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STUDIES ON LARVAL TREMATODES OF GANGAPUR PROJECT-GODAVARI RIVER: DISTOME CErCARIAE

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ABSTRACT

The earlier work on the snails and cercariae of this region was carried out by Karyakarte and Yadav (1974 to 1979) under PL. 480 Project on Control of Molluscan Agents of Helminth Parasites of Agricultural and Veterinary Importance (Project no. A-7- ADP-39) the account of their findings was published in the year 1981. They examined eight species of snails, viz. *Vivparous bengalensis, Melanoides tuberculatus, Melania scabra Lymnea acuminata, Lymnea auricularia, Lymnea luteola, Indoplanorbis exustus Anisus (Gyrulus) convexiusculus*. Out of these eight species they reported cercarial infection in six species of snails and the two species.*M. scabra* and *V. bengalensis* were free from larval infection. Their work included description of eleven species of freshwater cercariae belonging to Monostome Amphistome and Distome groups The work on larval trematodes was further continued in this region in the Trematology Laboratory and in all nineteen cecariae were collected and described. Out of these cercariae, two ceacarie belonging to Monostome and one belonging to Echinostome group are reported from the same host *Melania tuberculata*.(1986)Present paper deals with two species of freshwater cercariae which belongs to Distome cercariae. The collection was carried out at Gangapur Project, Godavari River, Darana river, Girnare ponds and ditches around Godavari river. The study is further extended to know various responses of the cercariae to various stimuli such as phototaxis, geotaxis, emrgence and survival of cercariae in various percentage of artificial media.

No key words :Molluscan Agents, Monostome, Amphistome ,Echinostome.

INTRODUCTION

Luhe (1990) made the first attempt to classify the cercariae in a comprehensice manner. He classifies various cercariae into five different groups. The groups were Monostome cercariae, Distome cercariae, Amphistome cercariae. Lophocercous cercariae, Gastrostome cercariae. Labour (1911) made a suvey of British marine cercariae and divided into two main groups Gastrostomata and Prosostomata. Cort (1914) made a survey of larval trematodes from North American freshwater snails Faust (1919b, 1921, 1924,1926) studies larval trematodes from South Africa and China. Miller (1936) made a comparative account of Furcocercus cerceriae and in 1936 he studied the North American cercriae. Sewell (1922) studied the freshwater cercriae from India and he modified the Luhe's classification and divided major groups into a number of smaller groups. Porter (1938) studied the freshwater Larval trematodos found in certain South American Mollusca. While classifying the cercariae he followed the ideas of Luhe (1909) and Sewell (1922). Soparkar (1921) gave a note on some Furcocercuos cercariae from Bombay. Chandler (1953) gave a key to the Furcocercuos cercariae. Khan (1960 to 1961) studied larval trematodes infecting freshwater snails in London and some adjoining area. Nasir (1964) gave a key to the cercariae from British freshwater Molluscs. In 1972 he gave some aspect of Xiphidocencarial classification and in 1973 he reported twenty new species of Venezuelan cercariae, *Ito et al*(1977) study on the freshwater cercarial in Leyte Island,

Philippines. After Sewell, few workers have described some cercariae from India (Singh 1952, Premavati 1956, Patki 1956, Srivastava 1958, Malaki and Singh 1962, Gupta and Taneja 1970, and 1970a, Mohands 1977 and 1979, Karyakarte and Yadav 1981, A Farahank 2006, 2007, Nkwengulila 1998, Gulam M.A. 2011, Eric 2005, Shimura 1980, Oleg Ditrich 1997, Sami Bdir 2011, Sey 2003, Todd 2004, Thapana 2011, Uthpala 2010,) Present paper deals with two species of freshwater cercariae which belongs to Distome cercariae. The classification followed in the paper is of Luhe (1909), Sewell (1922) and Porter (1938)

MATERIALS AND METHODS

(1) Collection and maintenance of snails

Studies on secure commenced with a collection of first intermediate host (snails). They were collected either hand handpicked or dragging a net through the water and were transported to the laboratory. The snails were then transferred to glass water bowls and well aerated acquaria already provided with a rich water plants such as Vallisineria, Hydrilla, Chara, Spiirgyra and fimbria etc. After a short period of acclimatization the snails were transferred to individual test tubes kept on wooden rocks in order to detect the cercariae. In the laboratory most preferably the same pond water was used for the snails from which they were collected as the purified tap water supplied to the laboratory proved unsuitable perhaps due to chemical purification

(2) Observations

The snails collected were kept under observation for some time. The snails which are fully grown showed larval infection while the young ones were normally free from larval infection. Due to the infection, it was observed that the snails grow in size and show a phenomenon of gigantism. Many a time the shell grow enormously and ballooning was observed. For the study of cercariae heavily infected snails were selected. Two methods were followed for the morphological observations.

1) Natual emerging method
2) Crushing method.

In natural emerging method the snails (2to3 at a time) were kept in separate test tubes. This was a constant source of living cercariae naturally emerging from the snails. The sunlight and artificial light play an important positive role in stimulating the emergence of cercariae . It was observed that some cercarie emerge only in darkness.

