammals, whereas the present form develops in mammals and birds, although its natural definitive host is not known. The cercariae of

the two species show differences in collar spine arrangement, number of cells in oesophagus and caeca, number of penetration glands and in flame cell formula. The hosts are also different, *Lymnaea luteola* is not the natural host of *E. malayanum*.

Cercariae indicae XLVIII Sewell, 1922 infecting *Indoplanorbis exustus* in Mananthody and Sultan Battery is the only other Indian cercaria with tail finfolds and 43 collar spines. But the presence of setae and number of oesophageal and caecal cells is neither mentioned in the text nor represented in the figure by Sewell (1922). Besides, he recorded the presence of only one pair of penetration glands in the text and figure. Therefore, with the available data it is not possible to compare the present cercaria with *C. indicae* XLVIII Sewell, 1922.

Since the present adult cannot be identified with any known species of *Echinostoma*, it is considered as new and named *Echinostoma* sp. III n. sp.

***Echinoparyphium* sp. I n. sp.**

Cercaria (Figs. 26a-b; Table 14)

Cercariae with 45 collar spines were found emerging from the pulmonate snails, *Lymnaea luteola*. The snails collected from Feroke and Ramanattukara in Kozhikkode, and Idimuzhikkal and Olippuramkadavu in Malappuram districts of

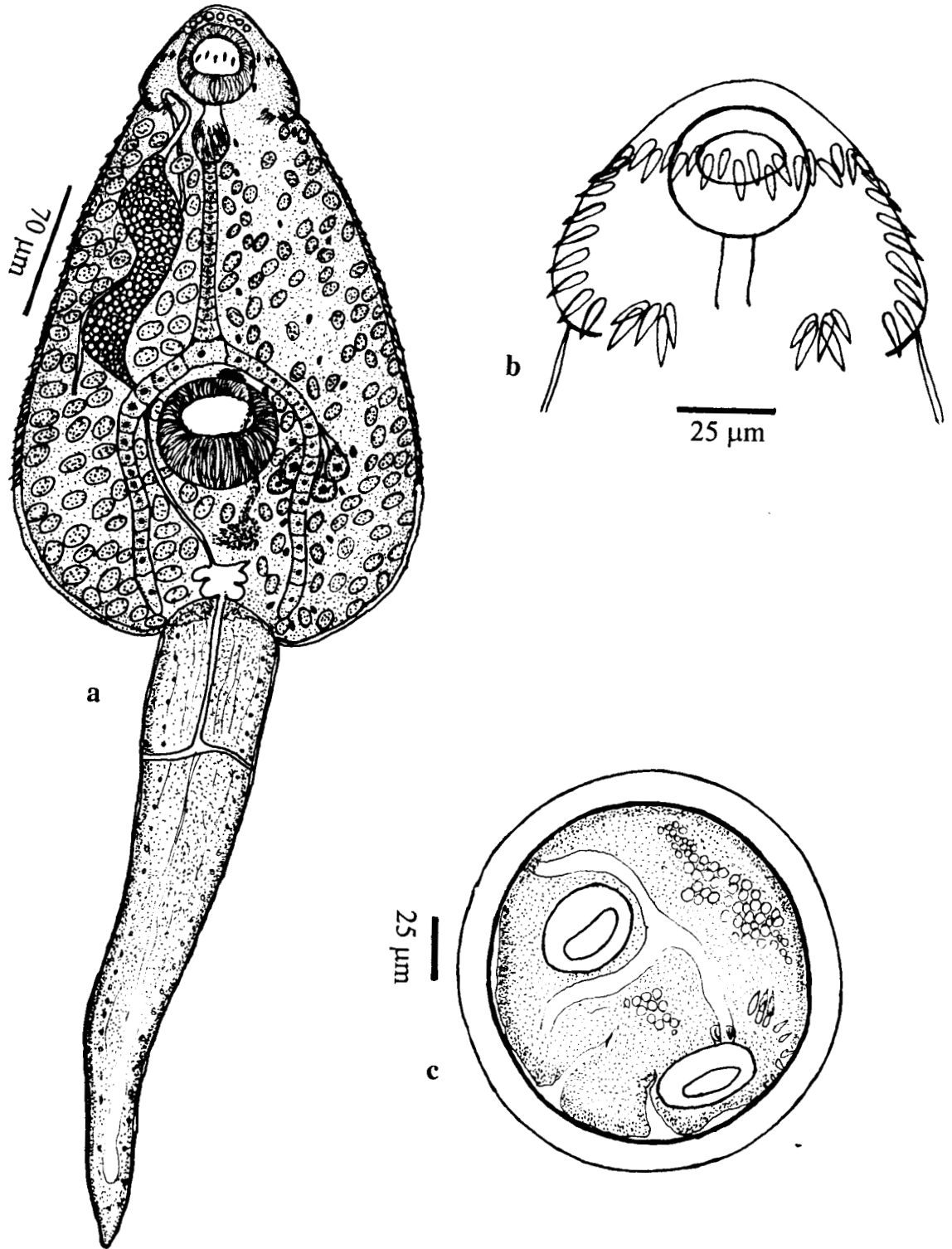


Fig. 26. *Echinoparyphium* sp. I n. sp.
a. Cercaria; b. Collar with collar spines; c. Encysted metacercaria.

Kerala were found to be infected. Infections were restricted to the period extending from September to February.

Behaviour : Cercariae emerged in large numbers in the morning hours, and emergence continued throughout day-time. They moved actively and remained alive for about 10 h in well-water.

Description: Body ovoid, 315-442 (398) x 154-193 (178) in size; cuticular spines arranged in transverse rows dorsally, extending from anterior end to posterior margin of acetabulum. Collar prominent, 90-126 (113) wide, with 45 spines, 9-16 (13) in length; spine arrangement characteristic: five corner spines on each ventral lappet, 3 oral and 2 aboral; 6 laterals on either side in single row; 23 dorsals, 12 oral and 11 aboral. Oral sucker circular, 40-56 (49) in diameter. Acetabulum post-equatorial, protrusible, ovoid, measured 58-76 (71) x 63-84 (79). Tail aspinose, conical without fin folds and sensory hairs; measured 306-467 (401) x 50-72 (68) in size.

Prepharynx short, narrow, 8-15 (13) long; pharynx well-developed ovoid, 27-39 (35) x 18-23 (21) in size. Oesophagus solid, narrow, 98-137 (112) long, consisting of 14-17 cells, with round nuclei and finely granular cytoplasm. Caeca solid, narrow, extending to posterior end of body, 102-149 (127) in length, each caecum consisting of 14 rectangular cells with an additional cell at bifurcation.

Penetration glands 4 pairs, situated posterior to or on the sides of acetabulum; duct outlets 8 in number, on dorsal lip of oral sucker. Cystogenous gland-cells numerous, distributed throughout body except at collar region; contain oval or round contents.

Genital primordia consist of two masses of cells, one at anterior margin of acetabulum and the other between acetabulum and base of tail, and connected by a string of cells passing dorsal to acetabulum. Excretory system stenostomate; excretory bladder bipartite, at posterior end of body; main collecting ducts dilated between pharynx and acetabulum, each containing 130-180 yellow-green concretions. Caudal excretory duct extends to anterior fifth of tail, then bifurcates, and opens out laterally. Flame cell formula: $2(3+3+3)+(3+3+3+3) = 42$.

Life Cycle

Metacercaria (Figs. 26c; 27a)

The cercariae encysted in the pericardial cavity, kidney, gonad and occasionally in the hepatopancreas of the host snail which liberated the cercariae. Metacercariae were also recovered from *Indoplanorbis exustus*, *Bellamya dissimilis*, *Thiara tuberculata* and *Paludomous tanschauricus*.

Table 14. Measurements of cercariae of *Echinoparyphium* sp. I n. sp.

Characters	Range	Mean
Body	315-442 x 154-193	398 x 178
Collar width	90-126	113
Collar spines (length)	9-16	13
Oral sucker	40-56	49
Acetabulum	58-76 x 63-84	71 x 79
Tail	306-467 x 50-72	401 x 68
Prepharynx (length)	8-15	13
Pharynx	27-39 x 18-23	35 x 21
Oesophagus (length)	98-137	112
Caeca (length)	102-149	127

Description: Cysts round to oval, 154-180 (165) x 137-169 (146) in size. Cyst wall double-layered with an outer, thin, transparent layer, 13-16 thick and an inner, translucent layer, 3-4 thick. Inside the cyst the larva remained curled up. Oral sucker, acetabulum, collar, excretory granules and digestive tract were visible through cyst wall.

Metacercariae exposed of digestive juice of white leg-horn chicks excysted in 4-5 hours. The excysted metacercariae measured 520-619 (566) x 147-202 (176). Five to 7 day-old metacercariae were found to be infective; albino rats fed with these metacercariae developed infection.

Adult (Figs. 27b, 28a-c; Table 15)

One-month-old albino rats force-fed with infective metacercariae developed mature adults in 8 days.

The 2-day-old flukes measured 610-706 (648) x 159-211 (173). Body divisible into a broad pre-acetabular region with minute spines or scales arranged in transverse rows and a narrow tapering post-acetabular region without spines. Oesophagus and gut increased in length and genital primordia appeared as aggregations of cells.

The 5-day-old specimens measured 1507-2136 (1718) x 276-342 (302). Differential growth of the anterior and posterior halves of body shifted the

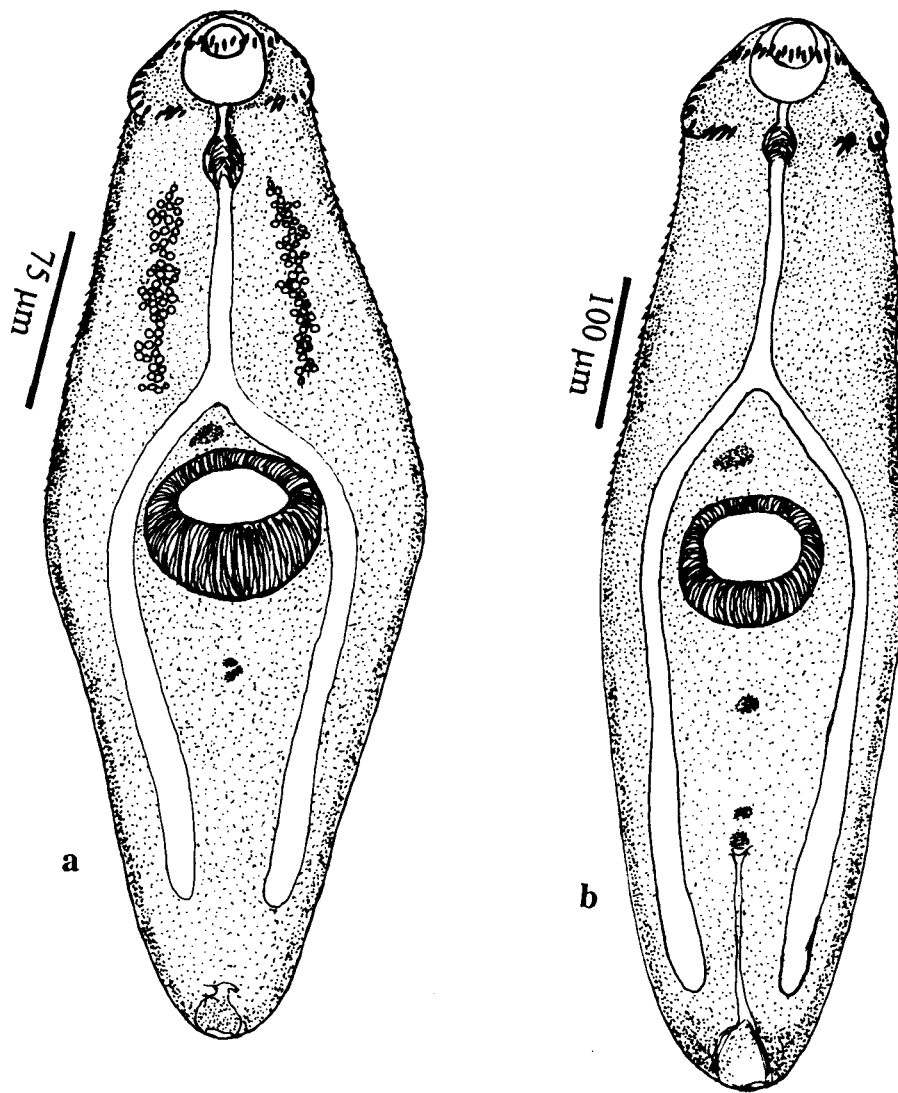


Fig. 27. *Echinoparyphium* sp. I n. sp.
a. Excysted metacercaria; b. 2-day-old adult.

acetabulum from its median to a more anterior position. The suckers increased in size; primordia of cirrus sac, ovary and testes visible.

Flukes recovered on the 8th day were fully mature, filled with a few eggs in their uterus. Body elongate with broad pre-acetabular and slender post-acetabular regions; measured 3154-4418 (3742) x 416-692 (523). Body spines limited to pre-acetabular region at ventral side, half of the body from anterior end at dorsal and lateral sides. Collar well developed, 266-327 (298) wide, with 45 conspicuous collar spines measuring 46-61 (54) x 6.3-9.5 (7.8); spine arrangement similar to that in cercaria. Latero-aboral of corner spines usually the largest (61 long) of all collar spines and latero-oral of dorsal spines the smallest (46 long). Oral sucker measured 107-136 (119) x 114-139 (123). Acetabulum muscular, protrusible, in anterior one-fourth of body; measured 320-432 (359) x 299-499 (416) in size.

Prepharynx 20-24 long; pharynx ovoid, 93-144 (114) x 90-122 (107) in dimensions; oesophagus 302-410 (382) long, bifurcating in front of acetabulum; caeca almost reaching posterior end of body. Genital pore immediately pre-acetabular, followed by genital atrium. Testes tandem, smooth, ovoid, intercaecal, post-equatorial. Anterior testis 415-479 (426) x 266-318 (296); posterior testis 455-598 (531) x 242-346 (316). Cirrus sac ovoid, antero-lateral to acetabulum, behind caecal bifurcation, measured 166-269 (238) x 81-130 (118); contained bipartite, coiled seminal vesicle and highly protrusible,

unspined cirrus, measuring 413-457 (431) in length. Pars prostatica apparently lacking. Genital pore immediately pre-acetabular, followed by genital atrium.

Ovary spherical or ovoid, equatorial, measured 246-378 (319) x 232-359 (299). Laurer's canal opening medially on dorsal surface posterior to ovary. Mehlis' gland complex well developed, between ovary and anterior testis. Uterine seminal receptacle filled with masses of sperms. Vitelline reservoir small, post-ovarian. Uterus inter-caecal, with 3 - 5 coils, containing as many as 27 eggs. Vitellaria large follicular, overlapping caeca in lateral fields, extending from the level of posterior margin of acetabulum to posterior end of body, often confluent dorsally posterior to testes, but seldom ventrally. Excretory bladder opening at posterior end.

Egg (Fig. 29a)

Eggs appear in faeces of albino rats 8 days after infection. Eggs ovoid, yellow-brown, with conspicuous operculum at narrow end; shell thickening with striated appearance at opposite end; measured 109-132 (123) x 69-86 (81). Eggs kept in well-water at room temperature (26°-28°C) hatched in 12 days. Exposure to light stimulated hatching.

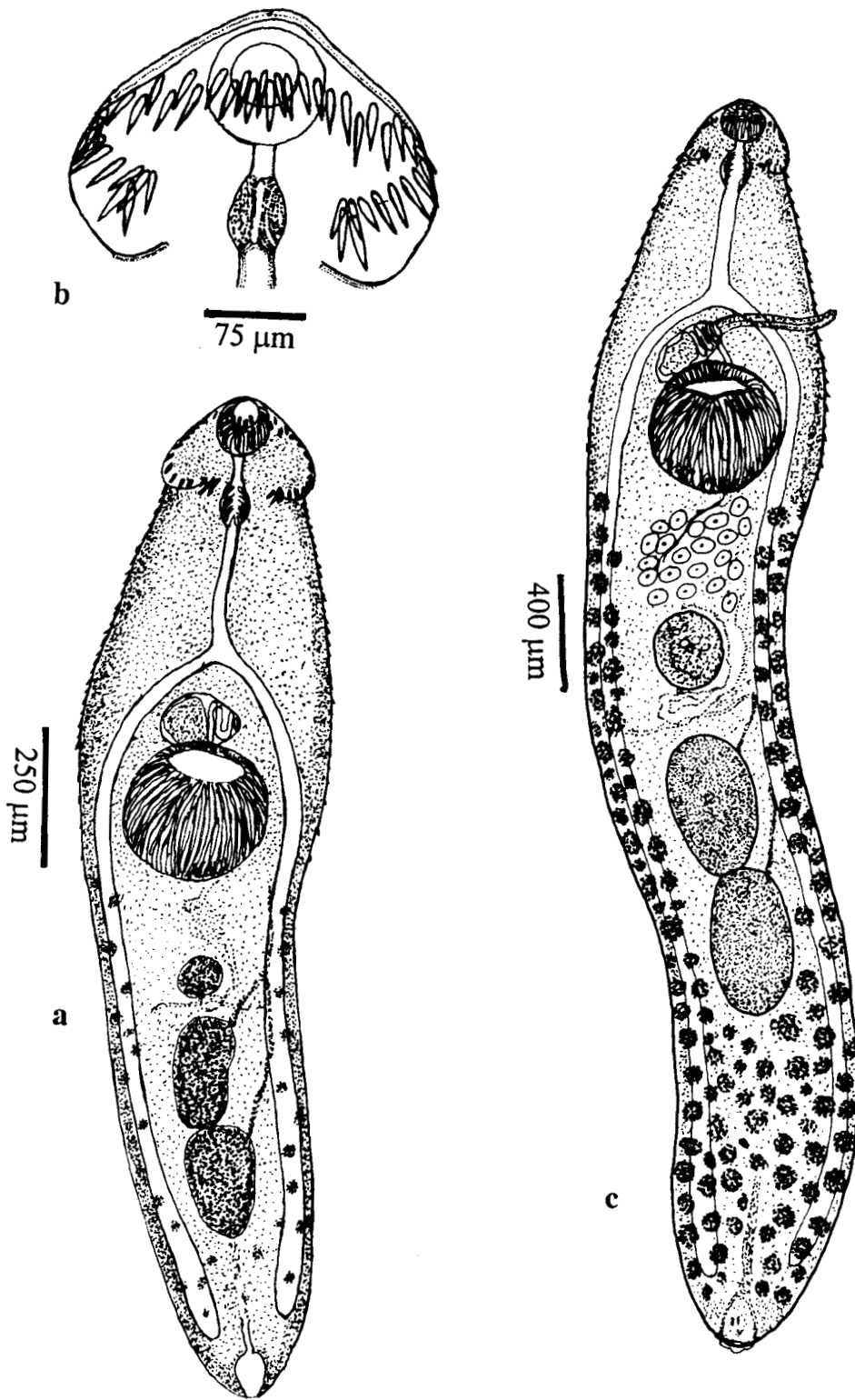


Fig. 28. *Echinoparyphium* sp. I n. sp.
a. 5-day-old adult; b. Collar with collar spines; c. Mature adult.

Table 15. Measurements of adults of *Echinoparyphium* sp. I n. sp.

Character	Range	Mean
Body	3154-4418 x 416-692	3742-523
Collar width	266-327	298
Collar spines	46-61 x 6.3 -9.5	54 x 7.8
Oral sucker	107-136 x 114-139	119 x 123
Acetabulum	320-432 x 299-499	359 x 416
Prepharynx (length)	20-24	-
Pharynx	93-144 x 90-122	114 x 107
Oesophagus (length)	302-410	382
Anterior testis	415-479 x 266-318	426 x 296
Posterior testis	455-598 x 242-346	531 x 316
Cirrus sac	166-269 x 81-130	238 x 118
Cirrus	413-457	431
Ovary	246-378 x 232-359	319 x 299
Egg	109-132 x 69-86	123-81

Miracidium (Figs. 29b-c)

Miracidia measured 116-129 (121) x 41-47 (43) in size. Body covered with 4 rows of ciliated epidermal plates consisting respectively 6,6,4, and 2 plates from anterior to posterior end; cilia 15-18 long. Apical papilla 11 x 6 in size with 2 bunches of setae. Lateral processes absent; primitive gut filled with refractile granules open at tip of apical papilla. Penetration glands 2, one on either side of gut and filled with hyaline contents. Eyespots anterior, median, consisting of 2 pairs of dark brown pigmented bodies lying side by side. Flame cells 2; left, ventral and posterior, right, dorsal and anterior with duct openings between third and fourth rows of epidermal plates. A few germ balls occupy the posterior half of body.

Sporocyst (Fig. 30a)

Sporocysts were recovered from the pericardial cavity of snails. Fully developed sporocysts colourless, elongated, sac-like bodies measuring 80-137 x 66-102 (112-87) in size; contained mature and immature rediae and some germ balls.

Redia (Fig. 30 b-c)

Mother rediae released from sporocysts were found in pericardial cavity and kidney of snails. Newly released mother rediae colourless, motile, with conspicuous collar and locomoter processes. Fully developed mother rediae measured 226-418 (340) x 106-129 (116) in size, filled with 6-8 daughter rediae and many germ balls.

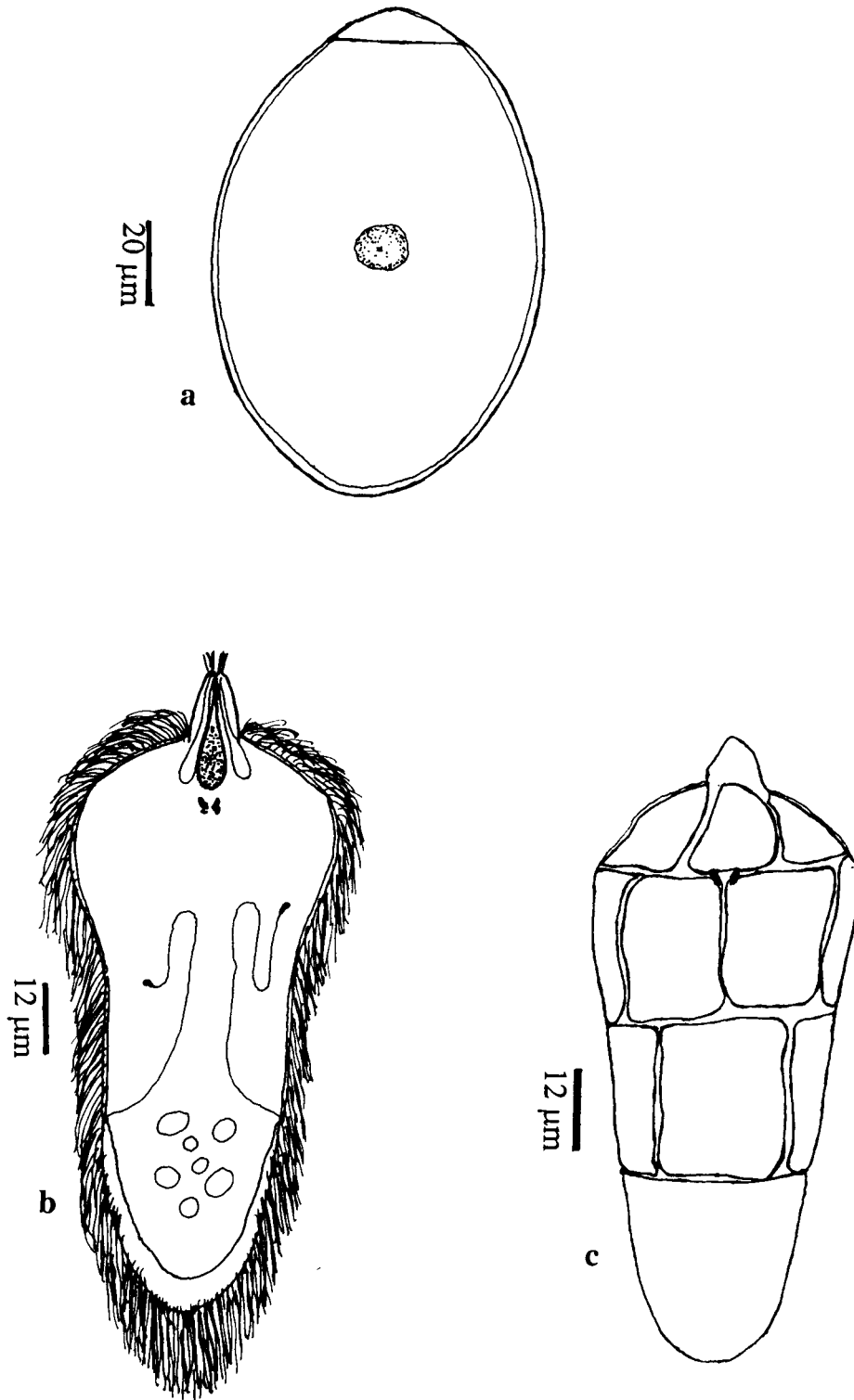


Fig. 29. *Echinoparyphium* sp. I n. sp.
a. Egg; b. Miracidium; c. Epidermal plates of miracidium.

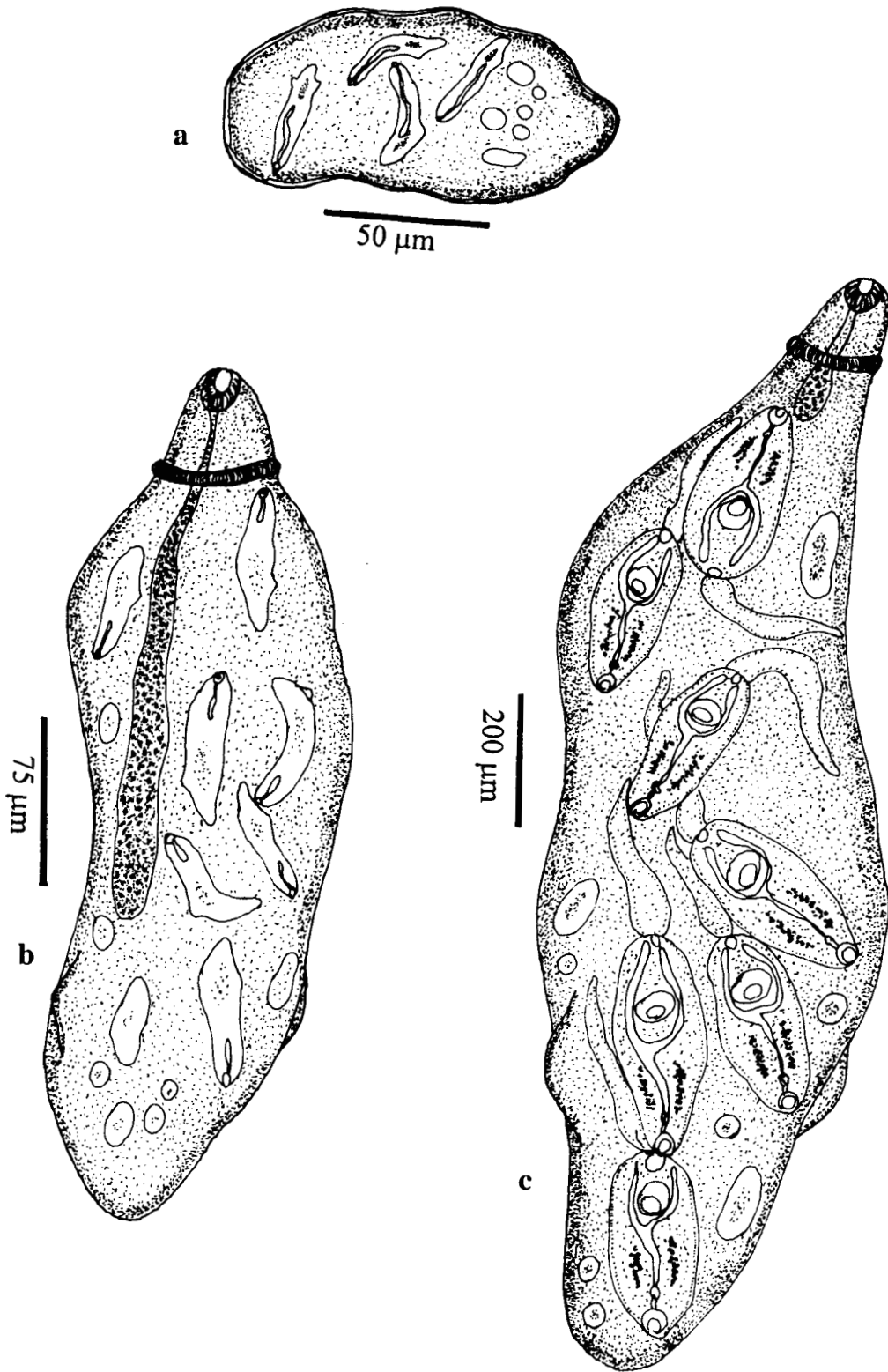


Fig. 30. *Echinoparyphium* sp. I n. sp.

a. Sporocyst; b. Mother redia with daughter rediae and germ balls; c. Daughter redia with cercariae and germ balls.

Daughter rediae were recovered from hepatopancreas, kidney and gonad of snails. Mature daughter rediae elongated, motile, yellow-brown, 766-2067 (1744) x 179-508 (348) in size; contained 2-8 cercariae and a few germ balls. Mouth terminal, pharynx 61-89 (78) x 58-84 (74) in size. Collar distinct, 94-133 (126) wide. Birth pore not discernible. Gut saccular, 166-403 (358) long, filled with dark-brown contents. Locomotor processes 2, situated 142-561 from posterior end of body.

Natural definitive host	:	Not known
Experimental host	:	Albino-rats
Location in the host	:	Duodenum and anterior region of ileum
First intermediate host	:	<i>Lymnaea luteola</i>
Locality	:	Feroke and Ramanattukara in Kozhikode district and Idimuzhikkal and Olippuramkadavu in Malappuram district.
Period of collection	:	September to November 1996, October 1997, January to February 1998, November and December 1998.
Prevalence	:	1.61 % (46 out of 2850 were infected)
Second intermediate hosts	:	<i>Lymnaea luteola</i> , <i>Indoplanorbis exustus</i> , <i>Bellamya dissimilis</i> , <i>Thiara tuberculata</i> and <i>Paludomous transchauricus</i>
Holotype		To be deposited in the parasite collections, Parasitology Laboratory, Department of Zoology, University of Calicut, Kerala (India).

Remarks

The present fluke developed experimentally in albino rats has slender body which is attenuated posteriorly. Head collar is well-developed, reniform, with double dorsally uninterrupted row of 45 spines. Spines in aboral row are larger than spines in oral row. Acetabulum is in anterior fourth of body. Testes are tandem, smooth, ovoid, intercaecal and post-equatorial. Cirrus sac is ovoid and antero-lateral to acetabulum behind caecal bifurcation. Pars prostatica feebly developed. These characters amply justify its conclusion under the genus *Echinoparyphium* Dietz, 1909 of the family Echinostomatidae Poche, 1926.

Cercaria of the present fluke is characterised by the presence of a conical tail without finfolds, which according to Fried *et al.* (1998) is found in the cercaria belonging to the genus *Echinoparyphium*. This cercarial character further justifies the inclusion of the fluke under *Echinoparyphium*.

Within the genus *Echinoparyphium* about 40 species have been described (Skryabin, 1956, Yamaguti, 1971). But according to the comprehensive key presented by Buscher (1978) only 25 species have been recognized as valid. This list did not include 3 species reported by Sing (1975a, 1975b and 1976). Later, Kiseliene and Grabda-Kazubska (1990) added another species making the total number of valid species 29. The present worm has spinose body with 45 collar spines, and therefore, only the species having these characters can be considered as relatives of the present form. These conditions fulfilled by other species are *E. recurvatum* (Linstow, 1873), *E. flexum* (Linton, 1892), *E. mordwilkoii* Skryabin,

1915, *E. ellisi* Johnston and Simpson, 1944, *E. baculoides* Dollfus, 1951, *E. hydromyos* Angel, 1966, *E. lanceolatum* Singh, 1975, *E. vitellocompactum* Singh, 1976 and *E. pseudorecurvatum* Kiseliene and Grabda-Kazubaska, 1990. The new species differs from *E. recurvatum*, *E. hydromyos*, *E. lanceolatum*, *E. pseudorecurvatum*, *E. mordowilkoi* and *E. baculoides* by body dimensions, collar spine arrangement, distribution of vitellaria position and size of cirrus sac and testes and lack of pars prostatica. Besides, all these species show differences in cercarial characters.

Comparative study of characters of the remaining 3 species, *Echinoparyphium ellisi*, *E. flexum* and *E. vitellocompactum*, with that of the present form (table 16) revealed that although differences among adults are not significant, the cercariae of the 4 species differ markedly. The present cercaria, differs from the other 3 in the arrangement of collar spines, number and position of penetration glands, number of cells in oesophagus and caeca, number and pattern of arrangement of flame cells and in body size. In contrast, the present cercaria correspond very well with the description of *Cercaria* sp. VII Kerala by Mohandas (1981), although there are minor differences in body proportions and dimensions of internal structures. These variations may be due to differences in fixing the material for collecting mensural data. Therefore, the present cercaria found in *Lymnaea luteola* collected from in Kozhikode and Malappuram districts is identical with *Cercaria* sp. VII Kerala Mohandas, 1981 reported from the same host species in Trichur, Palghat and Ernakulam districts of Kerala.

Table 16. Comparative characters and measurements of adults of *Echinoparyphium* sp.I n. sp. and of the 3 related species

Characters	<i>Echinoparyphium ellisi</i> Johnston & Simpson, 1944	<i>E. flexum</i> (Linton, 1892)	<i>E. vitellocompactum</i> Singh, 1976	<i>Echinoparyphium</i> I n..sp.
Body size	-	3800-4000 x 500-550	2650-2880 x 221-260	3154-4418 x 416-692
Body spination	-	Body spinose, dorsally, spine extend from posterior margin & collar to acetabulum; ventrally, spine extend to equator of body	Body spines extend upto acetabular level	Spinose
No. of Collar spines and arrangement	45 4 corner spines	45 (4+11+16+10+4)	45 (5+6+23+6+5)	45 (5+6+23+6+5)
Cirrus sac	Not extending to posterior edge of acetabulum	Between caecal bifurcation and acetabulum	Antero-lateral to acetabulum	Antero-lateral to acetabulum
Ovary		Spherical	Sub-spherical	Spherical to ovoid
Testes	-	Tandem, longitudinally elongate	Tandem, ovoid or elongate	Tandem, smooth, ovoid
Vitellaria	Extending from between acetabulum and ovary to body end.	Extending from posterior margin of acetabulum to the posterior end of body; not confluent	Extending the posterior margin of acetabulum to posterior end, confluent	Extending from posterior margin of acetabulum to posterior end of body; often confluent
Definitive host	Birds	Birds	Birds	Mammals

Considering the differences with the related *Echinoparyphium* species, the present fluke is recorded here as *Echinoparyphium* sp.I n.sp. This fluke is the adult of *Cercaria* sp. VII Kerala Mohandas, 1981.

Natural definitive host of the present fluke is not known. Metacercariae were found to develop experimentally in albino-rats; consistent attempts made to develop the metacercariae in white leg-horn chicks were futile.

***Paryphostomum giganticum* Rai and Agarwal, 1961**

Natural infections by cercariae of *Paryphostomum giganticum* Rai and Agarwal, 1961 were found in 76 of 2850 (2.99%) *Lymnaea luteola* collected from different localities. Maximum prevalence of infection was observed in the beginning of monsoon season (May-June).

The cercariae encysted in the same snail species or in other snails, *Indoplanorbis exustus* and *Bellamya dissimilis*, in the same biotopes where the cercarial infections were present. Metacercariae, obtained from natural infections or from laboratory-raised clean snails, *L. luteola*, *I. exustus* and *B. dissimilis* which had previously been exposed to cercariae, and fed to one-day-old white leg-horn chicks developed into adults. The life cycle of *Paryphostomum giganticum* has been established in the laboratory, and published in *Rivista di Parassitologia* (XVIII (LXII) 1: 46-51). A copy of the publication is included under Appendix.

Cercariae indicae XX Sewell, 1922

Cercaria (Figs. 31a, 32a; Table 17)

592.48
VEN/L

Infection by this cercaria which has 31 collar spines was found in the planorbid snails, *Indoplanorbis exustus*. The infected snails were collected from Thazhechovva in Kannur district of Kerala. Infection was fairly common during December-May.

Behaviour: The cercariae emerged from the snails immediately after sunrise, and emergence continued till evening. They were found swimming actively for some time and remained alive for about 12 h in well-water.

Description: Body ovoid, spinose, with 10-12 pairs of sensory hairs at anterior half; measured 303-360 (334) x 199-244 (216) in size. Collar inconspicuous, 89-106 (94) wide, with 31 spines, 9-17 (14) long. Spine arrangement characteristic: five corner spines on each ventral lappet, 3 oral and 2 aboral; 6 laterals in single row on each side; 9 dorsals, 5 oral and 4 aboral. Oral sucker slightly ovoid, 40-51 (46) x 43-54 (49) in size. Acetabulum post-equatorial, protrusible, measured 44-57 (49) x 51-62 (57). Tail cylindrical, aspinose, with 6 pairs of sensory hairs at anterior half; measured 316-380 (349) x 57-70 (66).

Prepharynx 19-25 (23) long. Pharynx oval, muscular, 26-33 (29) x 17-22 (20); oesophagus solid, 76-94 (87) long, consisting of 8 cells. Intestinal caeca solid, 142-176 (161) long, extending to the level of excretory bladder; each caecum with 6 rectangular cells and a medium cell at the point of bifurcation.

Penetration glands 6 pairs, along oesophagus with gland-duct openings on dorsal lip of oral sucker. Paraoesophageal gland-cells 12-16 in number, with duct openings around oral sucker and pharynx; cystogenous glands numerous, distributed from the level of pharynx upto body end, and contain rod-shaped contents arranged in parallel bundles.

Genital primordia consist of 2 masses of cells, one at anterior margin of acetabulum, the other behind acetabulum and connected by a string of cells passing dorsal to acetabulum. Excretory system stenostomate type; excretory bladder bipartite, at posterior end; main collecting ducts distended between pharyngeal and acetabular levels and filled with 20-40 excretory concretions. Caudal excretory duct extends to anterior-fourth of tail, then bifurcates into two branches opening laterally. Flame cell formula: $2[(3+3+3)+(3+3+3)] = 36$.

Metacercaria (Fig. 31 b)

Cercariae encysted on the gill arches and pharyngeal wall of the fish *Rasbora daniconius*. The snails, *Indoplanorbis exustus*, *Lymnaea luteola* and *Bellamya dissimilis* were not susceptible to metacercarial infection.

Cysts spherical, 99-130 (117) in size. Cyst wall double-layered; an outer, transparent layer, 7-9 thick, and an inner, opaque layer, 1.5-2.5 thick. Structures like suckers, collar spines and caeca were visible through cyst wall.

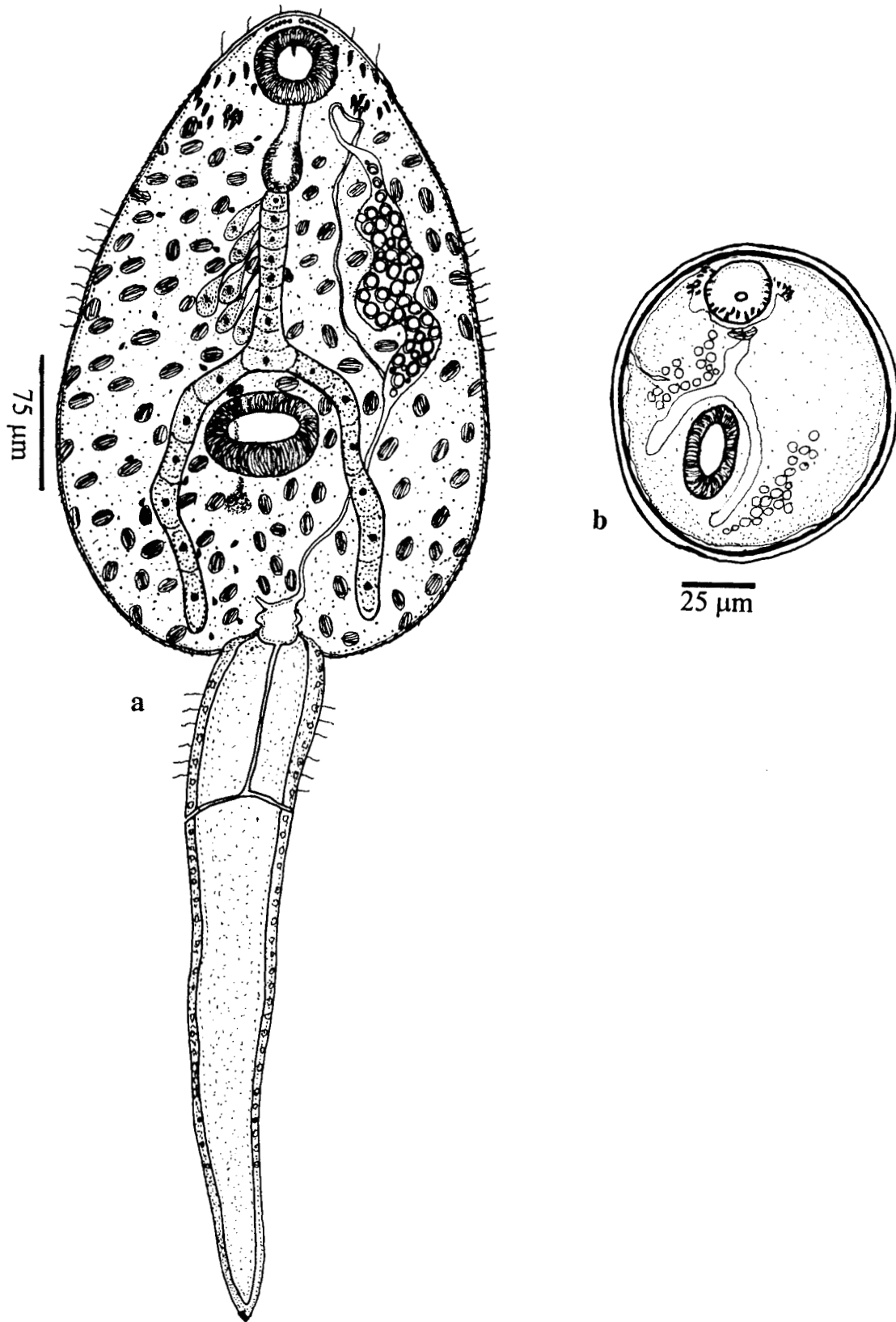


Fig. 31. *Cercariae indicae* XX Sewell, 1922
a. Cercaria; b. Encysted metacercaria.

Table 17. Measurements of *Cercariae indicae* XX Sewell, 1922

Character	Range	Mean
Body	303-360 x 199-244	334 x 216
Collar width	89-106	94
Collar spines (length)	9-17	14
Oral sucker	40-51 x 43-54	46 x 49
Acetabulum	44-57 x 51-62	49 x 57
Tail	316-380 x 57-70	349 x 66
Prepharynx (length)	19-25	23
Pharynx	26-33 x 17-22	29 x 20
Oesophagus (length)	76-94	87
Caeca (length)	142-176	161

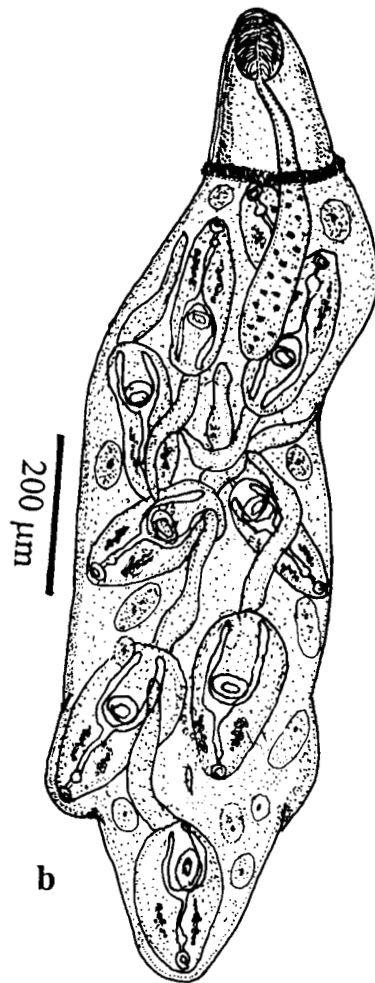
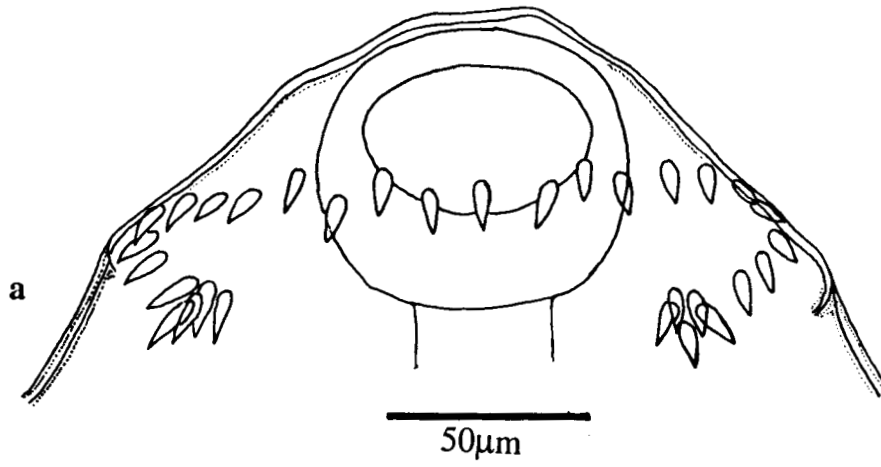


Fig. 32. *Cercariae indicae* XX Sewell, 1922

a. Collar and collar spines. b. Daughter redia with cercariae and germ balls.

Sporocyst : Not found.

Redia (Fig. 32b)

Mother rediae not found. Daughter rediae cylindrical, motile, yellow-brown, 691-1655 (1310) x 149-476 (311) in size and found in hepatopancreas and pericardial sac of snails. Collar conspicuous, 196-398 (280) wide. Mouth terminal; pharynx 110-179 (153) x 90-145 (127) in size. Gut extends to anterior one-fifth of body, filled with dark-brown contents. Fully grown daughter rediae contained 6-12 cercariae and a few germ balls at various stages of development. Birth pore lateral, immediately posterior to collar.

First intermediate host	: <i>Indoplanorbis exustus</i>
Locality	: Thazhechovva in Kannur district
Period of Collection	: December 1997 to May 1998, January 1999
Prevalence	: 1.5% (61 out of 4056 were infected)
Second Intermediate host	: <i>Rasbora daniconius</i>

Remarks

The present cercaria with 31 collar spines, recovered from *Indoplanorbis exustus*, resembles *Cercariae indicae* XX described by Sewell (1922) from the same host species in Calcutta. In body size, pattern of distribution of cystogenous glands and nature of their contents, and flame cell formula they are exactly identical. Sewell did not mention the number of penetration glands,

which during the present study has been found to be 6 pairs. Similarly, the exact number of collar spines and their groupings were not recorded by Sewell. The pattern of collar spine arrangement in the present larva is 5+6+9+6+5. As the characters of the present cercaria agreed fully with the description of *Cercariae indicae* XX given by Sewell (1922), it is identified and reported here as *Cercariae indicae* XX.

The cercaria has been redescribed adding more details and its intramolluscan stages described for the first time. Further, encystment of the cercaria was observed in the fish *Rasbora daniconius*.

Attempts made to develop metacercariae in snails of the locality and adults in chicks and albino-rats were unsuccessful.

***Cercaria* sp. VI Kerala Mohandas, 1981**

Cercaria (Fig. 33a-b; Table 18)

This cercaria was found emerging from 22 out of 509 *Lymnaea luteola* collected from Feroke and Kundayithode in Kozhikode, and Idimuzhikkal and Nilambur in Malappuram districts of Kerala. The infection was prevalent throughout the year.

Behaviour: Cercarial emergence was maximum during morning hours and continued in lesser numbers throughout day-time. They were negatively phototactic, swimming easily through water while curving the body ventrally

and surviving for about 24 hours. Most of the cercariae were found encysted at the bottom of the container.

Description: Body oval or pyriform, aspinose, with 12-14 pairs of sensory hairs at anterior half; measured 316-466 (402) x 172-241 (211) in size. Collar 98-127 (110) wide; with 47 spines, 12-19 in length. Spine arrangement characteristic: five corner spines on each ventral lappet, 3 oral and 2 aboral; 7 laterals on each side in single row; 23 dorsals, 12 oral and 11 aboral.

Oral sucker circular, 53-70 (67) in diameter. Acetabulum post-equatorial, protrusible, measured 66-90 (79) in diameter. Tail aspinose, slightly longer than body, 360-501 (450) x 59-80 (66) in dimensions, with 7 fin folds; 2 dorsal, 2 ventral, 2 small ventro-lateral and one small ventral. Posterior half of tail with 14 pairs of sensory hairs. Tail-tip finger-like, capable of independent movement.

Prepharynx slender, 28-34 (31) long. Pharynx globular, muscular, 26-35 (29) in diameter. Oesophagus solid, 90-120 (109) long, consisting of 9 to 10 cells. Caeca solid, 169-192 (188) long, extending to posterior end of body, each caecum consisting of 8-12 cells with an additional cell at the point of bifurcation.

Penetration glands 4 pairs, with finely granular contents; gland ducts open on dorsal lip of oral sucker. Paraoesophageal gland cells located in the region of oral sucker and pharynx, and open through 16-22 outlets. Cystogenous glands numerous, distributed throughout body and filled with oval or round bodies.

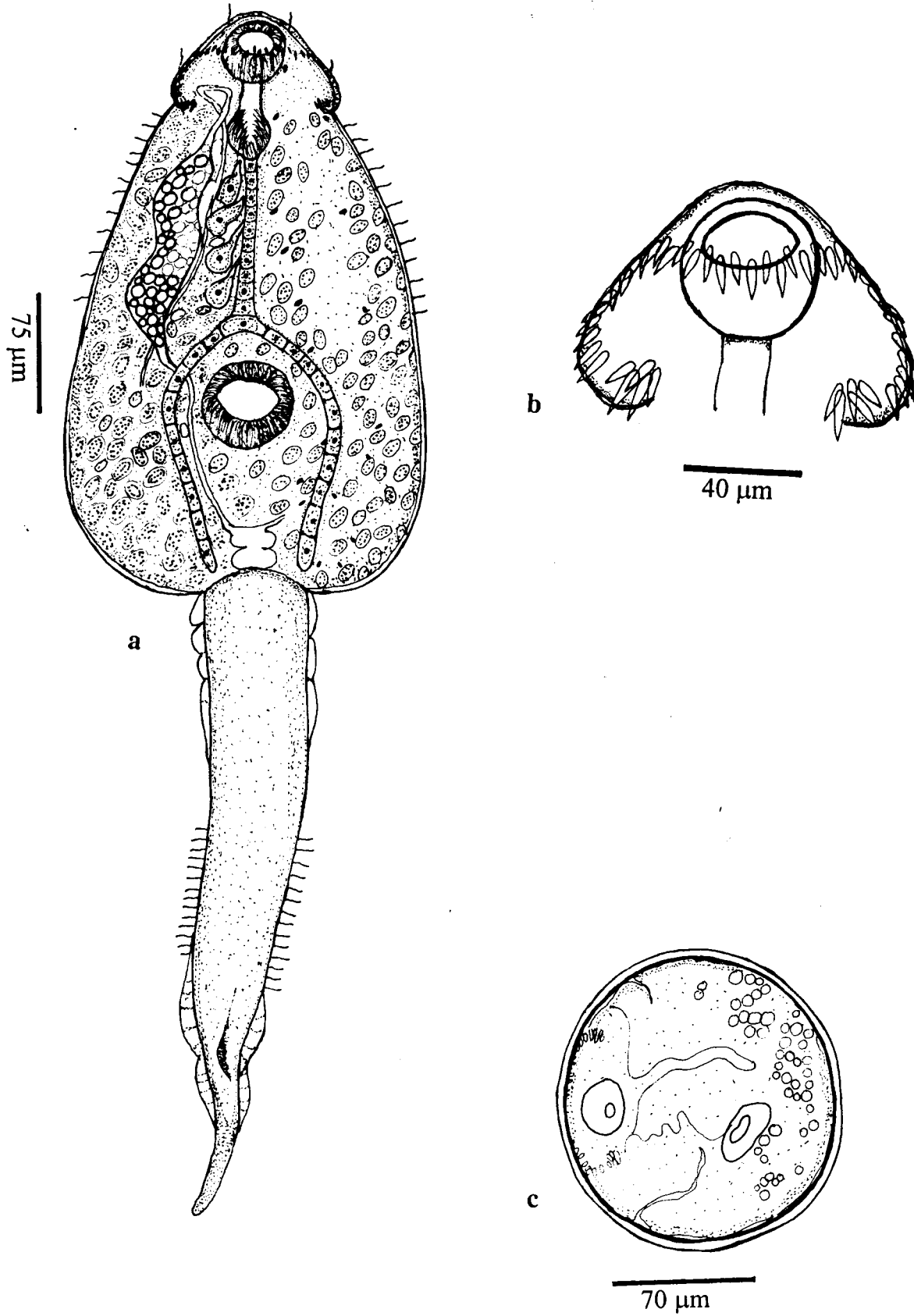


Fig. 33. *Cercaria* sp. VI Kerala Mohandas, 1981
a. Cercaria; b. Collar with collar spines; c. Encysted metacercaria.

Table 18. Measurements of *Cercaria* sp. VI Kerala Mohandas, 1981

Characters	Range	Mean
Body	316-466 x 172 x 241	402 x 211
Collar width	98-127	110
Collar spine (length)	12-19	16
Oral Sucker	53-70	67
Acetabulum	66-90	79
Tail	360-501 x 59-80	450 x 66
Prepharynx (length)	28-34	31
Pharynx	26-35	29
Oesophagus (length)	90-120	109
Caeca (length)	169-192	188

Genital primordia consist of two masses of cells, one at anterior margin of acetabulum, other between acetabulum and base of tail and connected by a ribbon of cells. Excretory system stenostomate, with main ducts extending from bipartite bladder to sides of oral sucker; main ducts distended between collar region and acetabulum, contain 40-80 excretory granules. Caudal excretory duct extends through anterior fourth of tail, then bifurcates and opens out laterally. Flame cell formula: $2[(3+3+3)+(3+3+3)] = 36$.

Metacercaria (Fig. 33c)

Natural infections by metacercariae were found in hepatopancreas, pericardial sac and mantle cavity of *Lymnaea luteola*, *Indoplanorbis exustus*, *Bellamya dissimilis* and *Thiara tuberculata*. *L. luteola* were found to be infected by maximum number of cysts (67). In experimental infections, cercariae developed into metacercarial cysts in all the four snail species.

Cyst spherical to oval, 170-198 (186) x 167-190 (179) in dimensions. Cyst wall consisting of an outer, transparent layer, about 9-13 thick, and an inner, opaque layer, about, 3-4.5 thick. Various structures such as oral sucker, acetabulum, parts of collar spines and excretory concretions were visible through cyst wall.

Experimental attempts to develop the metacercariae into adults in one-day-old white leg-horn chicks and one-month-old albino-rats were futile. Natural definitive host of the fluke not known.

Sporocyst: Not observed

Redia (Fig. 34a-b)

Fully grown mother radiae motile, pale-yellow, with conspicuous collar and locomotor organs; measured 692-1076 (879) x 193-335 (292) in size. Collar 135-188 (161) wide, pharynx oval, 39-68 (55) x 35-61 (52); gut extends to anterior two-third of body, filled with dark-brown contents. Fully mature mother radiae contained 12-16 daughter rediae and a few germ balls.

Daughter rediae cylindrical, motile, yellow-brown, 860-2095 (1416) x 198-494 (383) in size, found in hepatopancreas, kidney and gonads. Fully grown daughter rediae contained 3-6 cercariae and many germ balls.

First intermediate host	: <i>Lymnaea luteola</i>
Locality	Ferok and Kundayithode in Kozhikode district; Idimuzhikkal and Nilambur in Malappuram district.
Period of collection	: August to December 1996, February to May 1997 and April to November 1998
Prevalence	: 0.77 % (22 out of 2850 were infected)
Second intermediate host	: <i>L. luteola</i> , <i>Indoplanorbis exustus</i> , <i>Bellamya dissimilis</i> , <i>Thiara tuberculata</i>

Remarks

The present cercaria agrees fully with *Cercaria* sp. VI Kerala Mohandas, 1981, reported from *Lymnaea luteola* collected from Trivandrum district of Kerala. Host of the present form also is *L. luteola*. Therefore, the present cercaria and that described by Mohandas (1981) are same and recorded here as *Cercaria* sp. VI Kerala Mohandas, 1981. Mohandas reported encystment of the

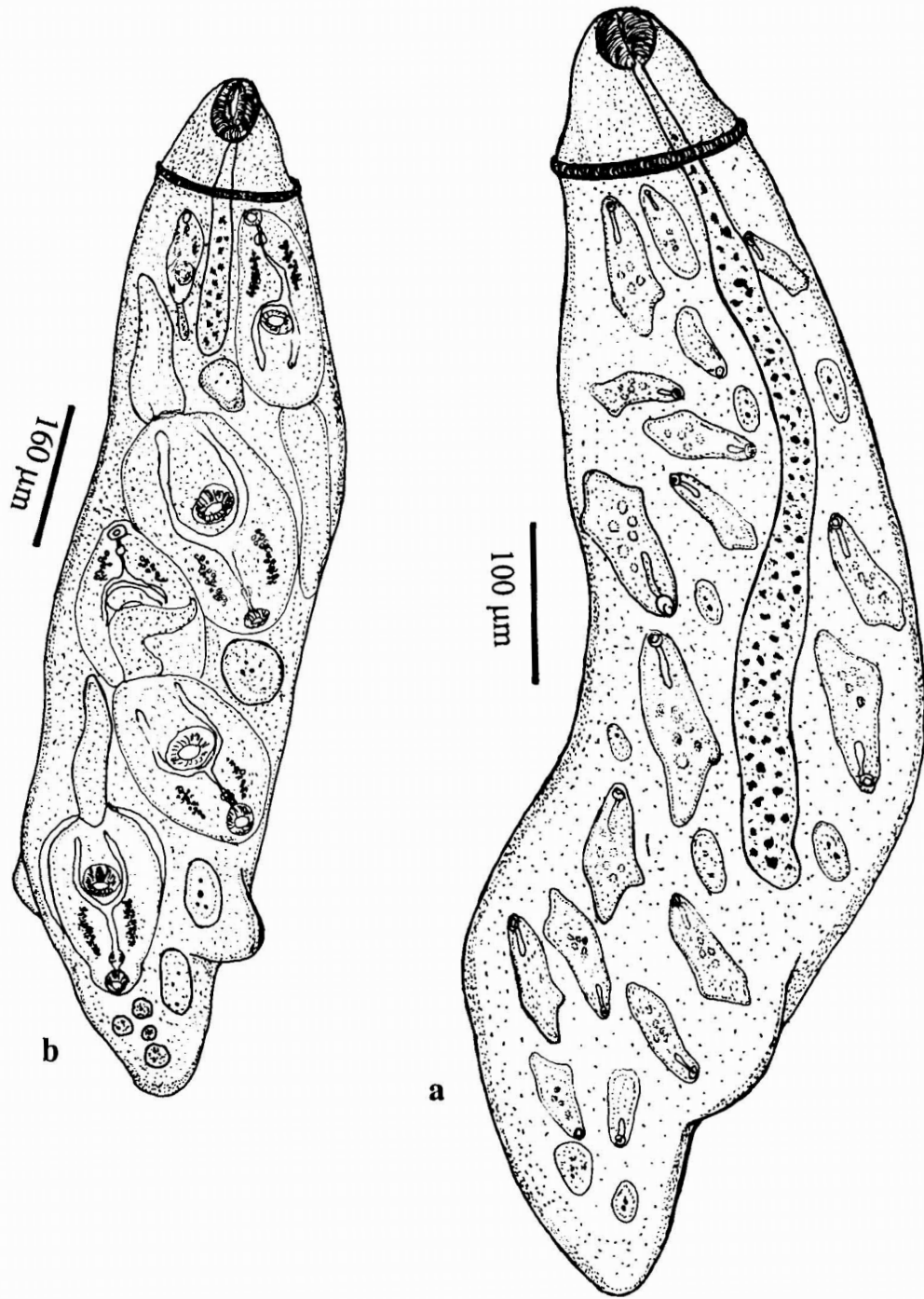


Fig. 34. *Cercaria* sp. VI Kerala Mohandas, 1981

- a. mother redia with daughter rediae and germ balls;
- b. Daughter redia with cercaria and germ balls.

cercaria in the same snail host. During the present investigation also the cercaria was found encysting in *L. luteola*. In addition, the metacercariae were found in snails like, *Indoplanorbis exustus*, *Bellamya dissimilis* and *Thiara tuberculata* collected from the same locality where infection by the cercariae were prevalent.

Attempts made to develop the adults from metacercariae in one-day-old white leg-horn chicks and one-month-old albino rats were unsuccessful.

Cercaria sp. VI Kerala Mohandas, 1981 exhibited dual infection by an unidentified furcocercous cercaria in 5 specimens of *L. luteola*

***Cercaria* sp. I Malabar n. sp.**

Cercaria (Figs. 35a-b; Table 19)

Infections by this cercariae were found in the pulmonate snails, *Lymnaea luteola* collected from Kanhangad in Kasargod district of Kerala. Infection was very rare, and only 3 of the 2850 snails examined were found to be infected.

Behaviour: The cercariae released by infected snails were active swimmers, showing no taxis. Cercarial emergence was maximum during morning hours, and continued in lesser numbers till evening. They remained alive for about 8 h in well-water.

Description: Body ovoid with anterior end narrow and posterior end broadly round, aspinose, with varying number of sensory hairs (10-14) at anterior half; measured 310-391 (346) long, 172-216 (193) wide. Collar not distinct, 93-117 (108) wide, with 19 spines in alternating, uninterrupted rows, and measuring

6-10 (8.3) in length. Oral sucker circular, 46-65 (53) in size. Acetabulum protrusible, post-equatorial, 53-81 (75) in diameter. Tail 266-347 (299) x 46-58 (51) in size, without fin folds and sensory hairs.

Mouth subterminal; prepharynx 26-34 (30) long with a small prepharyngeal body. Pharynx globular, 28-36 (33) in diameter. Oesophagus solid, 60-76 (69) long, consisting of 6 cells with round nuclei and finely granular contents. Caeca solid, extend to the level of excretory bladder, 156-183 (174) long, with 7 cells in each caecum and a median cell at the point of bifurcation.

Penetration glands not clearly visible, but 4 pairs of outlets open at the dorsal lip of oral sucker. Paraoesophageal glands present. Cystogenous glands numerous, distributed throughout body; filled with rod-shaped contents.

Genital primordia consist of two masses of cells, one at anterior margin and the other near posterior margin of acetabulum and connected by a string of cells passing dorsal to acetabulum. Excretory bladder, at posterior end of body; main collecting ducts distended between collar region and acetabulum, each with 30-60 excretory granules. The stenostomate excretory system composed of approximately 15 pairs of flame cells, the exact number of which was not discernible because they were covered by numerous cystogenous glands. The caudal excretory duct extends to one-fourth of tail length and then bifurcates into two lateral branches.

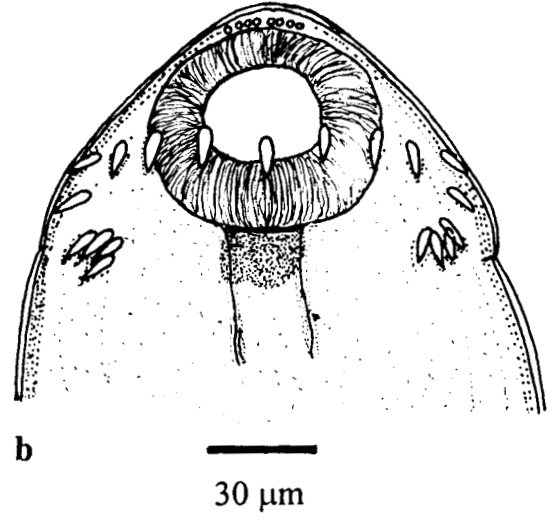
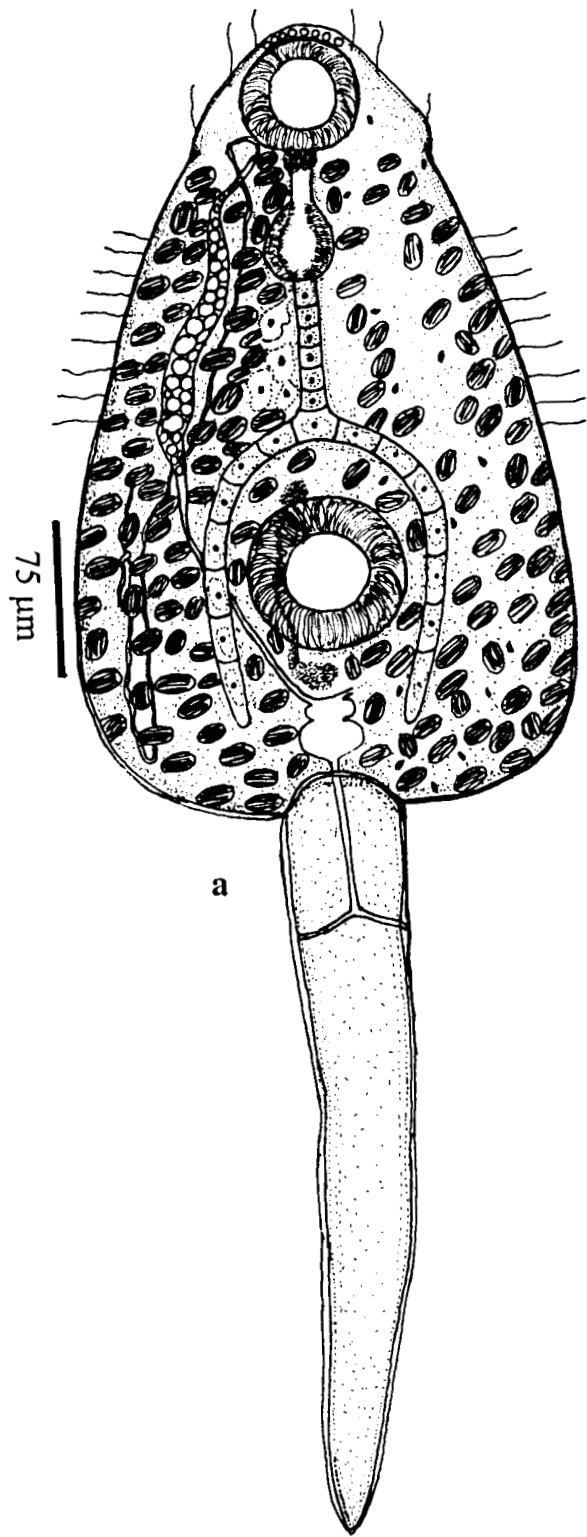


Fig. 35. *Cercaria* sp. I Malabar n. sp.
a. Cercaria; b. Collar with collar spines.

Table 19. Measurements of *Cercaria* sp. I Malabar n.sp.

Characterss	Range	Mean
Body	310-391 x 172-216	346-193
Collar width	93-117	108
Collar spines (length)	6-10	8.3
Oral sucker	46-65	53
Acetabulum	53-81	75
Tail	266-347 x 46-58	299 x 51
Prepharynx (length)	26-34	30
Pharynx	28-36	33
Oesophagus (length)	60-76	69
Caeca (length)	156-183	174

First intermediate host	: <i>Lymnaea luteola</i>
Locality	: Kanhangad in Kasargod district
Period of Collection	: January, 1998
Prevalence	: 0.11% (three out of 2850 were infected)

Remarks

The present cercaria resembles cercaria of *Petasiger grandivesicularis* Ishii, 1935 and *Cercaria rithorensis* Mukherjee, 1963 in the number of collar spines, which is 19. However both of them differs markedly in having small body and massive and very long tail. They differ from the present cercaria in several other respects particularly in the size and arrangement of collar spines, number of penetration glands and flame cells and number and size of excretory concretions in each excretory duct. Further, *C. rithorensis* has large number of small spines arranged in a triangular area posterior to pharynx and thus differs from the present cercaria beyond doubt.

Therefore, the present cercaria is considered new and is reported as *Cercaria* sp. I Malabar n.sp. It forms the first report of an Indian echinostome cercaria with 19 collar spines and from the lymnaeid snail, *Lymnaea luteola*.

***Cercaria* sp. II malabar n. sp.**

Cercaria (Figs. 36 a-b; Table 20)

Natural infections by the echinostome cercariae with 27-spined collar were found in the planorbid snails, *Indoplanorbis exustus*. Infected snails were collected from Kanhangad and Theerthankara in Kasargod; Cherukunnu in Kannur; Feroke in Kozhikode; Idimuzhikkal and Calicut University Campus in Malappuram and Malampuzha in Palakkad districts of Kerala. Out of a total of 875 snails collected 146 were infected.

Behaviour: Cercariae emerged from snails during day-time with peak emergence during morning hours. They were negatively phototactic, active swimmers and performed leech-like movements at the bottom of the container. They survived for 2 days in well-water.

Description: Body oval to elongate, aspinose 376-489 (431) x 176-260 (212) in size, with 8-10 pairs of sensory hairs at anterior region. Collar inconspicuous, 96-128 (113) wide, with 27 spines, 9-16 (13) in length; spine arrangement characteristic: four corner spines on each ventral lappet, 2 oral and 2 aboral; 6 laterals in single row on each side; 7 dorsals, 4 oral and 3 aboral. Oral sucker circular, 47-63 (56) in diameter. Acetabulum post-equatorial, protrusible, oval, 56-67 (59) x 63-74 (67) in size. Tail cylindrical, almost equal to body length, 341-450 (410) x 69-73 (72) in dimensions; sensory hairs and finfolds absent.

Mouth subterminal; prepharynx 19-23 (21) long; a prepharyngeal body found attached to prepharynx. Pharynx 31-42 (35) x 23-32 (28) in size. Oesophagus narrow, solid, 130-158 (142) long; consists of 8 cells with round nuclei and granular cytoplasm. Caeca solid, 211-254 (240) long, extending to the level of excretory bladder, each caecum consisting of 12 cells with an additional cell at the point of bifurcation.

Penetration glands 6 pairs, along oesophagus with four pairs of duct openings on dorsal lip of oral sucker. Paraoesophageal gland cells with 16-22 outlets, around oral sucker and 4 outlets in pharyngeal region. Cystogenous glands numerous, distributed throughout body, filled with rod-shaped contents.

Genital primordia consist of two masses of cells, one at anterior margin of acetabulum, the other between acetabulum and base of tail, and connected by a string of cells passing dorsal to acetabulum. Excretory system stenostomate type; excretory bladder bipartite, the main collecting ducts distended between pharynx and acetabulum, each containing 20-30 refractile granules of almost uniform size. Caudal branch of excretory duct extends to anterior fourth of tail, then bifurcates and opens out laterally. Flame cell formula $2[(3+3)+(3+3+3)] = 15$.

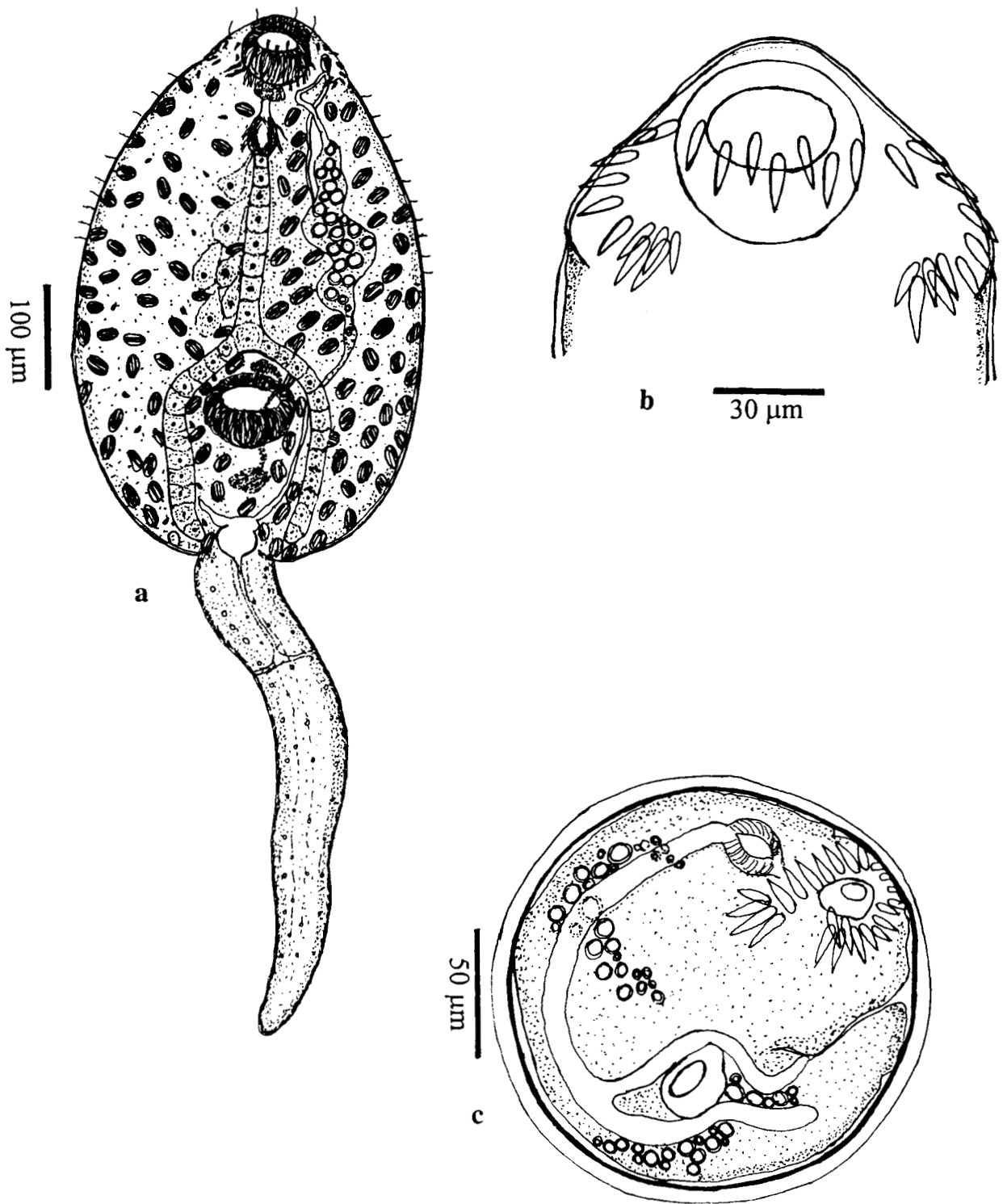


Fig. 36. *Cercaria* sp. II Malabar n. sp.
a. Cercaria; b. Collar with collar spines; c. Encysted metacercaria.

Table 20. Measurements of *Cercaria* sp. II Malabar n. sp.

Characters	Range	Mean
Body	376-489 x 176-260	431 x 212
Collar width	96-128	113
Collar spines (length)	9-16	13
Oral sucker	47-63	56
Acetabulum	56-67 x 63-74	59 x 67
Tail	341-450 x 69-73	410 x 72
Prepharynx (length)	19-23	21
Pharynx	31-42 x 23-32	35 x 28
Oesophagus (length)	130-158	142
Caeca	211-254	240

Metacercaria (Fig. 36c)

Metacercariae were found on the gills of freshwater fishes, *Aplocheilus panchax* and *Rasbora daniconius*, and in gill arches and pharyngeal wall of tadpoles of *Rana cyanophlyctis* and *Bufo bufo*. The cercariae did not encyst in *Lymnaea luteola*, *Indoplanorbis exustus*, *Bellamyia dissimilis* and *Thiara tuberculata* of the locality.

Cysts spherical, 133-168 (154) in diameter; cyst wall double-layered, consisting of an outer transparent layer, 6-7.5 thick and inner, opaque layer, 1.5-2.3 thick. Metacercaria remained folded inside the cyst cavity. Internal structures like collar spines, suckers, excretory granules and caeca were visible through cyst wall.

Consistent attempts made to develop metacercaria into adults in albino rats and white leg-horn chicks were futile.

Sporocyst: Not observed.

Redia (Figs. 37a-b)

Mother rediae usually remain in pericardial cavity, although a few migrated to haemolymph space surrounding caeca. Fully developed mother rediae motile, pale-yellow, measured 613-816 (771) x 205-319 (268). Collar inconspicuous; gut extends upto the level of locomotor processes, filled dark contents.

Daughter rediae were found in hepatopancreas. Fully developed daughter rediae cylindrical, motile, yellow-brown, 1308-2116 (1890) x 396-675 (518) in

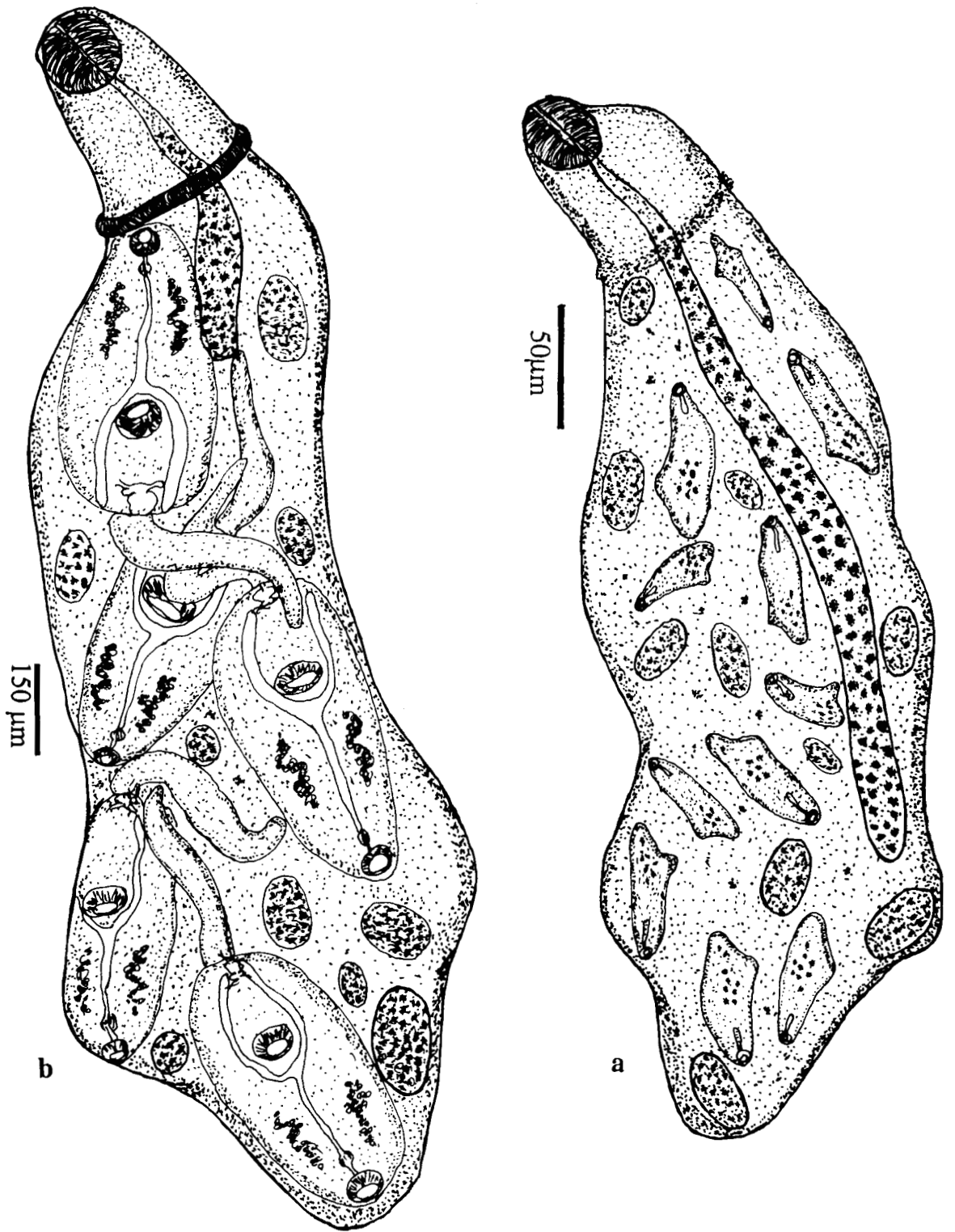


Fig. 37. *Cercaria* sp. II Malabar n. sp.

- a. Mother redia with daughter rediae and germ balls;
- b. Daughter redia with cercariae and germ balls

size. Collar 89-166 wide; birth pore inconspicuous. Mouth terminal; pharynx globular, muscular, 66-93 (79); gut extends up to anterior-fourth of body, filled with dark-brown contents. Locomotor processes well developed, located near, at posterior end. Daughter rediae contained 2-6 cercariae at different stages of development and a few germ balls.

First intermediate host	:	<i>Indoplanorbis exustus</i>
Locality	:	Kanhangad and Theerthankara in Kasargod district, Cherukunnu in Kannur district, Feroke in Kozhikode district, Idimuzhikkal and Calicut University Campus in Malappuram district and Malampuzha in Palakkad district.
Period of collection	:	May to October 1996, February to June 1997, November 1997 to June 1998.
Prevalence	:	3.6 % (146 out of 4056 were infected)
Second intermediate hosts	:	<i>Aplocheilus panchax</i> , <i>Rasbora daniconius</i> and tadpoles of <i>Rana cyanophlyctis</i> and <i>Bufo bufo</i> .

Remarks

Cercaria of *Paryphostomum radiatum* (Dujardin, 1845) Dietz, 1909, *P. segregatum* Lie and Basch, 1967, *Euparyphium melis* (Schrank, 1788) Beaver, 1941 and *Cercaria unnaoensis* IV Pandey and Lal, 1982 are the other cercariae characterized by the presence of 27 collar spines. All these cercariae have blunt tail without fanfold, and therefore, need comparison with the present

form. A comparison of characters and measurements of the four related cercariae with that of the present form is given in Table 21.

Cercariae of *Paryphostomum radiatum* and of *P. segregatum* (as described by Johnston and Angel, 1942 and Lie and Basch, 1967 respectively) have body without any setae, tails longer than, body and 15 and 18 pairs of flame cells. Cercaria of *Euparyphium melis*, as described by Beaver (1941), has also large spinose body without any setae and its tail is longer than body. The excretory system has 17 or 18 pairs of flames cells and cystogenous material is granular. In *Cercaria unnaoensis* IV, the group of corner spines on each side consists of only 3 spines, the number of flame cells and penetration gland cells are 12 pairs and 4 pairs respectively. Its tail is longer than body and setae are absent on body and tail. Further, all the cercariae use different species of snails as their hosts. On the basis of the above comparison the present species is regarded as new and recorded here as *Cercaria* sp. II Malabar n. sp.

Although this cercaria developed into metacercaria in fishes and tadpoles, attempts to raise its adults in white leg-horn chicks and albino rats were unsuccessful. Without a knowledge of the life cycle, it is not possible to assign the present cercaria to any of the echinostome genus.

Cercaria sp. II Malabar n. sp. exhibited mixed infections by cercaria of *Echinostoma malayanum* in 3 cases, and an unidentified furcocercous cercaria in 9 specimens.

Table 21. Comparative characters and measurements of *Cercaria* sp.II. Malabar n.sp. and of the 4 related cercariae species

Characters	<i>Cercaria</i> of <i>Paryphostomum</i> <i>radiatum</i> (Dujardin, 1845) Dietz 1909	<i>Cercaria</i> of <i>P.</i> <i>segregatum</i> Dietz, 1909	<i>Cercaria</i> of <i>Euparyphium melis</i> (Schrank, 1788) Beaver, 1941	<i>Cercaria unnaoensis</i> IV Pandey and Lal, 1982	<i>Cercaria</i> sp.II Malabar n.sp.
Body size	200-300 x 117-150	205-234 x 92-118	384-500 x 80-150	240-320 x 120-160	376-489 x 176-260
No. of collar spines	27	27	27	27	27
Body spination	Aspinose	Spinose	Spinose	Aspinose	Aspinose
Body setae	-	Present	-	-	8-10 pairs
Tail size	350-500 x 38-50	380-435 x 34-40	545 x 55	280-340 x 20-40	341-450 x 69-73
Tail finfold	Absent	Absent	Absent	Absent	Absent
Penetration gland	-	-	6 pairs	4 pairs	6 pairs
Oesophageal cells	7 or 8	-	-	-	8
Caecal cells	7+1+7	-	-	-	12+1+12
Cystogenous gland- cell content	Rod-shaped	Rod-shaped	Granular	-	Granular
Flame cells	15 pairs	18 pairs	17/18pairs	12 pairs	15 pairs
Snail host	<i>Amerianna</i> spp.	<i>Biomphalaria</i> <i>glabrata</i>	<i>Stagnicola</i> <i>emarginata</i>	<i>Lymnaea auricularia</i>	<i>Indoplanorbis</i> <i>exustus</i>

Cercaria* sp. III Malabar n. sp.*Cercaria** (Figs. 38a-b; Table 22)

Natural infections by the echinostome cercaria were found in the snails, *Lymnaea luteola* collected from Idimuzhikkal in Malappuram district of Kerala. Infected snails were found only during December-March.

Behaviour: Cercariae were released by snails throughout day-time with peak emergence during morning hours. They moved actively for about 4-8 hours, then sank to the bottom of the container and started creeping movements. Cercariae were positively phototactic; remained alive for about 2 days in well-water.

Description: Body oval, aspinose, 362-411 (400) x 166-227 (199) in size. Collar not distinct, 69-93(78) wide, with 31 spines, 8-13 (11) in length. Spine arrangement characteristic: four corner spines, 2 oral and 2 aboral on each ventral lappet; 6 laterals on each side in single row; 11 dorsals, 6 oral and 5 aboral. Latero-aboral among corner spines the largest, reaching upto 13 in length; dorso-oral spines usually smaller than the others, measuring 8 in length. Oral sucker oval, 27-36 (31) x 32-40 (36) in size. Acetabulum post-equatorial, protrusible, 78-97 (85) in diameter. Tail cylindrical, aspinose, 390-476 (438) x 54-77 (68) in size.

Mouth subterminal; prepharynx 14-22 (17) long. Pharynx 22-37 (26) x 12-17 (15) in size. Oesophagus narrow, solid, 86-118 (96) long, consisting of 8 cells. Caeca narrow, solid, 171-193 (186) long, extending to posterior end of

body; each caecum consists of 12 cells with an additional cell at the point of bifurcation.

Penetration glands 3 pairs, along oesophagus, filled with fine granules. Para-oesophageal gland cells opened through 18-20 outlets in oral sucker region and 4 outlets in pharyngeal region. Cystogenous glands numerous, fewer near oral sucker, filled with ovoid contents.

Genital primordia consist of two cell masses, one at anterior margin of acetabulum, and the other between acetabulum and base of tail and connected by a string of cells passing dorsal to acetabulum.

Excretory system stenostomate; excretory bladder bipartite, at posterior end of body; main collecting ducts distended between acetabulum and pharynx and filled with 40-60 refractile granules. Flame cells difficult to observe, probably 15 pairs arranged in groups of 3. Caudal excretory duct runs upto one-fourth of tail-length before bifurcating into two lateral branches.

Metacercaria (Fig. 38c)

Natural infections by metacercariae were found in hepatopancreas, kidney, gonad, intestine and muscles of *Lymnaea luteola* and *Indoplanorbis exustus*. In experimental infections also, the cercariae developed into metacercariae in both snail species.

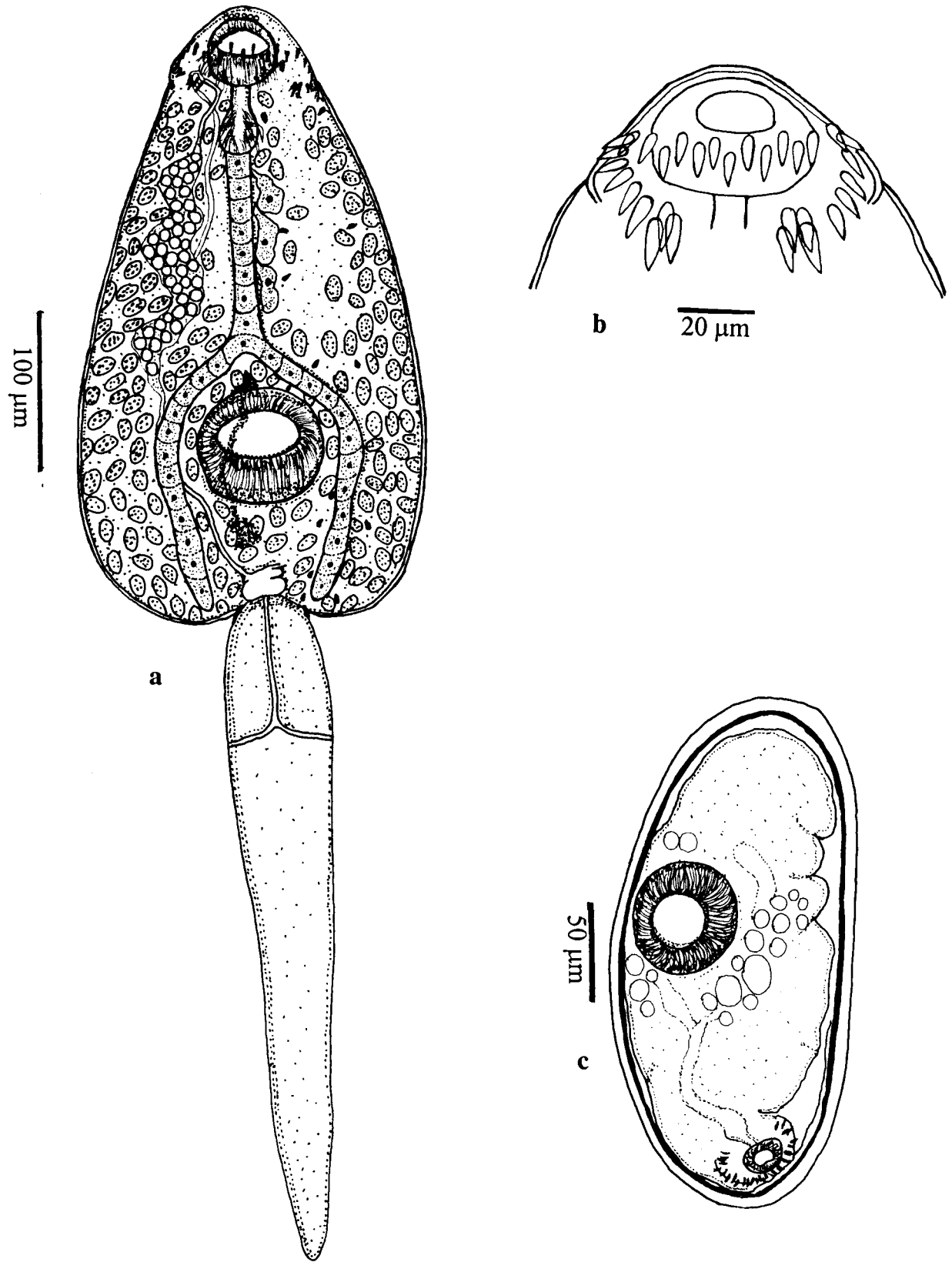


Fig. 38. *Cercaria* sp. III Malabar n. sp.
a. Cercaria; b. Collar with collar spines; c. Encysted metacercaria.

Table 22. Measurements of *Cercaria* sp. III Malabar n. sp.

Character	Range	Mean
Body	362-411 x 166-227	400 x 199
Collar width	69-93	78
Collar spines (length)	8-13	11
Oral sucker	27-36 x 32-40	31 x 36
Acetabulum	78-97	85
Tail	390-476 x 54-77	438 x 68
Prepharynx (length)	14-22	17
Pharynx	22-37 x 12-17	26 x 15
Oesophagus (length)	86-118	96
Caeca	171-193	186

Cysts elongate ovoid, 230-267 (240) x 132-156 (142) in size. Cyst wall double-layered; an outer, transparent layer, 7-9 thick and an inner opaque, layer 3.5-3.9 thick. Oral sucker, acetabulum, collar spine, excretory granules and caeca visible through cyst wall.

Experimental attempts made to develop the metacercariae into adults in one-day-old white leg-horn chick and one-month-old albino rats were futile. Natural definitive host of the fluke is not known.

Sporocyst: Not observed

Redia (Fig. 39a-b)

Mother redia were recovered from pericardial sac, kidney and gonad of the snail host. Body cylindrical, motile, pale-yellow, measured 637-950 (716) x 167-239 (198). Mouth terminal; pharynx globular, 31-46 (37) in diameter. Collar 126-139 (132) wide; gut 396-622 (510) long, filled with dark-brown contents. Locomotor processes paired, from posterior end of body. Birth pore inconspicuous. Fully grown mother rediae contained 10-20 daughter rediae and few germ balls.

Daughter rediae cylindrical, motile, yellow-brown, 945-1560 (1310) x 210-476 (385) in size, found in hepatopancreas. Mouth terminal; pharynx oval, 51-78 (63) x 47-71 (62) in size. Collar 141-167 (154) wide. Gut 190-380 (274) long, filled with dark-brown contents. Locomotor processes prominent, paired, from posterior end. Birth pore lateral, immediately posterior to collar. Daughter rediae contained 3-6 cercariae and a few germ balls.

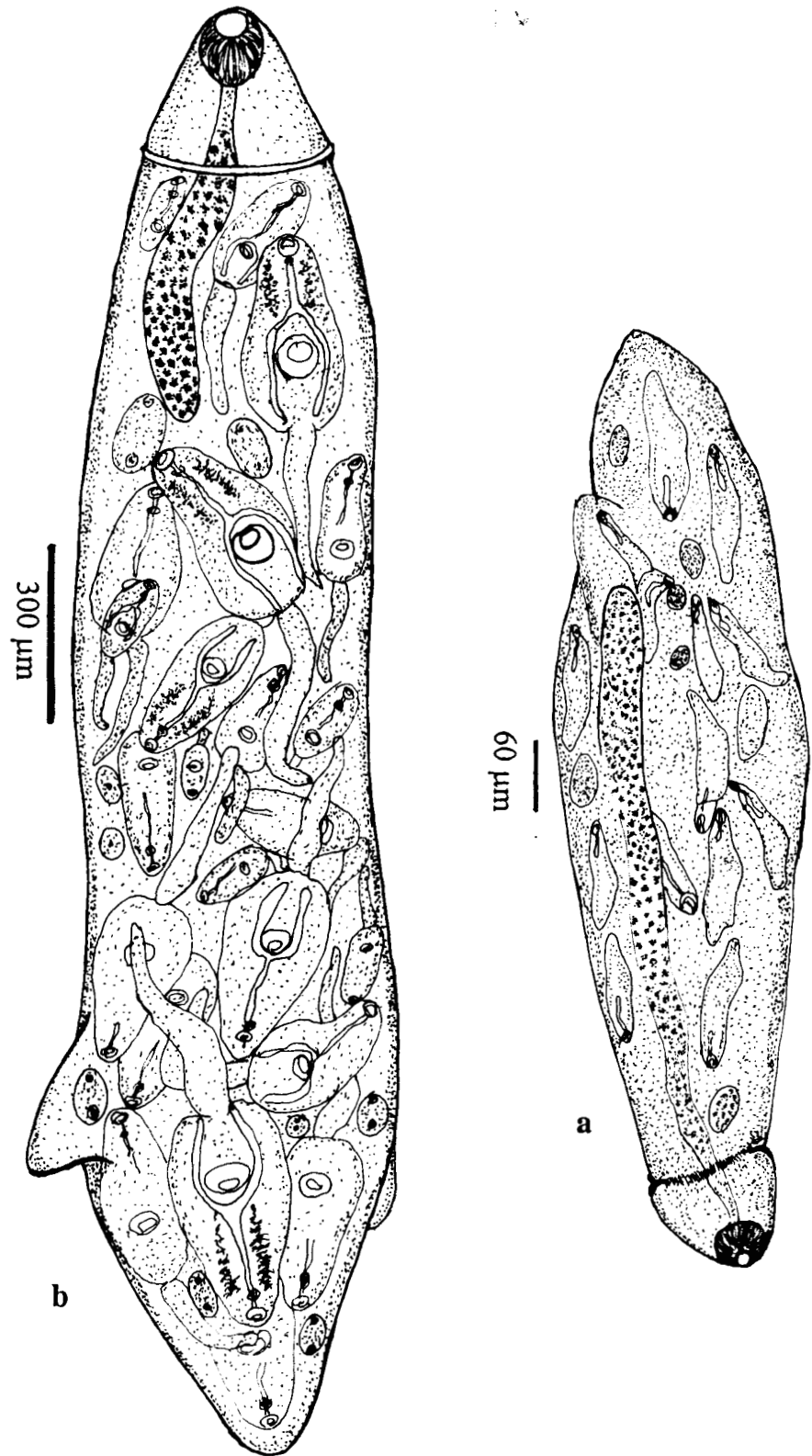


Fig. 39. *Cercaria* sp. III Malabar n. sp.
 a. Mother redia with daughter rediae and germ balls;
 b. Daughter redia with cercariae and germ balls.

First intermediate host	:	<i>Lymnaea luteola</i>
Locality	:	Idimuzhikkal in Malappuram district
Period of collection	:	February and March 1997; December 1998; January to March 1999.
Prevalence	:	0.95% (27 out of 2850 were infected)
Second intermediate hosts	:	<i>L. luteola</i> and <i>Indoplanorbis exustus</i>

Remarks

The cercaria under report is comparable to *Cercariae indicae* XX Sewell, 1922 infecting *Indoplanorbis exustus*, cercaria of *Echinostoma donosoi* Nasir, 1964 from *Marisa cornuarietis* and *Cercaria nawabganjensis* Pandey and Singh, 1984 from *I. exustus* in general morphology. A comparison of characters of the 3 species with that of the present form is given in Table 23.

Though *Cercariae indicae* XX resembles the present cercaria in having a blunt tail without finfolds, it differs in having spinose body, different number of penetration glands, flame cells and oesophageal and caecal cells and in the nature of cystogenous material.

Table 23. Comparative characters and measurements of *Cercaria* sp.III Malabar n. sp. and of the 3 related cercariae species

Characters	<i>Cercariae indicae</i> XX Sewell, 1922	Cercaria of <i>Echinostoma</i> <i>donosoi</i> Nasir, 1964	<i>Cercaria nawabganjensis</i> Pandey and Sing, 1984	<i>Cercaria</i> Sp.III Malabar n. sp.
Body size	230-540 x 70-210	528-721 x 286-403	240-300 x 80-110	362-411 x 166-227
Collar spines	29	31	32	31
Body spination	Spinose	Spinose	Spinose	aspinose
Body setae	Absent	Present	Absent	absent
Tail size	350 x 50	650-858 x 91-117	360-430 x 30-80	390-476 x 54-77
Tail finfolds	Absent	Present	Absent	absent
Penetration glands	-	-	5 pairs	3 pairs
Oesophageal cells	-	12	8	8
Caecal cells	-	22-24+1+2-24	14+1+14	14+1+12
Cystogenous gland-cell content	Rod-shaped	Granular	Oval and rod shaped	Granular
Flame cells	9 pairs	21 pairs	12 pairs	~15 pairs
Snail host	<i>Indoplanorbis exustus</i>	<i>Marisa cornuarietis</i>	<i>Indoplanorbis exustus</i>	<i>Lymnaea luteola</i>

Cercaria of *Echinostoma donosoi* differs from the present form in having different number of penetration glands, oesophageal and caecal cells and in the presence of setae over the body. Further, the tail possesses a dorso-ventral finfold.

Cercaria nawabganjensis has spinose body with 32 collar spines. There are 5 pairs of penetration glands, 14 cells in each caecum and 2 types of cystogenous glands making it different from the cercaria under discussion. Besides, the hosts of the two species are different. Considering the differences with the most similar species, the cercaria under report is regarded as a new species and named *Cercaria* sp. III Malabar n. sp.

***Cercaria* sp. IV Malabar n. sp.**

Cercaria (Figs. 40a-b; Table 24)

The viviparid snails, *Bellamya dissimilis* were found infected by echinostome cercariae with 35 collar spines. Infected snails were collected from Olipprumkadavu and Kizhisseri in Malappuram district of Kerala. The infection was found limited to the period from December to February.

Behaviour : Cercariae started emerging from snails during morning hours and continued to emerge throughout day-time. They were active swimmers, and showed negative phototaxis. They survived in well-water for almost 24 hours.

Description: Body elongate, spinose, 269-384 (335) x 160-211 (180) in size; 8-10 pairs of sensory hair present at anterior half of body. Collar prominent, 98-

130 (119) wide, with 35 spines, 8-12 (10.5) in length; spine arrangement characteristic: five corner spines, on each ventral lappet, 3 oral and 2 aboral; 7 laterals on each side in single row; 11 dorsals, 6 oral and 5 aboral. Oral sucker circular, 46-61 (54) in diameter. Acetabulum post-equatorial, circular, muscular, 66-91 (83) in diameter. Tail cylindrical, aspinose, 295-432 (368) x 51-78 (65) in size, with 6-8 pairs sensory hairs at anterior half.

Mouth subterminal; prepharynx 22 to 31 (27) long; pharynx ovoid, 24-35 (29) x 20-31 (26) in dimensions. Oesophagus solid, 106-144 (137) long, consisting of 8 cells. Caeca solid, extending to posterior end of body, 130-177 (161) long; each caecum consisting of 10-12 cells with an additional cell at the point of bifurcation.

Penetration glands 3 pairs, along oesophagus, with 3 pairs of duct openings on dorsal lip of oral sucker; each gland with round nucleus and granular cytoplasm. Para-oesophageal gland cells not discernible. Cystogenous glands numerous, fewer near oral sucker and pharynx; with rod-shaped contents.

Genital primordia consist of two masses of cells, one at anterior margin of acetabulum, the other between acetabulum and base of tail, and connected by a string of cells passing lateral to acetabulum. Excretory system stenostomate; excretory bladder bipartite, at posterior end of body; main collecting ducts distended between pharynx and acetabulum, each containing 30-40 refractile granules, 3-7 in diameter. Caudal excretory duct runs into anterior one-fifth of

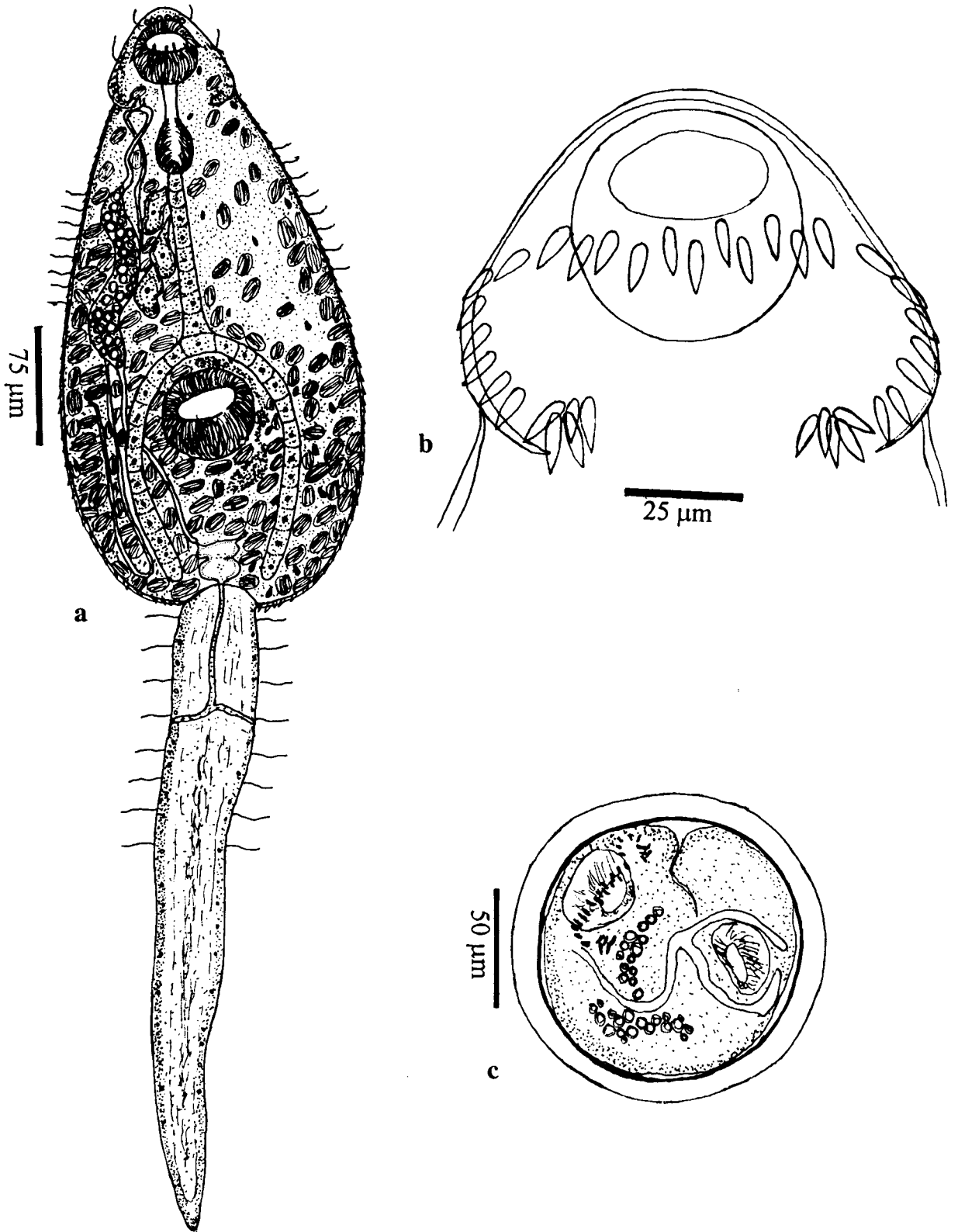


Fig. 40. *Cercaria* sp. IV Malabar n. sp.
a. Cercaria; b. Collar with collar spines; c. Encysted metacercaria.

tail, then bifurcates into two lateral branches. Flame cell formula:
 $2[(3+3+3)+(3+3+3)] = 36$.

Metacercaria (Fig. 40c)

Metacercariae were found in the hepatopancreas, kidney and muscles of *Lymnaea luteola*, *Indoplanorbis exustus* and *Bellamya dissimilis*.

Cysts spherical to oval, 122-165 (147) x 130-167 (155) in size. Cyst wall double-layered, with an outer, transparent, layer of 9-11 thickness and an inner, opaque layer of 2-3.5 thickness. Internal structures like collar spines, suckers, excretory granules and caeca were visible through cyst wall.

Attempts made to develop adults in one-day-old white leg-horn chicks and one-month-old albino-rats were unsuccessful.

Sporocyst: Not observed

Redia (Fig. 41)

Daughter rediae usually remain in hepatopancreas, although a few migrated to kidney and gonad. Redial body cylindrical, motile, yellow-brown, measured 1613-2406 (1911) x 355-512 (476). Collar prominent, 130-165 (149) in width. Mouth terminal; pharynx oval, 49-57 (52) x 41-52 (47); gut extends to the anterior-foth of body, filled with dark-brown contents. Locomotor processes paired, 302-478 (412) from posterior end. Birth pore lateral, just posterior to collar. Daughter rediae contained 3-6 cercariae and some germ balls.

Table 24. Showing measurements of *Cercaria* sp. IV Malabar n. sp.

Characters	Range	Mean
Body	269-384 x 160-211	335 x 180
Collar	98-130	119
Collar spines (length)	8-12	10.5
Oral sucker	46-61	54
Acetabulum	66-91	83
Tail	295-432 x 51-78	368 x 69
Prepharynx (length)	22-31	27
Pharynx	24-35 x 20-31	29 x 26
Oesophagus (length)	106-144	137
Caeca (length)	130-177	161

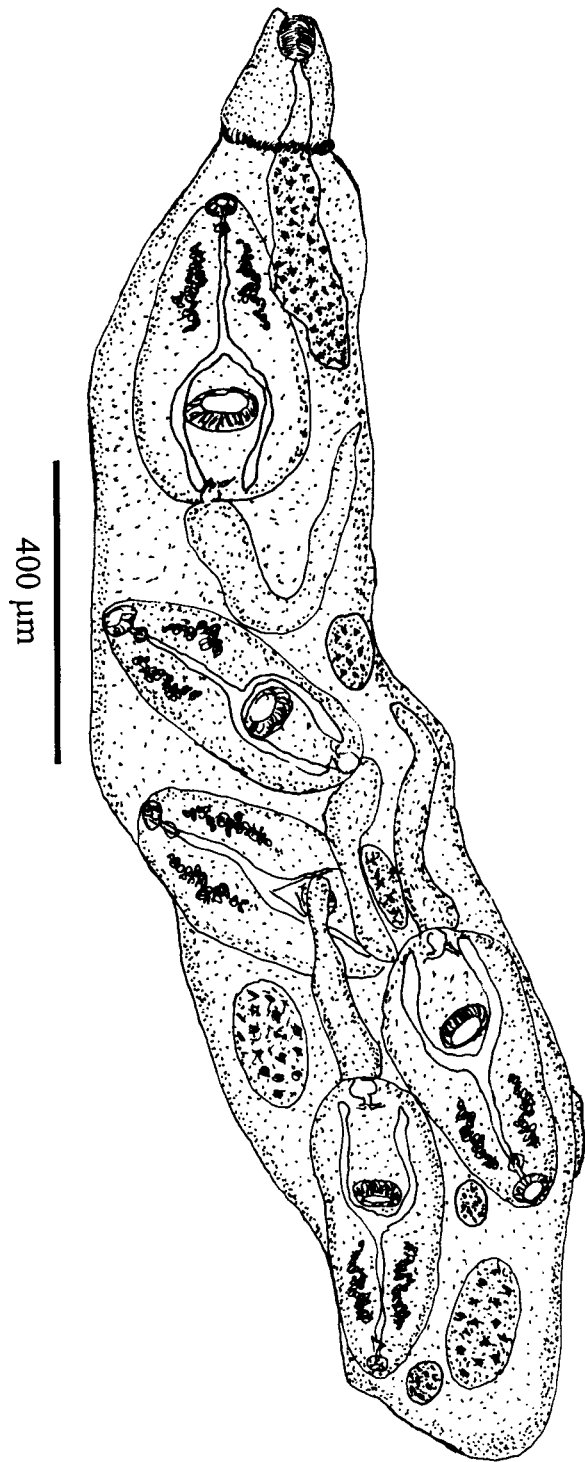


Fig. 41. *Cercaria* sp. IV Malabar n. sp.
a. Daughter redia with cercariae and germ balls.

First intermediate host	:	<i>Bellamya dissimilis</i>
Locality	:	Olippuramkadavu and Kizhisseri in Malappuram district
Period of collection	:	January 1996, December 1997 to February 1998
Prevalence	:	0.57 % (22 out of 3827 were infected)
Second intermediate hosts	:	<i>B. dissimilis</i> , <i>Indoplanorbis exustus</i> and <i>Lymnaea luteola</i>

Remarks

Echinostome cercariae recorded from *Bellamya* spp. in India are *Cercaria beaveri* Pandey and Agrawal, 1977, *C. komiyai* Pandey and Agrawal, 1977, *C. unnaoensis* III Pandey and Lal, 1982, *C. spinosa* Pandey and Singh, 1984 and *C. bellamyaii* Srivastava and Saxena, 1992. A comparison of characters of the 5 species with that of the present form is given in Table 25. Of these, *C. unnaoensis* III, *C. spinosa* and *C. bellamyaii* are characterised by the presence of tail finfolds making them distinct from the present form. The other two species, *C. beaveri* and *C. komiyai*, have aspinose body, and larger number of collar spines and penetration glands. Besides, in the arrangements of collar spines and in the nature of cystogenous gland contents they stand distinct from the present form. A comparison of the present cercaria with related cercariae from other snail species also revealed that it is distinctly different from the others. Therefore, the present cercaria is considered new, and is reported have as *Cercaria* sp. IV. Malabar n. sp.

Table 25. Comparative characters and measurements of *Cercaria* sp.IV Malabar n.sp. and of the 5 related species

Characters	<i>Cercaria beaveri</i> Pandey and Agrawal, 1977	<i>C. komiyai</i> Pandey and Agrawal, 1977	<i>C. unnaoensis</i> III Pandey and Lal, 1982	<i>C. spinosa</i> Pandey and Singh, 1984	<i>C. bellamyaii</i> Srivastava and Saxena, 1992	<i>Cercaria</i> sp. IV Malabar n. sp.
Body size	160-240 x 50-100	320-360 x 210- 270	260-380 x 100-70	270-40 x 110-180	130-280 x 70-150	269-384 x 160- 211
Collar spine	40 in single row	32 (in single row)	37 (double, dorsally interrupted)	37-38 (double, dorsally uninterrupted)	38 (double, dorsally uninterrupted)	35 (double, dorsally uninterrupted)
Body spination	Aspinose	Aspinose	Spinose	Spinose	Aspinose	Spinose
No. of setae	-	-	-	-	-	8-10 pairs
Tail length (µm)	160-260 x 10-20	-	300-500 x 30-50	400-520 x 30-50	130-220 x 20-30	295-432 x 51-78
Tail finfolds	Absent	-	Present	Present	Present	Absent
No. of penetration gland	7 pairs	6 pairs	3 pairs	3 pairs	Numerous	3 pairs
Cystogenous gland-cell content	Granular (oval, round)	-	Granular (oval, round)	-	Round	Rod-shaped
No. of caecal cell	-	-	-	-	-	10-12 cells
Flame cells	18 pairs	18 pairs	18 pairs	18 pairs	20 pairs	18 pairs
Snail host	<i>Bellamya</i> <i>bengalensis</i>	<i>B. bengalensis</i>	<i>B. bengalensis</i>	<i>B. bengalensis</i>	<i>B. bengalensis</i>	<i>B. disimilis</i>

***Cercaria* sp. V Malabar n. sp.**

Cercaria (Figs. 42a-b; Table 26)

Lymnaea luteola were found infected by a 41-collar-spined echinostome cercaria. Infected snails were collected from Payyanur in Kannur, and Chorode and Feroke in Kozhikode districts of Kerala. Infection was restricted to the monsoon period (June to September).

Behaviour: Cercariae emerged from snails during morning hours with peak emergence in the first 2 or 3 hours after sunrise. They were active swimmers, and exhibited photopositivity. They remained alive for about 12 h in well-water.

Description: Body elongate, aspinose, 470-532 (512) in length and 185-223 (205) in width, with 12-16 pairs of sensory hairs at anterior half. Collar well-developed, 139-166 (152) in wide, with 41 spines, 8.5-10.5 (9.7) in length. Spine arrangement characteristic: five corners on each ventral lappet, 3 oral and 2 aboral; 6 laterals in single row on each side; 19 dorsals, 10 oral and 9 aboral. Oral sucker circular, 52-67 (59) in diameter. Acetabulum post-equatorial, protrusible, 77-92 (87) in diameter. Tail aspinose, 416-524 (471) x 62-85 (73) in size.

Mouth subterminal; prepharynx 21-26 (23) in length; pharynx muscular, elongate-oval, 31-42 (35) x 27-36 (34) in size; oesophagus 148-185 (167) long, solid, consisting of 8 cells with round nuclei and finely granular cytoplasm. Caeca 231-262 (249) long, solid, reaching nearly to posterior end of body; each caecum consisting of 10-12 rectangular cells with an additional cell at bifurcation.

Penetration glands, 4 pairs, along oesophagus with four pairs of duct openings on dorsal lip of oral sucker; first two pairs of glands contain fine granules and the other two pairs coarse granules. Para-oesophageal gland cells found at pharyngeal region, with 12-14 duct outlets around oral sucker and 6 outlets in pharyngeal region. Cystogenous glands numerous, fewer near oral sucker and pharynx, filled with rod-shaped contents.

Genital primordia consist of two masses of cells, one at anterior margin of acetabulum, the other behind acetabulum and connected by a string of cells passing dorsal to acetabulum. Excretory system stenostomate, with main ducts extending from bipartite bladder to sides of oral sucker; main collecting ducts dilated between pharyngeal and acetabular levels, each containing 90-130 refractile granules, up to 4 in diameter. Caudal excretory duct extends to anterior fourth of the tail, where it bifurcates and opens through lateral pores. Flame cell formula: $2[(3+3+3)+(3+3+3+3)] = 42$.

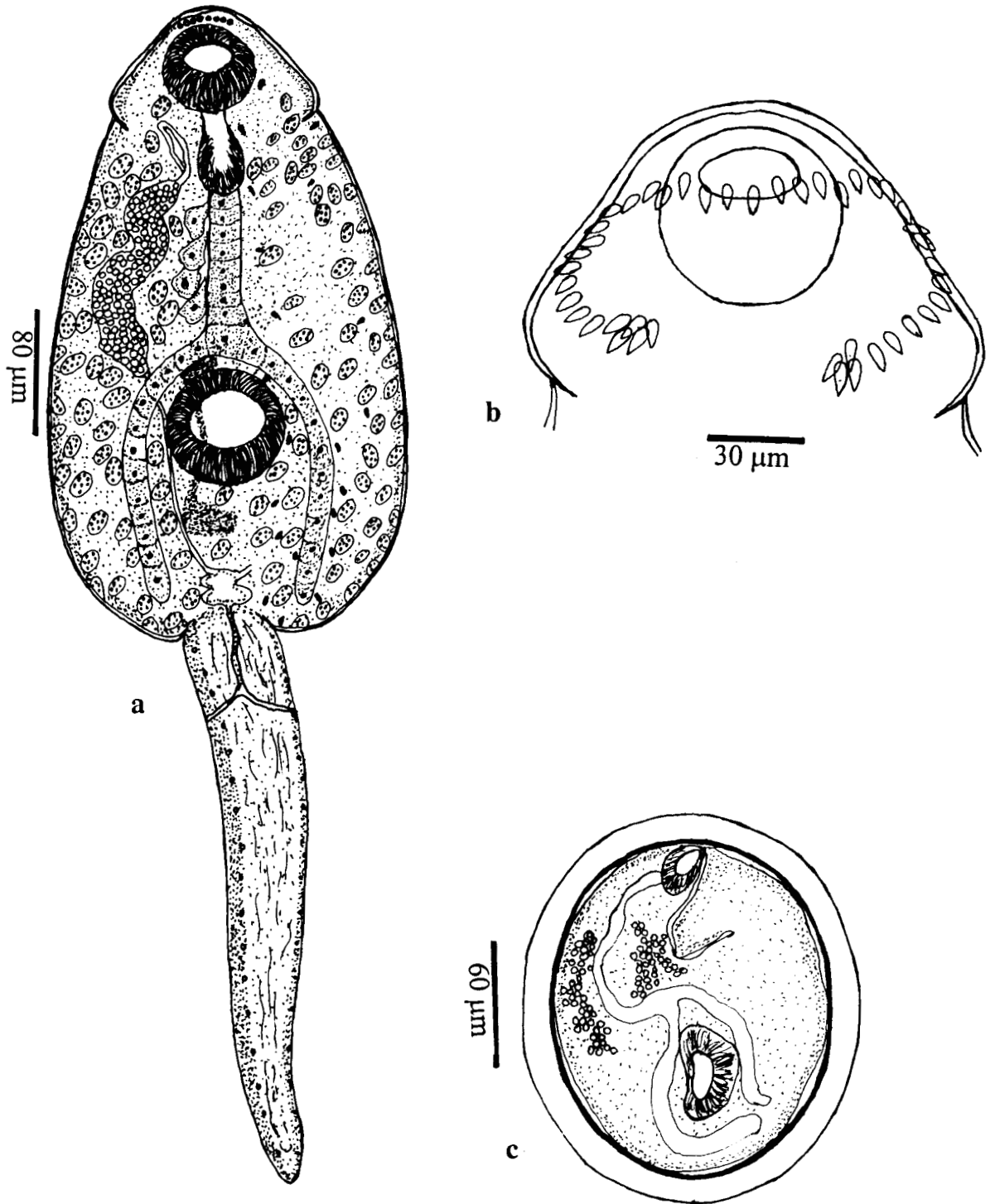


Fig. 42. *Cercaria* sp. V Malabar n. sp.
 a. Cercaria; b. Collar with collar spines; c. Encysted metacercaria.

Table 26. Measurements of *Cercaria* sp.V Malabar n. sp.

Characters	Range	Mean
Body	470-532 x 185-223	512-205
Collar Width	139-166	152
Collar spine (length)	8.5-10.5	9.7
Oral sucker	52-67	59
Acetabulum	77-92	87
Tail	416-524 x 62-85	471 x 73
Prepharynx (length)	21-26	23
Pharynx	31-42 x 27-36	35 x 34
Oesophagus (length)	148-185	167
Caeca (length)	213-262	249

Metacercaria (Fig. 42c)

Cercariae encysted in the hepatopancreas of *Lymnaea luteola* and *Indoplanorbis exustus*. Cyst elongated, 182-217 (193) x 157-169 (161) in size. Cyst wall double-layered, with an inner, opaque layer, about 3-4.5 thick and outer, transparent layer, 12-14 thick. Internal structures like, oral sucker, acetabulum, collar spines and caeca were distinct.

Attempts to develop metacercariae to adults in one-day-old white leg-horn chicks and one-month-old albino rats were futile.

Sporocyst: Not observed

Redia (Fig. 43)

Daughter rediae were recovered from hepatopancreas, kidney and gonad of *Lymnaea luteola* and *Indoplanorbis exustus*. Rediae cylindrical, motile, yellow-brown, measured, 820-1350 (1154) x 299-410 (376). Collar prominent, 110-127 (119) wide. Mouth terminal, Pharynx globular, 67-81 (72) in diameter. Gut extends beyond the collar. Locomotor processes paired near posterior end. Birth pore inconspicuous. Rediae contain 1-2 full developed cercariae, some developing cercariae and a few germ balls.

First intermediate host	:	<i>Lymnaea luteola</i>
Locality	:	Payyanur in Kannur district and Cherode and Feroke in Kozhikode district
Period of Collection	:	August and September 1997 and June and August 1998.

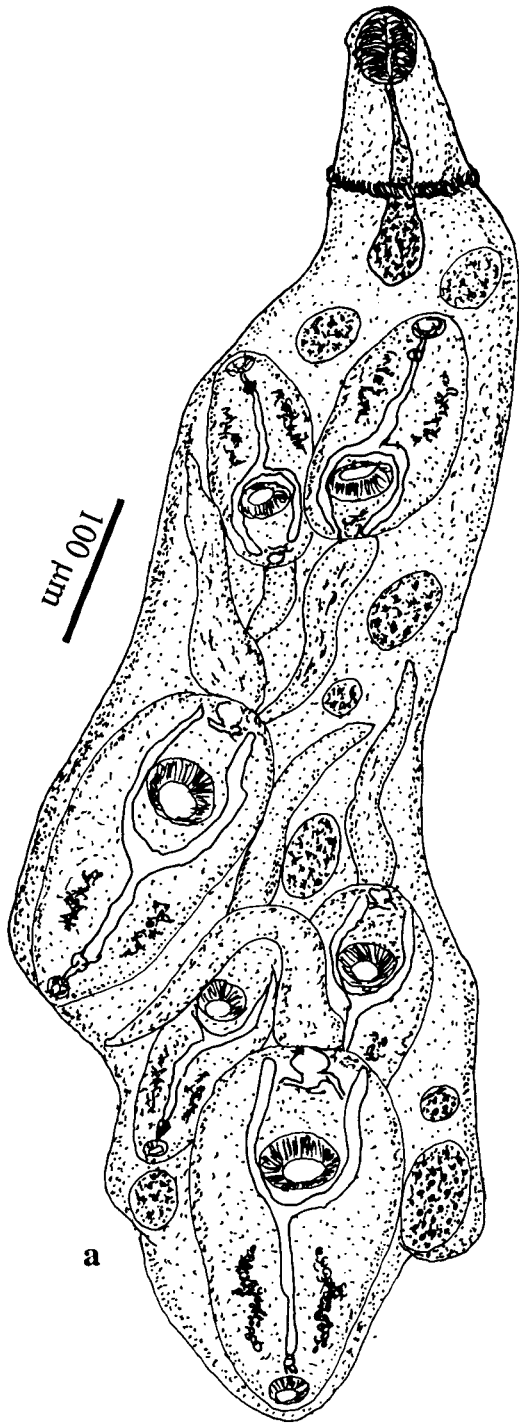


Fig. 43. *Cercaria* sp. V Malabar n. sp.
a. Daughter redia with cercariae and germ balls.

- Prevalence : 0.56%, (16 out of 2850 were infected).
Second intermediate hosts : *L. luteola* and *Indoplanorbis exustus*

Remarks

Of the various echinostome cercariae with more than 40 collar spines, the present form comes close to *Cercariae indicae* XX Sewell, 1922, *Cercaria tetraglandulata* Srivastava, 1968, *C. triglandulata* Baugh, 1975 and *C. beaveri* Pandey and Agrawal, 1977 in overall morphology. But from a comparison of characters of the present cercaria with that of the 4 species presented in table 27, it is evident that the present form differs from the others in morphometry, collar spine arrangement and in the presence of 12-16 pairs of body setae, 21 pairs of flame cells. The present form differs further from *C. tetraglandulata* and *C. triglandulata* in having tail without any finfolds and from *Cercariae indicae* XXIII and *Cercaria tetraglandulata* in lacking body spination.

Since the present cercaria cannot be identified with any known echinostome cercariae, it appears reasonable to consider the present species as new and the name *Cercaria* sp. V Malabar n. sp. is proposed to it.

Table 27. Comparartive characters and measurements of *Cercaria* sp.V Malabar n. sp. and of 4 related species

Characters	<i>Cercariae indicae</i> XXIII Sewell, 1922	<i>C. tetraglandulata</i> Srivastava, 1968	<i>C. triglandulata</i> Baugh, 1975	<i>C. beaveri</i> Pandey and Agrawal, 1977	<i>Cercaria</i> sp. V. Malabar n .sp
Body size	320-440 x 100-140	220-270 x 70-100	350-360 x 180-190	160-240 x 50-100	470-532 x 185-223
Collar spines	43	42	41	40	41
Body spination	Present	Present	Absent	Absent	Absent
No. of body setae	-	-	-	-	12-16 pairs
Tail Size	440 x 40	300-370 x 40	380-400 x 50	160-260 x 10-20	416-524 x 62-85
Tai finfolds	Absent	Present	Present	-	Absent
Penetration glands	2 pairs	4 pairs	3 pairs	14 pairs	4 pairs
Cystogenous gland-cell contents	-	Rod-shaped	Rod-shaped	Round/oval	Rod-shaped
No. of excretory concretions in each duct	-	6-9	6-9	-	90-130
Oesophageal cells	-	-	-	-	8
Caecal cells	-	-	-	-	10-12
Flame cells	18 pairs	8 pairs	18 pairs	18 pairs	21 pairs
Snail host	<i>Lymnaea</i> sp.	<i>Indoplanorbis</i> <i>exustus</i>	<i>I. exustus</i>	<i>Vivipara</i> <i>bengalensis</i>	<i>Lymnaea luteola</i>

***Cercaria* sp. VI Malabar n. sp.**

Cercaria (Figs. 44 a-b; Table 28)

The lymnaeid snails, *Lymnaea luteola* were found infected by echinostome cercariae with 49 collar spines. The infected snails were collected from Feroke and Ramanattukara in Kozhikode district. Out of 2850 snails examined 19 were found infected by this cercaria showing an overall prevalence of 0.66%.

Behaviour: The cercariae emerged from snails immediately after sunrise, and emergence continued till noon. They were active swimmers, and survived up to 14 h in well-water.

Description: Body elongate, spinose, with 10-12 sensory hairs at anterior half; measured 350-478 (411) x 177-243 (206). Collar conspicuous, 102-139 (123) wide, with 49 spines, 7 to 9 (8.3) long; spine arrangement characteristic: five corner spines on each ventral lappet, 3 oral and 2 aboral; 6 laterals in single row on either side; 27 dorsals, 14 oral and 13 aboral. Oral sucker oval, 38-56 (49) x 47-59 (54). Acetabulum protrusible, post-equatorial, 59-73 (66) in diameter. Tail aspinoe, without fin folds, 344-460 (395) x 56-78 (69) in size. Anterior region of tail with 12-14 pairs of sensory hairs.

Mouth subterminal; prepharynx 12-17 (14)long; oesophagus 77-94 (83)long, solid, consisting of 8 distinct cells containing round conspicuous nuclei. Caeca 180-201 (194) long, solid, extending upto anterior end of excretory bladder; each caecum consists of 8 cells, with an additional cell at the point of bifurcation.

Penetration glands 4 pairs, lobate, filled with fine granules. Para-oesophageal gland cells present. Cystogenous glands with rod-shaped contents, in lateral fields of body.

Genital primordia consist of two masses of cells, situated at anterior and posterior margins of acetabulum, and connected by a string of cells passing dorsal to acetabulum.

Excretory system stenostomate, with bipartite excretory bladder; main collecting ducts distended between prepharyngeal and acetabular levels, each containing 190-240 excretory granules. Caudal branch of excretory system extends to anterior-fourth of tail and bifurcates before opening to lateral sides. Flame cell formula: $2[(3+3+3)+(3+3+3)] = 36$.

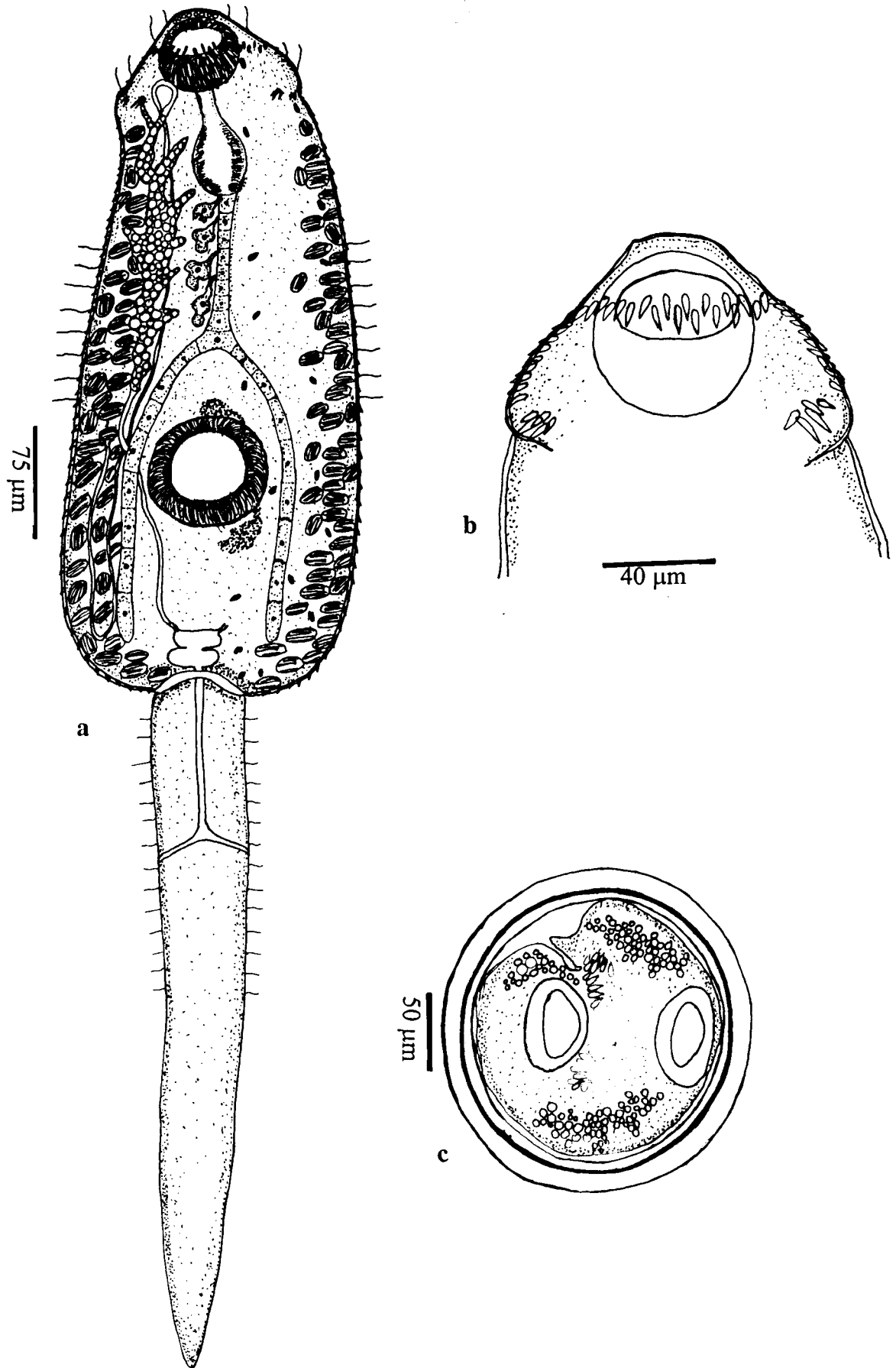


Fig. 44. *Cercaria* sp. VI Malabar n. sp.
a. Cercaria; b. Collar with collar spines; c. Encysted metacercaria.

Table 28. Measurements of *Cercaria* sp.VI Malabar n.sp.

Characters	Range	Mean
Body	350-478 x 177-243	411-206
Collar Width	102-139	123
Collar spines (length)	7-9	8.3
Oral sucker	38-56 x 47-59	49 x 54
Acetabulum	59-73	66
Tail	344-460 x 56-78	395 x 69
Prepharynx (length)	12-17	14
Pharynx	47-59 x 40-53	54 x 47
Oesophagus (length)	77-94	83
Caeca (length)	180-201	194

Metacercaria (Fig. 44c)

Natural infections by the metacercariae were found in hepatopancreas, kidney and gonad of *Lymnaea luteola*. Cyst spherical, 183-206 (196) in diameter; cyst wall double-layered, with an inner, opaque layer, about 3.5 - 6.5 thick and outer, transparent layer, about 9-10.5 thick. Collar spines excretory concretions, suckers and caeca were visible through cyst wall. In experimental infections, cercariae penetrated and developed into metacercariae in *Indoplanorbis exustus* and *Bellamya dissimilis*.

Sporocyst: Not observed.

Redia (Figs. 45a-b)

Mother rediae, were found in pericardium and kidney of snails. They are elongate, light yellow, measured 676–1059 (944) x 296–410 (319). Mouth terminal, pharynx globular, 121-165 (143) in size. Collar prominent, 190 – 240 (112) wide. Gut extend upto the level of locomotor organs, filled with dark granules. Birth pore inconspicuous. Fully-grown mother rediae contained 12–16 daughter rediae and many germ balls.

Daughter rediae recovered from hepatopancreas and kidney of the infected snail host were cylindrical, motile, yellow-brown. Mature daughter rediae contain 3-6 mature, 2-10 immature cercariae and a few germ balls; measured 1900-2740 (2318) long and 390-576 (498) wide. Mouth terminal, pharynx globular, 180-270 (235) in diameter. Collar 246-321 (276) wide. Gut

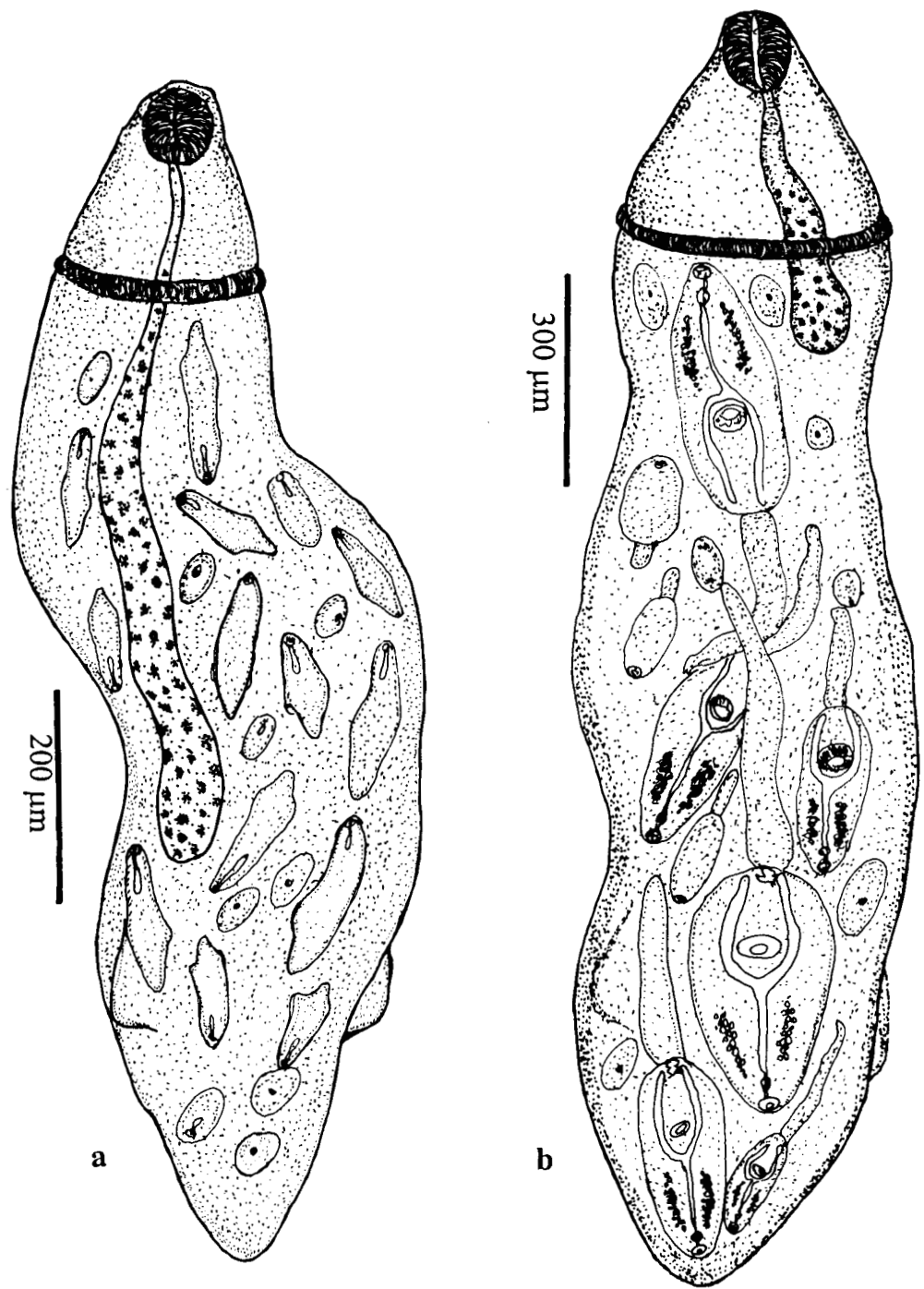


Fig. 45. *Cercaria* sp. VI Malabar n. sp.
a. Mother redia with daughter redia and germ balls;
b. Daughter redia with cercariae and germ balls.

extends upto anterior-fourth of the body, filled with dark granules, measured 350-510 (466) long. Birth pore conspicuous, lateral, lies immediately posterior to collar.

First intermediate host	: <i>Lymnaea luteola</i>
Locality	: Feroke and Ramanattukara in Kozhikode district
Period of Collection	: September and October 1997, February and March 1998
Prevalence	: 0.66 % (19 of 2850 were infected)
Second intermediate host	: <i>L. luteola</i> , <i>Indoplanorbis exustus</i> and <i>Bellamya dissimilis</i>

Remarks

This cercaria is related to *Cercaria affinis* Wesenberg-Lund, 1934, *C. densi* Fain, 1953 and *C. essexensis* Khan, 1960 in general morphology and in having a five corner spines. But comparative characters presented in table... show that *C. densi* differs in having larger number of spines (53 or 54), different body dimensions and in that the caudal excretory duct continues posteriorly after giving out its lateral branches. *C. affinis* has probably 47 spines and differs from the present spine in body dimensions and in being strongly attenuated behind the collar. The present cercaria is different from *C. essexensis* in having 18 pairs of flame cells, four pairs of penetration glands, 8 cells in oesophagus, and 10-12 setae on body. Its tail is shorter than the body and cystogenous material is rod-shaped.

Among the 21 echinostome cercariae reported from lymnaeid snails in India, the present form comes close to *Cercaria* sp. VI Kerala Mohandas, 1981, *Cercaria* sp. VIII Kerala Mohandas, 1981 and *C. unnoaensis* III Pandey and Lal, 1982.

Cercaria sp. VI Kerala has aspinose body with 47 collar spines, 11-14 pairs of body setae, finfolds on tail, which is larger than the body, granular cystogenous material, 3 pairs of penetration glands, and 10 cells in oesophagus. *Cercaria* sp. VIII Kerala differs from the present form in having aspinose body with larger number of collar spines (51), 2 pairs of setae at oral sucker region, granular cystogenous material, 21 pairs of flame cells, and 2 pairs of penetration glands. Further its tail is almost equal to the body. *C. unnoaensis* III has a different collar spine number and flame cell formula that of the present form. In addition, the present form having different number of penetration gland, and caecal and oesophageal cells.

On the basis of the above comparison, the present species as new and named *Cercaria* sp. VI Malabar n.sp.

DISCUSSION

Echinostomes in Kerala have not been the subject of any comprehensive study. The present investigation on larval and adult echinostomes in Malabar was aimed at studying the systematics, biology and host-parasite relations of echinostome cercariae infecting freshwater snails in Malabar, and elucidating the life cycles of a few favourable species using cercaria as the starting point. This study makes it possible to reveal the significance of freshwater snails in the spreading of echinostomes among mammals, birds and other lower vertebrates. The data collected could be used as biological grounds in organizing control measures against the spread of echinostomes.

Eleven species of freshwater snails representing 9 genera, collected from various aquatic bodies in Malabar, were used for this investigation during the period, from March 1996 to May 1999. The snail species proved positive for infection were *Bellamya dissimilis*, *Pila virens*, *Lymnaea luteola* and *Indoplanorbis exustus*. Out of 12,963 snails (4 species) examined, 1085 were found infected, indicating an overall prevalence of 8.37%. Altogether 15 species of echinostome cercariae were recovered from these snails: 9 from *L. luteola*, 3 from *I. exustus*, 2 from *B. dissimilis* and one from *P. virens*. The species of cercariae recovered, their hosts and prevalence of infection are summarised in Table 29.

Table 29. Summary of echinostome cercariae recovered, their hosts and prevalence of infection

Sl. No.	Cercariae	Host	No. of Host		Prevalence (%)
			Infected	Examined	
1	Cercaria of <i>Echinostoma revolutum</i> (Frölich, 1802) Dietz, 1909	<i>Lymnaea luteola</i>	65	2850	2.28
2	Cercaria of <i>Echinostoma</i> sp.I n. Sp	<i>Pila virens</i>	131	2230	5.87
3	Cercaria of <i>Echinostoma</i> sp. II n. sp.	<i>Bellamyia dissimilis</i>	353	3827	9.22
4	Cercaria of <i>E. malayanum</i> Leiper, 1911	<i>Indoplanorbis exustus</i>	61	4056	1.5
5	Cercaria of <i>Echinostoma</i> sp.III n. sp.	<i>L. luteola</i>	37	2850	1.3
6	Cercaria of <i>Echinoparyphium</i> sp.I n.sp	<i>L.luteola</i>	46	2850	1.61
7	Cercaria of <i>Paryphostomum giganticum</i> Rai and Agrawal, 1961	<i>L.luteola</i>	76	2850	2.6
8	<i>Cercariae indicae</i> XX Sewell, 1922	<i>I. exustus</i>	61	4056	1.5
9	<i>Cercaria</i> sp.VI Kerala Mohandas, 1981	<i>L.luteola</i>	22	2850	0.77
10	<i>Cercaria</i> sp.I Malabar n.sp.	<i>L.luteola</i>	3	2850	0.11
11	<i>Cercaria</i> sp.II Malabar n.sp	<i>I. exustus</i>	146	4056	3.6
12	<i>Cercaria</i> sp.III Malabar n. sp.	<i>L.luteola</i>	27	2850	0.95
13	<i>Cercaria</i> sp. IV Malabar n.sp.	<i>B. dissimilis</i>	22	3827	0.57
14	<i>Cercaria</i> sp. V Malabar n.sp.	<i>L.luteola</i>	16	2850	0.56
15	<i>Cercaria</i> sp.VI Malabar n.sp.	<i>L.luteola</i>	19	2850	0.66

Life cycles of 4 new species have been successfully established in the laboratory. These include *Echinostoma* sp. I n.sp., *Echinostoma* sp. II n.sp., *Echinostoma* sp. III n.sp., *Echinoparyphium* sp. I n.sp. Among these, the cercariae of *Echinostoma* sp. II n.sp. and *Echinoparyphium* sp. I n.sp. have already been reported; these are *Cercaria spinosa* Pandey and Singh, 1984 and *Cercaria* sp. VII Kerala Mohandas, 1981 respectively. Observations were made on further course of development of 3 species of cercariae of previously known life cycles. These are *E. revolutum* (Frölich, 1802) Dietz, 1909, *E. malayanum* Leiper, 1911 and *Paryphostomum giganticum* Rai and Agarwal, 1961. All the stages in the life cycles of these flukes, from egg to egg-producing adults, have been recovered and reported.

The remaining 8 cercarial species encountered during the present investigation could not be developed into their adults even after consistent attempts. Among these, the cercariae already reported from elsewhere are, *Cercariae indicae* Sewell, 1922 infecting *I. exustus* and *Cercaria* sp. VI Kerala Mohandas, 1981 from *L. luteola*. All the known cercariae have been redescribed in the present study furnishing additional information in certain cases.

Six species could not be identified with any of the known cercariae and are, therefore, tentatively reported as new species. The new species are,

Cercaria sp. I Malabar n.sp. from *L. Luteola*, *Cercaria* sp. II Malabar n.sp. from *I. exustus*, *Cercaria* sp. III Malabar n.sp. from *L. luteola*, *Cercaria* sp. IV Malabar n.sp. from *B. dissimilis*, *Cercaria* sp. V Malabar n.sp. from *L. luteola* and *Cercaria* sp. VI Malabar n.sp. from *L. luteola*.

Discussion on systematics and life cycles of individual species of adult and larval echinostomes have been made while describing them in the thesis. However, a general discussion on systematics and certain aspects of host-parasite relations of echinostomes is presented here.

Systematics

We know that species determination of closely related echinostomes, based on adult characters, is mostly misleading and confusing, often producing a number of synonyms. For eg. *Echinostoma paraulum*, treated as synonym of *E. revolutum* by Beaver (1937), was regarded by Supperer (1959) as distinctly different based on larval characters, intermediate hosts and on the size of metacercaria. Beaver (1937) and Fried (1984), noted variations in the characters of adult *E. revolutum* raised in different hosts. Berrie (1960), Ching (1961), Arora *et al.* (1962), Lie (1963) Huffman and Fried (1990), Kanev *et al.* (1994) and Kanev *et al.* (1995) also observed intraspecific variations in other trematodes. Systematic position of 7 species of adults could be established without any doubt because I was able to

elucidate their life cycles experimentally and compare their larval characters. The 8 cercarial species recovered could not be related to their adults, and without a knowledge of life cycles, it is not possible to assign these cercariae to any of the known genera.

As much confusion prevails in the literature about the classification of echinostome cercariae, it is desirable to discuss the subject in the light of the present findings. Lühe (1909) used the presence or absence of caudal finfolds in cercaria and length of gut in redia for division of echinostome cercariae. Sewell (1922) disregarded the length of gut in redia as a criterion for classification as he thought it to be a variable character. He divided echinostome cercariae into 3 subgroups based on the presence or absence of caudal finfolds in combination with the type of excretory system. These are Echinata, Coronata and Echinatoides. Faust (1924) suggested, yet another classification based solely on the flame cell pattern. He recognized Echinata and Coronata groups of Sewell and included Agilis and Reflexae groups which were placed as an appendix to the echinostome cercariae by Sewell (1922). Johnson (1920), Beaver (1937) and Kanev *et al.* (1995), even after studying a large number of echinostome cercariae of a single species, were still doubtful about the exact pattern of flame cells, and concluded that the correct pattern of flame cells could not be traced because of the numerous cystogenous gland-cells that cover them; this was experienced during the

present study also. Therefore, the use of flame cell pattern in the classification of echinostome cercariae cannot be considered as valid.

Sewell's classification has been accepted by most of the later workers. Recent studies have brought to light some interesting facts about his classification. The knowledge of a number of cercariae studied so far has shown that cercaria with tail finfolds or without tail finfolds may develop into species of the same genus. For example, cercaria of *Echinoparyphium spinigerum*, as described by Ahmed (1959), has a tail-finfold, whereas the cercariae of *E. recurvatum*, as described by Wesenberg-Lund (1934), that of *E. flexum*, as described by Najarin (1953), and of another *Echinoparyphium* species reported by Fried *et al.* (1998) have no tail finfolds. During the present study I recovered *Cercaria* sp.VII Kerala Mohandas, 1981 from *Lymnaea luteola* and developed it in experimental hosts into a new species of *Echinoparyphium*. This cercaria also does not possess tail finfolds. Similarly, different species of one genus of echinostome have cercariae with either echinata or coronata type of excretory system. It is evident in the case of the genus *Echinoparyphium* in which cercaria of *E. flexum* has the echinata type of excretory system while that of *E. recurvatum* has the coronata type.

Mukherjee (1992) retained Sewell's 3 subgroups and added one more subgroup, the Megacaudata, to include cercariae with tails which are massive and many times longer than the body. These cercariae undoubtedly form a compact natural group, which can easily be separated from other echinostome cercariae. Hence the view expressed by Cort (1915), Dubois (1929), Wesenberg-Lund (1934) and Miller (1936) that the echinostome cercaria cannot be subdivided into smaller natural groups cannot be accepted. However, in view of the facts stated above, characters like the pattern of flame cells and presence or absence of caudal finfolds are not suitable for dividing echinostome cercariae into different natural groups; these could only be of specific value. As suggested by Khan (1960), echinostome cercariae should remain in a single group until more suitable characters for their subdivision are discovered.

Host - parasite relations

The echinostome cercariae recovered during the present investigation, their hosts and prevalence of infection are summarised in Table 29. From an analysis of the data, it is evident that all the 15 species of cercariae recovered and reported are species-specific, each having its own snail as host. Results of cross infection studies conducted with miracidia of 3 species of 37-collar-spined worms (*Echinostoma revolutum*, *Echinostoma*

sp. I n. sp. and *Echinostoma* sp. II n. sp.) using laboratory-raised clean snails, *Pila virens*, *Bellamya dissimilis* and *Lymnaea luteola*, also confirmed that the cercariae are strictly species-specific. Out of a total of 11 snail species, collected and examined, only 4 were positive. These snails were not equally preferred by cercariae. The most preferred one was *L. luteola* with 9 species infecting it. Of the 2850 specimens examined 2539 were found to be free from infection. Next to this was *Indoplanorbis exustus* harbouring 3 species. *B. dissimilis* was found infected by 2 cercarial species and *P. virens* by only one species. An examination of list of echinostome cercariae reported from Indian freshwater snails till date (Table. 1) reveals that out of the 6 genera of snails infected, *Lymnaea* sp. is the most preferred host with 23 cercariae infecting it. This is followed by *Indoplanorbis* sp. with 20 cercariae and *Bellamya* sp. with 5. *Gyraulus* sp. carries 3 cercariae and *Pila* sp. and *Amnicola* sp. one each. It is interesting to note that the data on number of species of cercariae infecting each snail species, collected during the investigation, is in agreement with that accumulated over the years. In either case *L. luteola* is the most preferred host, and is followed by *I. exustus*. It is a well known fact that lymnaeid snails in general act as good intermediate hosts for trematodes. A satisfactory explanation cannot be offered for the highest frequency of infection observed in *L. luteola*. I would agree with Mohandas (1974) and note that the family Lymnaeidae

may be phylogenetically very old and well adapted to withstand the effects of infection because of the long-term association between the host and the parasite. The same explanation can probably be offered for planorbids as well.

Mixed infections

Mixed infections were observed in 3 of 4 snail species infected by echinostome cercariae (Table 30). Double infections were found in 38 specimens (21 *I. exustus*; 14 *L. luteola*; 3 *P. virens*) and two snails exhibited triple infections (one *L. luteola*; one *P. virens*). In all the mixed infections one of the infections involved an echinostome cercaria which is *Echinostoma malayanum* in 22 of 40 cases, *Cercaria* sp. II Malabar n.sp. in 9, *Cercaria* sp. VI Kerala in 5 and *Echinostoma* sp. I n. sp. in 4. Among the double infections the superimposed infection was also with another echinostome in 3 instances, with 3 species of furcocercous cercariae in 21, *Cotylophoron cotylophoron* in 11 and *Cercaria pilai* I in 3 cases. The single *I. exustus* exhibiting triple infection harboured *E. malayanum*, *C. cotylophoron* and furcocercous cercaria sp.No.1 and the single *P. virens* served as host for *Echinostoma* sp.I n. sp., *C. pilai* 1 and *C. pilai* II.

There were several instances of snails exhibiting mixed infections by cercariae (Cort, 1915; Sewell, 1922; Dubois, 1929; Cort *et al.*, 1937;

Lie *et al.*, 1965; Mukherjee, 1966; Probert, 1966; Jain, 1970; Robinson and Williams, 1970; Donges, 1972; Choubisa and Sharma, 1986; Jourdane and Mounkassa, 1986 and Jourdane *et al.*, (1990). Sewell (1922) recorded 18 cases of double infections; 16 of which occurred in *Thiara tuberculata* and 2 in *I. exustus*. In the present study, 40 out of 1085 infected snails exhibited mixed infections. Of these, 31 occurred in *I. exustus*, 5 in *L. luteola* and 4 in *P. virens*. Mixed infections were not observed in *Bellamya dissimilis* although this snail is a host for 2 echinostomes. The reason for this is not known.

It is an established fact that mixed infections by larval Digenea in snails are rare. Sewell (1922) suggested that the entry of the first miracidium renders the snail host unfit physiologically for second invasion, either by destroying the chemotactic stimulus normally present or by damaging the host tissue and thus preventing development of another parasite. He believed that mixed infections occur when 2 or more species of miracidia penetrate a snail simultaneously or within a short period of time. Dubois (1929) and Probert and Erasmus (1965) also agreed with this view. Cort *et al.* (1937) provided evidence for the presence of some sort of immunity or antagonism between 2 species of plagiorchiid cercariae they studied. Lie *et al.* (1965) demonstrated that the occurrence of antagonism between species of larval echinostomes infecting *L. rubiginosa* results in the reduction or absence of

double infection. Jourdane and Mounkassa (1986) showed interspecific competition between the mother sporocysts of *Schistoma mansoni* and *Echinostoma caproni* infecting *Biomphalaria pfeifferi* resulting in a shifting of most *S. mansoni* mother sporocysts out of their usual sites into deeper snail tissues; also noted was a degeneration of some of the mother sporocysts of *S. mansoni* in the normal site. The authors speculated that if the interspecific competition favoured the echinostome, during early development some of the *S. mansoni* mother sporocysts would be able to protect themselves by migrating to deeper tissues within the snail. Of the 15 cercarial species recovered during this study, only two echinostome species, *Echinostoma malayanum* and *Cercaria* sp.II Malabar n. sp., exhibited concurrent infection. This was observed in *I. exustus* which harboured 3 species of echinostomes. Although 1085 snails were found infected by echinostomes, only 40 specimens exhibited mixed infections. Whether this rarity of multiple infection is due to physiological changes produced in snails or due to the presence of some sort of immunity or antagonism existing between larval digeneans is not understood. This is a profitable area for further investigations.

Table 30. Incidence of various combinations of mixed infections in snail hosts

Host	Combination of infection		No. of snails, with mixed infection/ Total infected
	Echinostome cercaria	Other species	
<i>Indoplanorbis exustus</i>	Cercaria of <i>Echinostoma malayanum</i>	Cercaria of <i>Cotylophoron cotylophoron</i> (Amphistome cercaria)	11/61
	Cercaria of <i>E. malayanum</i>	Furcocercous cercaria sp.No. 1 (unidentified)	7/61
	Cercaria of <i>E. malayanum</i>	Cercaria of <i>C. cotylophoron</i>	1/61
		Furcocercous cercaria sp. No.I (unidentified)	
	Cercaria of <i>E. malayanum</i>	<i>Cercaria</i> sp. II Malabar n. sp.	3/204
		<i>Cercaria</i> sp. II Malabar n. sp.	Furcocercous cercaria sp. No.2 (unidentified)
<i>Pila virens</i>	Cercaria of <i>Echinostoma</i> sp. I n. Sp.	<i>Cercaria pilai</i> II (Xiphidiocercaria)	3/131
	Cercaria of <i>Echinostoma</i> sp.I n. sp.	<i>C. pilai</i> I (Xiphidiocercaria) <i>Cercaria pilai</i> II	1/131
<i>Lymnaea luteola</i>	<i>Cercaria</i> sp. VI Kerala	Furcocercous cercaria sp. No.3 (unidentified)	5/22

S U M M A R Y

The present investigation on larval and adult echinostomes in Malabar, carried out for a period of 3 years and 3 months (March 1996 to May 1999), was aimed at studying the systematics, biology and host-parasite relations of echinostome cercariae infecting freshwater snails in Malabar, and establishing the life cycles of a few favourable species using cercaria as the starting point. A total of 23,536 freshwater snails belonging to 9 genera and 11 species, collected from different aquatic bodies in Malabar, were examined for cercarial infections. Of these, 4 species were found infected by 15 species of cercariae; 9 from *Lymnaea luteola*, 3 from *Indoplanorbis exustus*, 2 from *Bellamyia dissimilis* and one from *Pila virens*. Life cycles of 4 new species have been successfully worked out. These include *Echinostoma* sp.I n.sp., *Echinostoma* sp.II n.sp., *Echinostoma* sp.III n.sp. and *Echinoparyphium* sp.I n.sp. Observations were made on further course of development of 3 species of cercariae of previously known life cycles. These are *E. revolutum* (Frölich, 1802) Dietz, 1909, *E. malayanum* Leiper, 1911 and *Paryphostomum giganticum* Rai and Agarwal, 1961. All the stages in the life cycles, from egg to egg-producing adults, have been recovered and reported.

Echinostoma sp. I n.sp. has a 3-host life cycle: the 37-collar-spined cercariae shed by the apple snails, *P. virens*, encysted in *I. exustus*, *B. dissimilis* and *L. luteola* and occasionally in *P. virens*. The metacercariae developed into adults in the intestine of white leg-horn chicks and albino-rats.

The life cycle of *Echinostoma* sp. II n. sp., infecting the whitebreasted waterhen, *Amaurornis phoenicurus*, was established using the 37-spined cercaria liberated by *B. dissimilis*. The cercaria is identified as *Cercaria spinosa* Pandey and Singh, 1984. The metacercariae, obtained from *B. dissimilis*, *L. luteola*, *P. virens*, *I. exustus*, *Thiara tuberculata* and tadpoles of *Bufo bufo* developed into adults in *A. phoenicurus* and albino-rats. This forms the first report of an echinostome from *A. phoenicurus* in India.

The 43-collar-spined cercariae released by *L. luteola* encysted in *L. luteola*, *I. exustus*, *B. dissimilis* and *Paludomous tanschauricus*. The adult developed in albino-rats is identified and reported as *Echinostoma* sp. III n. sp.

Echinoparyphium sp. I n.sp. has 43-collar-spined cercaria which was reported by Mohandas (1981) as *Cercaria* sp. VII Kerala. Metacercariae recovered from *I. exustus*, *B. dissimilis*, *T. tuberculata* and *P. tanschauricus* developed into adults in albino-rats.

The life cycle stages of *E. revolutum*, *E. malayanum* and *P. giganticum*, established in the laboratory, were identical with those reported for these worms by Mohandas (1973), Kanev (1994), Lie (1963) and Rai and Agarwal (1961).

The remaining 8 species of cercariae could not be developed into their adults. Of these, the cercariae encountered during the present study, but already reported from elsewhere are, *Cercariae indicae* XX Sewell, 1922 infecting *I. exustus* and *Cercaria* sp. VI Kerala Mohandas, 1981 infecting *L. luteola*. All the known cercariae have been redescribed in the present study furnishing additional information in certain cases. Six species could not be identified with any of the known cercariae and are, therefore, described and reported as new species. These are, *Cercaria* sp. I Malabar n.sp. from *L. luteola*, *Cercaria* sp. II Malabar n.sp. from *I. exustus*, *Cercaria* sp. III Malabar n.sp. from *L. luteola*, *Cercaria* sp. IV Malabar n.sp. from *B. dissimilis*, *Cercaria* sp. V Malabar n.sp. from *L. luteola* and *Cercaria* sp. VI Malabar n.sp. from *L. Luteola*.

Data on prevalence of infection have been collected and presented. Data collected on mixed infections, host-specificity and other aspects of host-parasite relations have been presented and discussed in the light of available information.

REFERENCES

- Agarwal, S.M. 1959. Studies on two new species of the genus *Paryphostomum* (Dietz, 1909) (Trematoda: Echinostomatidae) from *Bubulcus ibis*. Indian J. Helminthol., 10 (1): 19-30.
- Agrawal, N. 1974. Cercariae from Lucknow – II. On a new echinostome cercaria and its excretory system. Indian J. Zool., 15(3): 131-134.
- Agrawal, N. and Sharma, S.K. 1980. *Cercaria chillavanensis* n.sp. A new echinostome cercaria from *Lymnaea acuminata* (L). J. Inst. Sci., 3 (1): 215-220.
- Ahmed, Z. 1959. Die Cercarien fauna der Umgebung von Munster (Westf.) und der experimental ermittelte Individual cyclus von *Echinoparyphium spiniferum* La vallette (Trematoda)." Z. Parasitenk., 19: 67-99.
- Arora, S., Agarwal, M.M. and Agarwal, S.M. 1962. Studies on some intraspecific variations in *Paradistomum orientalis* Narain and Das. Collected from the liver and intestine of *Calotes vesicolor* Daud Part II (Dicrocoelidae: Trematoda). Indian Helminthol., 14: 5-15.
- Bashkirova, E.E. 1941. Echinostomatidae of birds of the USSR and observations of their life cycle. Trudy Bashkiroskii nauchnich Issledovaleski Veterinarni Opytnich Stantsii 3: 243-300.
- Baugh, S.C. 1950. On *Paryphostomum horai* sp. nov. (Trematoda: Echinostomatidae) with a note on the systematic position of *Paryphostomum novum* Verma, 1937. Rec. Indian. Mus., 47: 99-106.
- Baugh, S.C. 1975. Studies on some echinostome cercariae. Rev. Iber. Parasitol., 35: 311-328.
- *Beaver, P.C. 1937. Experimental studies on *Echinostoma revolutum* (Frölich) a fluke from birds and mammals. Illinois Biol. Monogr., 15: 1-96.
- Beaver, P.C. 1941. Studies on the life history of *Euparyphium melis* (Trematoda: Echinostomatidae) J. Parasitol., 27: 35-43.
- Berrie, A.D. 1960. The influence of various definitive hosts on the development of *Diplostomum phoxini* (Strigeidae: Trematoda). J. Helminthol., 34: 205-210.

- Bisseru, B. 1953. Some stages on the development of larval echinostomes recovered from mollusks acting as carriers of schistosomes in Central America. *Trans. Royal Soc. Trop. Med. Hyg.*, **47**: 262-263.
- Bisseru, H. 1967. Stages in the development of larval echinostomes recovered from schistosome transmitting mollusks in Central Africa. *J. Helminthol.*, **2**: 89-108.
- Buscher, H.N. 1978. *Echinoparyphium speotyto* sp. n. (Trematoda: Echinostomatidae) from the burrowing owl in Oklahoma, with a discussion of the genus *Echinoparyphium*. *J. parasitol.*, **64** (1): 52-58.
- Cantwell, G.E. 1981. Methods for invertebrates. In: *Staining procedures* (Ed. Clark, G.). Williams and Wilkins, Baltimore, 255-280.
- *Chatterjee, R.C. 1933. On an echinostome cercariae, *Cercaria palustris*, with notes on its life history, *Bull. Acad. Sci. U.P. Agra and Oudh.*, **2**(3):193-201.
- Ching, H.L. 1961. The development and morphological variation of *Philophthalmus gralli* Mathias and Leger, 1960 with a comparison of species of *Philophthalmus* Looss. 1899. *Proc. Helminthol. Soc. Wash.*, **28**: 130-138.
- Chishti, M.Z. and Tanveer. S. 1992. On new species of trematode genus *Echinoparyphium* Dietz, 1909 from domestic fowl in Kashmir. *Indian J. Parasitol.*, **16** (2): 155-157.
- Choubisa, S. L. and Sharma, P.N. 1986. Incidence of larval trematodes infection and their seasonal variations in the freshwater molluscas of south Rajasthan. *Rec. Zool. Surv. India*, **83**: 69-80.
- Choubisa, S.L. and Sharma, S.L. 1984. *Cercaria tewarii* n.sp. (Echinostomatid cercaria) from the fresh water snail, *Indoplanorbis exustus* (Deshayes). *Bio-Sci. Res. Bull.*, **1** (1-2)B: 50-53.
- *Cobbold, T.S. 1860. Synopsis of the Distomidae. *J. Proc. Linn. Soc., London. Zool.*, **17** (5): 10-56.
- *Cort, W.W. 1915. Some North American Larval Trematodes. *Illinois Biol. Monogr.*, **1** (4): 1-70.
- *Cort, W.W., McMullen, D.B. and Sterling, B. 1937. Ecological studies on the cercariae in *Stagnicola emarginata angulata* (Sowerby) in the Douglas Lake region, Michigan. *J. Parasitol.*, **323**: 504-532.

- *De Fillipi. 1857. Mémoire pource Servir á l' histoire génétique des Trematodes. Mem. Acad. Sci. Turin., 15 (2): 331-358.
- *De Fillipi. 1859. Troisieme Mémoire pour servir á l'histoire gé né tique des Tre 'matodes. Mem. Acad. Sci. Turn., 18 (2): 201-232.
- Dhanumkumari,C.H., Rao, K.H. and Shymasundari, K. 1991. The life cycle of *Echinochasmus bagulai* (Trematoda: Echinostomatidae). Inter. J. Parasitol., 21(2): 259-263.
- *Dietz, E. 1909. Die Echinostomatiden der vogel. Zool. Anz., 34: 180-192.
- Dollfus, R.P. 1953. Sulla forma adulta diun Echinostomide offenuta sperimentalmente net ratto bianco di laboratorio. Atti Akad. Zad. Lincei, Rendic. Cl . sc. fis. mat. e nat. s. 8, 14 (5): 658-665.
- Donges, J. 1972. Double infection experiments with echinostomatids (Trematoda in *Lymnaea stagnalis* by implantation of rediae and exposure to miracidia. Intl. J. Parasitol., 2: 409-423.
- Dube, J.P. 1964. On some helminthes parasites in domestic duck (*Anas platyrhynchos*). Indian J. Helminthol. 16 (1): 33-43.
- *Dubois, G. 1928. Les cercaires de la region de Neuchatel. Bull. Soc. Neuchatel Sci. nat., 53: 3-177.
- *Dubois, G. 1929. Les cercaires de laregion de neuchatel. Bull. Soc. Neuchatel. Sci. Nat., 53 (2): 2-177.
- *Ercolani, G. 1881. Dell' adattamento delle specie all'ambiente, nuove ricerche sulla storia genetica di Trematodi. Mem. della Reale Acad. della Sci. dell Inst. di Bologna., 2: 237-334.
- Faruqui, A.J. 1930. On a new species of *Cercariae indicae* found in *Indoplanorbis exustus* (Deshayes). Indian J. Med. Res., 17: 1205-1214.
- *Faust, E.C. 1924. Notes on larval flukes from China. II. Studies on some larval flukes from the Central and South Coast provinces of China. American J. Hyg., 4: 241-301.
- Fried B., Frazer, B.A. and Kanev, I. 1998: Comparative observations on cercarial and metacercariae of *Echinostoma trivolvis* and *Echinoparyphium* sp. J. parasitol., 84 (3): 623-626.
- Fried, B. 1984. Infectivity, growth and development of *Echinostoma revolutum* (trematoda) in the demestic chick. J. Helminthol., 58: 241-244.

- *Frölich, J.A. 1802. Beitrage zur Naturgeschichte der Eingewedewurmer. Der Naturforscher., 29: 5-96.
- Ganapati, P.N. and Rao, K.H. 1968. On anomalous emission of echinostome larval stages and their inter-redial encystment of cercariae in the snail, *Pila globosa*, Swainson. Curr. Sci., 37: 19-20.
- Ganapati, P.N. and Rao, K.H. 1969. Some observations on the structure and life-history of *Cercaria andhraensis* n.sp. (Trematoda: Echinostomatidae) from the apple snail *Pila globosa* Swainson of Waltair. Proc. Indian. Acad. Sci., 69 (13): 277-283.
- Ghosh, R.K. and Chauhan, B.S. 1977. *Echinostoma eduardoi* sp. nov. (Trematoda: Echinostomatidae) from Indian mole rat, *Bandicota bengalensis* (Gray). Institute de Biologia publicaciones Especiales, 4: 165-168.
- *Goeze, J.A.E. 1782. Versuch einer Naturgeschinte der Eingeweidewürmer tierischer Körper. 1 – 471.
- Gupta, V. and Jahan, A. 1976. On four new trematodes of Echinostomatidae Poche, 1926 from birds. Indian J. Helminthol., 29 (1 & 2): 57-72.
- Haseeb, M.A. and Khan, D. 1983. Studies on larval trematodes infecting freshwater snails in Pakistan. VII. Echinostome cercariae. Trans. Am. Microsc. Sco., 102 (3): 251-257.
- Hsu, K.C., Lie, K.J. and Basch, P.F. 1968. The life history of *Echinostoma rodriguessi* sp. n. (Trematoda: Echinostomatidae). J. Helminthol., 54 (2): 333-339.
- Huffman, J.E. and Fried, B. 1990. "Echinostoma and Echinostomiasis". Adv. Parasitol., 29: 215-269.
- Jain, G.P. 1960. On the genus *Artyfechinostomum* (Lane, 1915) (Echinostomatidae: Trematoda). Parasitology., 50: 1-5.
- Jain, S .P. 1970. Double infection of larval trematodes in mollusca. Agra Univ. J. Res. (Sci.), 21: 47-48.
- *Johnson, J.C. 1920. The life cycle of *Echinostoma revolutum* (Frölich). Univ. California Public. Zool., 19:335-388.
- *Johnston, T.H. and Angel, L.M. 1942. The life cycle of the trematode *Paryphostomum tenuicollis* (S.J. Johnston). Tr. Roy. Sco. S. Austral., 66:119-123.

- Jourdane, J. Mounkassa, J.B. 1986. Topographic shifting of primary sporocysts and *Schistosoma mansoni* in *Biomphalaria pfeifferi* as a result of coinfection with *Echinostoma caproni*. *J. Inverte. Pathol.*, **48**: 269-274.
- Jourdane, J., Mounkassa, J.B. and Imbert-Establet, D. 1990. Influence of intramolluscan larval stages of *Echinostoma liei* on the infectivity of *Schistosoma mansoni* cercariae. *J. Helminthol.*, **64**: 71-74.
- Kanev, I. 1990. A checklist of the helminth parasites of *Echinis*, *Echinostoma*, *Echinostomatidae* (Trematoda) with reference for their renaming, replacement and reclassify. Publish. House Bulgarian. Acad. Sci., Sofia.
- Kanev, I. 1994. Life-cycle, delimitation and redescription of *Echinostoma revolutum* (Frölich, 1802) (Trematoda: Echinostomatidae). *Syst. Parasitol.*, **28**: 125-144.
- Kanev, I. and Odening, K. 1983. Further studies on *Cercaria spinifera* La valette, 1855 in Central Europe. *Khelminthologia.*, **15**: 24-34.
- Kanev, I., Fried, B., Dimitrov, V. and Radev, V. 1995. Redescription of *Echinostoma trivolvis* (Cort, 1914) (Trematoda: Echinostomatidae) with a discussion on its identity. *Syst. Parasitol.*, **32**: 61-70.
- Kanev, V. Radev, I., Vassilev, V. and Dimitrov, D.M. 1994. The life cycle of *Echinoparyphium cinctum* (Rudolphi, 1803) (Trematoda: Echinostomatidae) with re-examination and identification of its allied species from Europe and Asia. *Helminthologia*, **31**: 73-82.
- Kanev, I., Fried, B., Dimitrov, V. and Radev, V. 1995. Redescription of *Echinostoma trivolvis* (Cort, 1914) (Trematoda: Echinostomatidae) with a discussion on its identity. *Syst. Parasitol.*, **32**: 61-70.
- Khan, D. 1960. Studies on larval trematodes infecting freshwater snails in London (U.K.) and some adjoining areas. Part I. Echinostome cercariae. *J. Helminthol.*, **34**: 277-304.
- Kiseliene, V. and Grabda-Kazubska, B. 1990. *Echinoparyphium pseudorecurvatum* sp. n. (Trematoda: Echinoastomatidae) and its life cycle.
- Kosupko, G.A. 1972. Studies on the morphological and biological peculiarity of *Echinostoma revolutum* (v.Förlich, 1802) and *Echinostoma miyagawai* Ishii, 1932 (Trematoda: Echinostomatidae) on experimental material. Ph.D. Thesis, Moscow, 1-258. pp.

- *La Valette de., George. 1855. *Symbolae ad trematodum evolutionis historiam* (Berolini, 39 pp).
- Lal, M.B. 1939. Studies in Helminthology. Trematode parasites of bird. Proc. Indian Acad. Sci., **10** (2): 111-200.
- *Lane, C. 1915. *Artfechinostomum sufratyfex*. A new parasitic Echinostome of man. Indian J. Med. Res., **2**: 977-983.
- *Leiper, R.T. 1911. A new Echinostome parasite in Man. J. London School Trop. Med., **1**: 27-28.
- Leiper, R.T. 1915. Report on the results of the Bilharzia mission in Egypt. J. Royal Army Med. Corps. **25**: 1-267.
- Lie, K.J. 1963a. The life history of *Echinostoma malayanum* Lieper, 1911 (Trematoda: Echinostomatidae) Trop. Geogr. Med., **15**: 17-24.
- Lie, K.J. 1963b. Studies on Echinostomatidae in Malaya IV. The animal hosts of *Echinostoma malayanum* Leiper, 1911 (Trematoda). Zool. Parasit., **23**: 136.
- Lie, K.J. 1964. Studies on Echinostomatidae (Trematoda) in Malaya VII. The life history of *Echinostoma lindoense* Sandground and Bonne, 1940. Trop. Geogr. Med., **16**: 72-81.
- Lie, K.J. 1965. Studies on Echinostomatidae (Trematoda) in Malaya VIII. The life history of *Echinostoma audyi* sp. n. J. Parasitol., **51** (5): 781-788.
- Lie, K.J. and Basch, P.F. 1967. The life history of *Paryphostomum segregatum* Dietz, 1909. J. parasitol., **53** (2): 280-286.
- Lie, K.J. and Umathevy, T. 1965a. Studies on Echinostomatidae (Trematoda) in Malaya VIII. The history of *Echinostoma audyi* sp.n. J. parasitol., **51**: 781-788.
- Lie, K.J. and Umathevy, T. 1965b. Studies on Echinostomatidae (Trematoda) in Malaya. X. The life history of *Echinoparyphium dumni* sp.n. J. parasitol., **51** (5): 793-799.
- Lie, K.J. and Umathevy, T. 1966. Studies on Echinostomatidae (Trematoda) in Malaya. IX. The life history of *Echinostoma hystricosum* sp.n. J. parasitol., **52** (3): 449-453.
- Lie, K.J., Basch, P.F. and Umathevy, T. 1965. Antagonism between two species of larval trematodes in the same snail. Nature, **206**: 422-423.

- *Linstow, O.F. Von. 1873. Einige new Distomen and Bemerkungen uber die weiblichen Sexual organe der Trematoden. Archiv for Naturg., 39: 95-108.
- *Lühe, A. 1909. Parasitische plattwarmer, I: Trematodes Die Süßwasser Fauna Deutschlands. Heft. 17.
- *Lutz, A. 1924. Estudos sobre a evolucao dos endotrematodes Brasileiros. Mem. Inst. Osw. Cr., 14: 95-103.
- Lynch, J.E. 1933. The miracidium of *Heronimus chelydrae* MacCallum. Quart. J. Micros. Soc., 76: 13-34.
- McDonald, M.E. 1981. Key to trematodes reported in waterfowl. US Department of the Interior. Fish and Wild life Service. Resour. Public., Washington, 142: 1-156.
- Mehra, H.R. 1980. The Fauna of India and adjacent countries – Platyhelminthes; I, Trematoda, Zool. Surv. India, Calcutta. i-xii + 418.
- Mendheim, H. 1940. Zbeitrage Zur Systematik und biologie der Familie Echinostomatidae (Trematoda). Nova Acta Leopoldina, 8: 489-688.
- Mendheim, H. 1943. Beitrage Zur Systematik and Biologie der Familie Echinostomatidae. Archiv. For Naturg., 12: 175-302.
- Miller, E.L. 1936. Studies on North American Cercariae. Illinois, Biol. Monogr., 14: 1-125.
- Mogh, M.A. 1932. Two new species of trematodes from an Indian ruff (*Philomachus pugnax* Gray). parasitol., 2 (1): 54-59.
- Mohandas, A. 1971. *Artyfechinostomum sufratyfex* Lane, 1915 a synonym of *Echinostoma malayanum* Leiper, 1911 (Trematoda, Echinostomatidae). Acta Parasitol. Pol., 19: 361-368.
- Mohandas, A. 1973. Studies on the life history of *Echinostoma ivaniosi* n. sp. J. Helminthol., 47: 421-438.
- Mohandas, A. 1974. Studies on the freshwater cercariae of Kerala. I. Incidence of infection and seasonal variation. Folia Parassitologia, 21: 311-317.
- Mohandas, A. 1981. Studies on the freshwater cercariae of Kerala VIII. Echinostomatid cercariae. Proc. Indian Acad. Sci. Animal Sci., 90: 433-455.

- Moravec, F., Barus, V. and Yousif, F. 1974. Observations on the development of two echinostomes, *Echinoparyphium recurvatum* and *Echinostoma revolutum*, the Antagonists of Human Schistosomes in Egypt. *Folia Parasitol.*, **21**: 107-126.
- Mukherjee, R.P. 1963. Studies on two new cercariae from Bareilly, India. *Indian J. Helminthol.*, **15**: 77-84.
- Mukherjee, R.P. 1966. Seasonal variations of cercarial infections in snails. *J. Zool., Soc. India*, **18**: 39-45.
- Mukherjee, R.P. 1992. The fauna of India and the adjacent countries – Larval trematodes of India part II Echinostome cercariae. *Zool. Surv. India. Calcutta*, 10-100.
- Mukherjee, R.P. and Ghosh, R.K. 1968. On the synonymy of the genus *Artyfechinostomum* Lane, 1915 (Trematoda: Echinostomatidae). *Proc. Indian Acad. Sci.*, **68**: 52-58.
- Mukherjee, R.P. and Ghosh, R.K. 1968. On the synonymy of the genus *Artyfechinostomum* Lane, 1915 (Trematoda: Echinostomatidae). *Proc. Indian Acad. Sci.*, **48**: 52-58.
- Mukherjee, R.P. and Ghosh, R.K. 1977. Studies on the larval trematodes of West Bengal Part II. On a new echinostome cercaria. *Rec. Zool. Surv. India*, **72**: 277-280.
- Najarin, H.H. 1953. The life cycle of *Echinoparyphium flexum* (Linton) Dietz, 1910 (Trematoda: Echinostomatidae) *Science.*, **177**: 564-565.
- Nambiar, M.V. and Janardanan, K.P. 2001. The life cycle of *Paryphostomum giganticum* Rai and Agarwal, 1961 (Trematoda: Echinostomatidae). *Riv. Parassitol.*, **18** (42): 45-51.
- Nasincova, V. 1986. Contribution to the distribution and the life history of *Echinostoma revolutum* (Trematoda) in central Europe. *Vestník Československé společnosti Zoologické.*, **50**: 70-80.
- Nasir, P. 1960. Studies on the life history of *Echinostoma nudicaudatum* n. sp. (Echinostomatidae: Trematoda). *J. Parasitol.*, **46**: 833-847.
- Nath, D. 1973. Observations on the echinostomid metacercarial fauna encountered in Indian freshwater snail. *Indian Vet. J.*, **50** (3): 292:293.

- Odening, K. 1964. What is *Cercaria spinifera* La valette? Some remarks on the species identity and biology of some echinostome cercariae In: Ergens, G. and Rysavy, B. (eds). Parasitic worms and aquatic conditions. Czech Acad. Sci., 91-97.
- *Odhner, T. 1911. "Zum naturlichen system der digenen Trematoden I". Zool. Anz., 37: 181-191.
- Pandey, K.C. 1965. On an echinostome cercaria, *Cercaria mainpurensis* n. sp. from Mainpuri, U.P. (India). Nat. Acad. Sci. India, 35 (4): 390-392.
- Pandey, K.C. 1973. Studies on cercarial fauna of Lucknow II. On two new cercaria from *Lymnaea stagnalis* (L.) and their excretory system. Indian J. Zool., 14 (3): 149-154.
- Pandey, K.C. and Agarwal, N. 1977. Studies on cercarial fauna of Kathauta Tal, Lucknow. Indian J. Zool., 18: 1-50.
- Pandey, K.C. and Singh, H.S. 1984. Observations on a new echinostome cercaria. Indian J. Helminthol., 1 (1&2): 45-59.
- Pandey, K.C. and Singh, K.S. 1982. Observation on a new echinostome cercariae. B.K. Tandon Commen., 1: 70-74.
- Patnaik, M.M. and Ray, S.K. 1966. On the life history and distribution of *Echinostoma revolutum* (Frölich, 1802) in Orissa. Indian Vet. J., 43 (7): 591-600.
- Peter, C.T. 1955. Studies on the cercarial fauna in Madras. Part II. A new species of echinostome cercaria. Indian. J. Vet. Sci. Anim. Husband., 25: 219-224.
- *Poche, F. 1926. Das System der Platyhelminthes. Arch. Naturg. A. 91 (2-3): 1-458.
- Probert, A.J. 1966. Studies on the incidence of larval trematodes infection the freshwater mollusca of Llangorse Lake, south wales. J. Helminthol., 40: 115-130.
- Probert, A.J. and Erasmus, D.A. 1965. The migration of *Cercaria* X Baylis (*Strigeida*) within the molluscan intermediate hosts *Lymnaea stagnalis*. Parasitology., 55: 77-92.
- Rai, P. and Ahluwalia, S.S. 1958. A note on common helminths of pigs at Aligarh (Part I). Proc. Nat. Acad. Sci. India, 28(B): 195-199.

- Rai, S.L. 1962. On a new species of the genus *Echinoparyphium* Dietz, 1909 (Trematoda: Echinostomatidae). Proc. Nat. Acad. Sci., India, **32** (3)B: 209-212.
- Rai, S.L. and Agarwal, S.M. 1961. *Paryphostomum giganticum* sp. nov. (Trematoda: Echinostomatidae). Indian J. Helminthol., **13** (1): 23-24.
- Ramalingam, K. 1960. The morphology and life history of *Echinochasmus bagulai* Verma, 1935 (Trematoda; Echinostomatidae) with ecological observations on its larval forms. J. Mar. Biol. Ass. India, **2**(1): 35-50.
- Ramanujachari, G. and Alwar, V.S., 1953. *Paryphostomum sufratryfex* (Lane, 1915) Bhalerao, 1931 in pigs (*Sus scrofa domestica*) in Madras. Indian Vet. J., **30**: 498-580.
- Rao, M.A. 1933. A Preliminary report on the adult trematode obtained from *Cercaria indicae* XXIII Sewell. Indian J. Vet. Sci. and Animal Husband., **3**: 317-320.
- Reddy, G.D. and Verma, K. 1950. *Paryphostomum sufratryfex* (intestinal fluke) infection in Man. Indian Med. Gaz., **85**: 546-547.
- Robson, E.M. and Williams, I.C. 1970. Relationships of some species of *Degenea* with the marine prosobranch *Littorina littorea* (L.). I. The occurrence of larval digenea in *L. littorea* on the north Yorkshire coast. J. Helminthol., **44**: 153-168.
- *Rudolphi, C.A. 1809. Entozoorum sive vermium intestinalium historia naturalis. 2 Amstelacdami., 1-560.
- *Rudolphi, C.A. 1819. Entozoorum synopsis cui accedunt mantissa duplex et indices locupletissimi. Berolini., 1-811.
- Sewell, R.B.S. 1922. *Cercariae indicae*. Indian. J. Medical Res., **10** (supplement): 1-370.
- *Siebold, K.T. 1837. Zur Entwicklungsgeschichte der Helminthen. In: Burdach, K.F. (Ed.) Die Physiologischen Erfahrungswissenschaft. Leipzig., **2**: 183-213.
- Singh, D.P. and Bali, H.S. 1991. Studies on fresh water cercariae of Punjab – III Distome cercariae – (subgroup – Echinostome) J. Adv. Zool., **12** (2): 121-130.

- Singh, U. 1975a. Studies on Echinostomes in India –III. Morphology and life history of *Echinoparyphium hymani* n.sp. (Trematoda: Echinostomatidae) Proc. Nat. Acad. Sci., India, **45** (4)B: 249-255.
- Singh, U. 1975b. Studies on Echinostomes in India – IV. Morphology and life history of *Echinoparyphium lanceolatum* n.sp. (Trematoda: Echinostomatidae) Proc. Nat. Acad. Sci., India, **45** (4)B: 261-268.
- Singh, U. 1976. Studies on Echinostomes in India – V. Morphology and life history of *Echinoparyphium vitellocompactum* n.sp. (Trematoda: Echinostomatidae) Proc. Nat. Acad. Sci., India, **46** (4)B: 519-526.
- Singh, U. 1977. Studies on echinostome in India VI. Morphology and life history of *Echinostoma dietzi* n.sp. (Trematoda: Echinostomatidae). Proc. Nat. Acad. Sci. India, **47**: 17-24.
- Skryabin, K.I. 1947. Family Echinostomadtidae Dietz, 1909. *Snovy Trematologii.*, **1**: 310-384.
- Skryabin, K.I. 1956. Trematodes of animals and man. Principles of trematodology. Vol. XII. Izdatelstvo AN SSSR, (English) Moskva.
- Skryabin, K.I. and Shul'ts, R.E.S. 1937. *Echinostoma paraulum* – Nouveau parasite de l'homme. *Med. Parasot. Parasitar. Bolenzi.*, **7** (1): 129-138.
- Srivastava, C.B. 1982. The Fauna of India and the adjacent countries Platyhelminthes I. (supplement) Trematoda-Diegenea. *Zool. Surv. India, Calcutta*, 1-163.
- Srivastava, S. 1968. Studies on larval trematodes. On a new echinostomes cercaria. *Cercaria tetragladulata* n. sp. from *Indoplanorbis exustus*. Proc. Nat. Acad. Sci. India, **38**: 230-234.
- Srivastava, S. 1978. Larval trematodes. Part II. On two new cercariae from *Indoplanorbis exustus* (Deshayes). Proc. Indian Acad. Sci., **87**: 329-337.
- Srivastava, S. and Solomon, V. 1992. On two new echinostome cercariae from *Indoplanorbis exustus* (Deshayes) from Lucknow, India. *Indian J. parasit.*, **16** (2): 139-144.
- Srivastava, S. and Saxena, V. 1992. Two new cercariae from the snail, *Bellamya bengalensis*. *Indian Vet. Med. J.*, **16**: 127-135.
- *Stossich, M. 1895. Notizie elminthologiche. *Boll. Soc. Adriat. Sci. Nat. Trieste.*, **16**: 33-46.

- Supperer, R. 1959. Untersuchungen uber Parasiten der Hausente. *Anas Platyrhynchos domesticus*. Zeitschrift fur Parasitenk., 19: 259-277.
- Tharpar, G.S. 1969. Studies on the life history of trematode parasites. II. Some new and little known cercariae from Lucknow and its environments. Indian J. Helminthol., 21: 119-146.
- *Travassos, L.P. 1922. Informacoes sobre a fauna helminthojica de matto Grosso. Folha medica., 3 (24): 187-190.
- Verma, S.C. 1936. Notes on trematode parasites of Indian birds. Part I. Allahabad Univ. Stud., 12: 147-188.
- Wesenberg-Lund, C. 1934. Contribution to the Trematoda Digenea. Part II. The biology of the freshwater cercariae in Danish freshwaters. Kongelige Danske Videnskabernes Selskab Skrifter, Naturv. Og. Math., Afd., 9 Raekke., 3: 1-223.
- Yamaguti, S. 1958. Systema helminthum. Vol.I. The digenetic trematodes of vertebrates. Part I and II Inter Science Publish. Inc., New York, 1-1575.
- Yamaguti, S. 1971. Synopsis of digenetic trematodes of vertebrates. Keigaku Publishing Co., Tokyo, 1-1074.
- Yamaguti, S. 1975. A synoptical review of life histories of digenetic trematodes of vertebrates. Tokyo. Keigaku, 1-590 pp.

* Not referred to original

APPENDIX

THE LIFE CYCLE OF *PARYPHOSTOMUM GIGANTICUM* RAI AND
AGARWAL, 1961 (TREMATODA: ECHINOSTOMATIDAE)

M. VENUGOPALAN NAMBIAR K.P. JANARDANAN

The life cycle of *Paryphostomum giganticum* Rai and Agarwal, 1961 (Trematoda: Echinostomatidae) has been established using natural and experimental hosts. Echinostome cercariae were shed by the lymnaeid snail, *Lymnaea luteola*. Metacercariae occurred in hepatopancreas, kidney, mantle and head regions of *L. luteola*, *Indoplanorbis exustus* and *Bellamya dissimilis*. Metacercariae fed to one-day-old white leg-horn chicks developed into mature adults in 12 days.

INTRODUCTION

Dietz (1909) established the genus *Paryphostomum* to receive *P. radiatum* (Dujardin, 1845) Dietz, 1909 as its type species. Subsequently, 10 species have been added to the genus by several workers all over the world (Yamaguti, 1971; Mehra, 1980; Srivastava, 1982). Of these, 6 species have been reported from India. These are *P. radiatum* (Dujardin, 1845) Dietz, 1909; *P. pentadactylum* Verma, 1936; *P. horai* Baugh, 1950; *P. dollfusi* Agarwal, 1958; *P. giganticum* Rai and Agarwal, 1961, and *P. testitri-fo- lium* Gogate, 1934 (Prudhoe, 1944; Verma, 1936; Baugh, 1950; Agarwal, 1958; Rai and Agarwal, 1961). Life cycles of only 2 species have been elucidated. Johnston and Angel (1942) described the life cycle of *P. radiatum* and Lutz (1924) and Lie and Basch (1967) illustrated that of *P. segregatum*.

During an explorative study on the cercarial fauna of Kerala, we recovered an echinostome cercaria from the lymnaeid snails, *Lymnaea luteola* collected from paddy fields, ponds and rivulets at Melechovva in Kannur and Feroke in Kozhikode districts of Kerala. The cercariae encysted in the host snail itself and other snails of the locality and became infective metacercariae. The metacerca-

Received: December 2000; Accepted: February 2001

Department of Zoology, University of Calicut, Kerala 673635, India.

riae fed to one-day-old white leg-horn chicks, developed into adults of *Paryphostomum giganticum*. This forms the first report on the life cycle of the genus *Paryphostomum* from India.

MATERIALS AND METHODS

The lymnaeid snails, *Lymnaea luteola* were collected from paddy fields, ponds and rivulets in Kannur and Kozhikode districts of Kerala from February 1997 to November 1998. The snails were screened for cercarial infection, and infected snails were isolated and kept in separate containers. The cercariae emerging from infected snails were studied alive with or without vital stains. Collar spines were studied by using silver impregnation technique of Lynch (1933). Study of excretory system was made by exposing the cercariae to 0.01% urea solution. Genital primordia were observed in acetic orcein stained cercariae. A few infected snails were later crushed and examined for intramolluscan stages of development.

Metacercarial development was observed in infection-free *Lymnaea luteola*, *Indoplanorbis exustus* and *Bellamya dissimilis* exposed to fresh cercariae. Natural infections by metacercariae were found in snails collected from the water bodies where infections by the cercariae were present. In order to follow the development of adults, the metacercariae were fed to one-day-old white-leghorn chicks. The fed chicks were dissected at regular intervals, and the flukes at different stages of development were recovered. The metacercariae and adults were observed under a phase-contrast microscope for their structural details. Those used for permanent preparations were fixed in hot 5% formalin and stained with Mayer's carmalum (Cantwell, 1981). The eggs of the flukes, appeared in faeces of chicks 12 days post-feeding by metacercariae, were cleaned and kept in a petridish containing well-water for miracidial development. The emerged miracidia were studied alive, with or without vital staining. The silver impregnation technique of Lynch (1933) was used to study epidermal plates.

The descriptions are based on measurements of a minimum of 15 specimens. Measurements are in micrometres; the range is followed by the mean value in parentheses. Illustrations were made with the aid of a camera lucida and details added free-hand from observations on live specimens.

RESULTS

Egg:

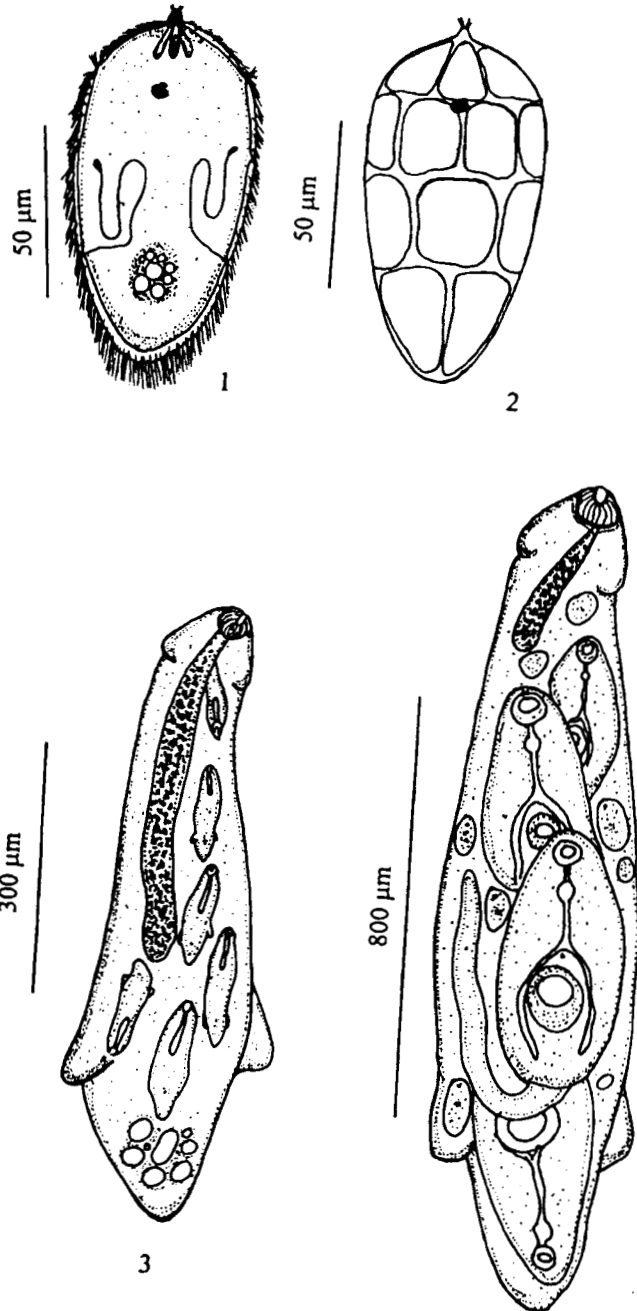
Eggs oval, yellowish brown, operculate with a thickening at the anoperculate end; measured 116-129 (124) x 86-99 (93).

Miracidium (Figs.1 - 2):

Eggs kept in water started to hatch and liberate miracidia in 7 days; exposure to light stimulated hatching process and the miracidia were attracted by light. Newly emerged miracidia measured 96-112 (104) x 52-69 (65) in size. Apical papilla with two pairs of setae measured 10 x 6. Body covered with 4 rows of ciliated epidermal plates; first row with 6 triangular plates; second row with 6 square plates; third row with 4 roughly rectangular plates; fourth row with two more or less triangular plates. Cilia 12 -14 long. Primitive gut filled with refractile granules opens at the tip of apical papilla. Penetration glands two, one on either side of gut. Eye spots 2, with dark-brown pigmented bodies. Two flame cells present; left ventral and posterior; right dorsal and anterior.

Redia (Figs.3 - 4):

Mother rediae, found in pericardial cavity, are motile, colourless with conspicuous collar and locomotor organs; measured 863-1476 (1124) x 228-390



Figs. 1 - 4: *Paryphostomum giganticum*
1. Miracidium. 2. Miracidium with epidermal plates. 3. Mother redia. 4. Daughter redia

(364). Collar 177-194 (182) wide; pharynx globular, measured 40-57 (51) in diameter; gut extends upto the level of locomotor organs. Mother rediae contain 4-8 daughter rediae and a few germ balls.

Daughter rediae are released on 10th day post-exposure to miracidia, and migrate to hepatopancreas where they start producing cercariae. Mature daughter rediae elongate, cylindrical, yellowish-brown, contain 1-2 mature cercariae, 2-6 immature cercariae and a few germ balls; measured 1364-2870 (1660) x 350-486 (420).

Cercaria (Figs. 5 - 6):

Natural infections by the cercariae were found in 76 of 362 (20.99%) *Lymnaea luteola*. Maximum prevalence of infection was noticed during the beginning of monsoon season (May-June).

Description: Body elongate, spinose, with 8 pairs of sensory hairs at the anterior half; measured 435-523 (468) x 205-261 (247) in size. Collar distinct, 130-159 (141) wide, with 47 spines, 11-13 long. Spine arrangement characteristic: five corner spines on each side, 3 oral and 2 aboral; 12 laterals on each side in double row; 13 dorsals, 6 oral and 7 aboral. Oral sucker subterminal, measured 46-58 (53) x 50-49 (56). Acetabulum protrusible, situated in post-equatorial region; measured 67-92 (83). Tail cylindrical, aspinose, almost equal to body length, without finfolds. Anterior region of tail with 6 pairs of sensory hairs; measured 399-512 (478) x 64-88 (77).

Mouth sub-terminal, prepharynx 19-25 (23) long. Pharynx muscular, 34-42 (39) x 26-32 (29); oesophagus solid, 167-184 (175) long; consisting of 10 cells; intestinal caeca solid, 190-260 long, extending to the level of excretory bladder.

Penetration glands 4 pairs, along oesophagus; gland ducts open on dorsal lip of oral sucker. Para-oesophageal gland cells opened in the region of oral sucker and pharynx. Cystogenous glands numerous, densely distributed in lateral fields, filled with oval to spherical granules.

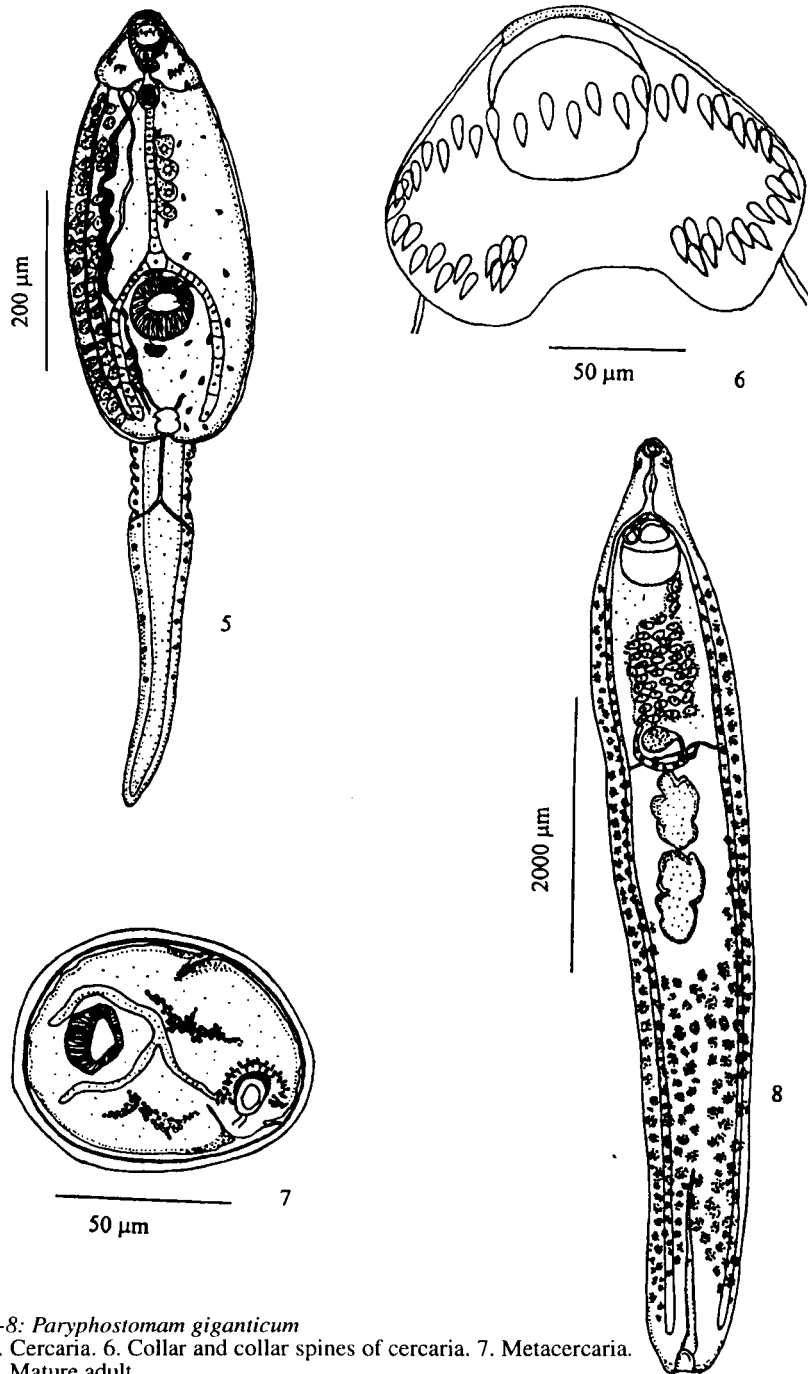
Genital primordia consist of two masses of cells, one at anterior margin of acetabulum, other between acetabulum and base of tail and connected by a string of cells passing dorsally to acetabulum. The excretory system is of the "Echinata" type; excretory bladder bipartite, at posterior end of body; main collecting tubes distended between acetabular and collar region, each containing 120-180 excretory granules. Flame cell formula, 2 [(3+3+3+3)+(3+3+3)] = 42.

Metacercaria (Fig. 7):

In natural and experimental infections, metacercariae were found in the hepatopancreas, kidney, mantle and head regions of *Lymnaea luteola*, *Indoplanorbis exustus* and *Bellamya dissimilis*. *L. luteola* carried the maximum number of metacercariae (upto 130) showing that they are the most susceptible hosts. Experimentally raised metacercariae became infective in 3 days.

Cysts slightly oval, measured 167-189 (179) x 135-148 (141). Cyst wall double-layered with an outer 10.5 - 12 thick transparent layer and an inner, opaque, 2.3-2.8 thick layer. Oral sucker, acetabulum, collar and collar spines, pharynx, oesophagus and caeca are distinct.

Excysted metacercaria measured 486-698 (627) x 241-379 (312).



Figs. 5-8: *Paryphostomum giganticum*
5. Cercaria. 6. Collar and collar spines of cercaria. 7. Metacercaria.
8. Mature adult.

Adult (Fig. 8):

Adult flukes at different stages of development were recovered from the duodenum of white leg-horn chicks. Three-day-old flukes were almost similar to metacercariae in their structural organization, but primordia of testes, ovary and cirrus pouch started developing.

On the 7th day of exposure, the flukes increased in size and measured 1678-2840 (1904) long, and 410-708 (632) wide. Testes and ovary also increased in size; ootype and uterine coils were visible. Cirrus sac with unspined cirrus and pars prostatica differentiated. Vitelline follicles made their appearance.

The 12-day-old flukes were fully mature, measured 4505-9728 (7692) x 1029-2355 (1960), with their uterine coils filled with 30-40 eggs. These flukes resembled *Paryphostomum giganticum* Rai and Agarwal, 1961 infecting the purple moorhen, *Porphyrio poliocephalus* in most respects except for the slight difference in dimensions. As we had no permission to shoot wild birds in Kerala, we had no opportunity to confirm whether *P. giganticum* reported by Rai and Agarwal is present in the purple moorhen of this locality.

DISCUSSION

The genus *Paryphostomum* Dietz, 1909 belongs to family Echinostomatidae Poche, 1926 and it includes 10 valid species. The adult flukes obtained experimentally from white leg-horn chicks are identified as *P. giganticum* described by Rai and Agarwal (1961) from the purple moorhen, *Porphyrio poliocephalus* (= *Porphyrio porphyrio*) in Jabalpur. In the present locality where infected snails were collected, the purple moorhen, *P. porphyrio* are abundant, and in all probability they are the definitive hosts. This could not be ascertained since we had no permission to collect birds.

Life cycles of only two species of *Paryphostomum* have been established. Johnston and Angel (1942) illustrated the life cycle of *P. radiatum*, and Lutz (1924) and Lie and Basch (1967) that of *P. segregatum*. Both exhibited typical three-host life cycle pattern. *P. radiatum* used *Amerianna* sp. as first intermediate host, fishes such as *Carassius auratus*, *Phalloceros caudomaculata*, *Barbus* spp., *Gambusia affinis* and *Oryzias latipes* and tadpoles of *Pseudophryne bibroni* as the second intermediate hosts and different species of cormorants as the definitive hosts. In the case of *P. segregatum*, several species of *Biomphalaria* and *Planorbis* served as first, and mullets and tadpoles as second intermediate hosts. The definitive hosts are urubu vulture, *Coragyps astratus* and the owl, *Oenops aura*. The life cycle of *P. giganticum* differs from that of the above species in that it has a two-host life cycle involving only molluscan and bird hosts. The echinostome cercariae emerged from the lymnaeid snail, *Lymnaea luteola*, encyst and develop into metacercariae in the same snail host or in other snails of the locality. The metacercariae became infective in 3 days and developed into mature adults in 12 days in the intestine of the experimental host.

Four species echinostome cercariae with 47 collar spines, and without finfolds on tail have previously been described. These are *Cercaria affinis* Wessenberg-Lund, 1934; *Cercaria essexensis* Khan, 1960; *Cercaria leyteensis* no.24 Ito, 1977 and *Cercaria paraspinicephala* Haseeb and Khan, 1983.

Cercaria affinis shows marked differences from the present cercaria in that its body is strongly attenuated behind collar, collar spine arrangement and body-tail ratio are different, and it has a different type of excretory system. *C. essexensis* is significantly different from the present form in having 47-49 collar spines (according to Khan, these probably are 49 in number) arranged differently, larger number of penetration glands, and lesser number of oesophageal cells and flame cells. Besides, *C. essexensis* is smaller in size and its body-tail ratio is different. *C. leyteensis* no.24 can be distinguished by its spinose body and tail, and different collar spine arrangement, and body-tail ratio. Further, it has a different type of excretory system. *C. paraspinicéphala* is a smaller species, and it differs from the present form in the number of penetration glands and oesophageal cells, and in body-tail ratio. Moreover, its excretory system is different.

As far as is known, cercariae of only two species of *Paryphostomum* have been reported. The present cercaria is distinct from that of *P. segregatum* reported by Lutz (1924) and Lie and Basch (1967) and that of *P. radiatum* described by Johnston and Angel (1942) in the number of collar spines (both have 27 collar spines), morphometry and structural details. Therefore, the present cercaria is new, and is reported for the first time.

This forms the first report on the life cycle of *P. giganticum*.

ACKNOWLEDGEMENT

One of the authors (M.V.N) is thankful to the University Grants Commission, New Delhi for financial assistance in the form of Senior Research Fellowship.

REFERENCES

- AGARWAL S.M. (1958) - Studies on two new species of the genus *Paryphostomum* (Dietz, 1909) (Trematoda: Echinostomatidae) from *Bubulcus ibis*. *Ind. J. Helminthol.*, 10: 19-30.
- BAUGH S.C. (1950) - On *Paryphostomum horai* sp. nov. with a note on the systematic position of *Paryphostomum novum* Verma, 1936. *Rec. Ind. Mus.*, 47: 99-106.
- CANTWELL G.E. (1981) - Methods for invertebrates. In: Staining procedures (Ed. Clark G.). Williams and Wilkins, Baltimore, 255-280.
- DIETZ E. (1909) - Die Echinostomiden der Vögel. *Zool. Anz.*, 34: 180-192.
- JOHNSTON T.H., ANGEL L.M. (1942) - The life cycle of the trematode *Paryphostomum tenuicollis* (S.J. Johnston). *Tr. Roy. Soc. S. Austral.*, 66: 119-123.
- LIE K.J., BASCH P.F. (1967) - The life history of *Paryphostomum segregatum* Dietz, 1909. *J. Parasitol.*, 53: 280-286.
- LUTZ A. (1924) - Estudos sobre a evolução dos endotrematodes Brasileiros. *Mem. Inst. Osw. Cr.*, 14: 95-103.
- LYNCH J.E. (1933) - The miracidium of *Heronimus chelydrae* MacCallum. *Qurt. J. Micros. Soc.*, 76: 13-34.
- MEHRA H.R. (1980) - The Fauna of India and the adjacent countries - Platyhelminthes Vol. I. *Trematoda*. *Zool. Sur. India, Calcutta*, i-xii + 1-418 pp.
- PRUDHOE S. (1944) - On some trematodes from Ceylon. *Ann. Mag. Nat. Hist.*, Illinois. 73: 1-13.
- RAI S.L., AGARWAL S.M. (1961) - *Paryphostomum giganticum* sp. nov. (Trematoda: Echinostomatidae). *Ind. J. Helminthol.*, 13: 23-24 (1).
- SRIVASTAVA C.B. (1982) - The Fauna of India and the adjacent countries-Platyhelminthes Vol. I. (supplement), Trematoda: Digenea. *Zool. Sur. India, Calcutta*, 1-163 pp.
- VERMA S.C. (1936) - Notes on trematode parasites of Indian Birds. Part I. *Allahabad Univ. Stud.*, 12: 147-188.
- YAMAGUTI S. (1971) - Synopsis of digenetic trematodes of vertebrates. Keigaku Publishing Co., Tokyo, 1-1074 pp.

THNB 2943

592.48

VEN/S

60