1) Crushing method

This method of investigation of cercariae found suitable for morphological observation on various developmental stages such as sporocysts and rediae. This quick method was useful for studying the seasonal percentage of infections of cercariae. The carcariae collected were subjected to various artificial methods for the study of various internal structures.

(3) Movement relaxation

Sometimes cercariae were found to be so active that observation under power was impossible without some method interfering with or controlling their movement. Hence dilute solutions of gum, starch, and gelatin were used to slow down their movements.

(4) Vital stains

For the study of structural details in live condition vital stains were used such as Neutral red, Methyl green, Nile blue, Azur II and Nile blue sulphate. In the study of flame cells Indian ink and Amphibian ringer solution were found to be suitable. For the preparation of permanent mounts the cercariae were fixed in 1% hot formalin, stained with Delafieid's haematoxylin, cleared in clove oil and mounted in D.P.X.

(5) Measurments

Most of the specimens were measured in a live state. In the preset work the measurements given for two species of cercariae and their parthenitae represent averages of twenty specimens of each species. The diagrams have been made with the aid of a camera lucida. Sketches were drawn at different magnification using oil immersion objectives if necessary. This method gave the most uniform results. All the measurements are in millimeters. The most suitable time making the diagrams for morphological study of living cercariae was immediately after they emerged from the snails without vital staining otherwise became opaque after remaining in water for half an hour.

Responses

The responses of cercariae to various stimuli were studied in the laboratory conditions at temperature 28®C

(A) For the study of phototaxis a glass apparatus was fabricated and used. The cercariae allowed moving into four limbs of the apparatus. Three limbs were subjected to various light intensities and fourth the dark one.

(B) For geotaxis U tube was used.

(C) Emergence of cercariae was concluded after series of such observations.

(D) For studying the survival of cercariae in sagar and salt solutions, of 0.5%, 0.7%, 0.9%, 1.0%, 1.4%, 1.6%, 1.8%, 2.0%, 2.2% concentrations were used and the survival time was noted at laboratory temperature 28®C.

Cercaria darna n.sp.

Collection Data

Percentage of infection during the years 2012 and 2013

Sr. No	Month	Locality	No.of snails examined	No.of snails infected	% infection
1	January 2012	Gangapur Project, Dharna river, Godavari river, Nashik District Maharashtra. India	40	8	20.00
2	February 2012	--do--	42	12	27.90
3	March 2012	--do--	167	53	31.73
4	April 2012	--do--	243	24	9.87
5	May 2012	--do--	49	3	6.12
6	June 2012	--do--	43	2	4.65
7	July 2012	--do--	211	--	--
8	August 2012	--do--	67	--	--
9	September 2012	--do--	125	--	--
10	October 2012	--do--	80	--	--
11	November 2012	--do--	40	--	--
12	December 2012	--do--	50	--	--
Annual percent age of infection 2012		**Total**	**1158**	**102**	**8.35**
Sr. No	**Month**	**Locality**	**No.of snails examined**	**No.of snails infected**	**% infection**
1	January 2013	Gangapur Project, Dharna river, Godavari river, Nashik District Maharashtra. India	60	9	20.00
2	February 2013	--do--	102	31	30.39
3	March 2013	--do--	198	65	32.82
4	April 2013	--do--	45	4	8.88
5	May 2013	--do--	10	-	-
6	June 2013	--do--	52	-	-
7	July 2013	--do--	22	-	-
8	August 2013	--do--	100	-	-
9	September 2013	--do--	82	-	-
10	October 2013	--do--	50	1	2.00
11	November 2013	--do--	67	4	5.97
12	December 2013	--do--	40	6	15.00
Annual percent age of infection 2013		**Total**	**828**	**120**	**9.17**

Percentage of infection (Mean) 8.76

DISTOME CERCARIAE OF "PARAPLEROLOPHOCERCA" GROUP

1) CERCARIAE DHARANA n.sp

In the present work Distome cercariae are represented by two species. These cercariae were collected from the snail host *Melania scabra* and *Lymnea luteola*. The snails collected at Gangapur project – Godavari river, Dharana river and nearby places. As indicated in collection data of snails and carcariae it is evident the larval forms of *Cercaria dharana n.sp.* are abundant during the month of February and march. The percantage of infection of *Cercaria dimorpha n.sp* was not very heavy during the both years 2012 and 2013 two new cercariae belonging to a Parapleurolophocarca group of Leptocercariae in Distome cercariae are described here. The collection constituted two new species and hence described

The cercaria is yellowish in colour with black granular rod-shaped masses spread over the entire body. These structures are more in the acetabular and postacetabular regions. The cystogenous glands occupy the entire body region of the cercaria. The main body of cercaria is elongated and spinose, tapering at both the ends. The tail is narrower than the body and nearly double the length of main body. The main body measures 0.65 (0.57 to 0.73) in length and 0.17 (0.13 to 0.21) in width. The tail is 0.95 (0.90 to 1.00) long and 0.06 (0.03 to 0.09) wide. The finfold is observed on the tail. The tail tapers at the posterior end, the finfold in this region is slightly broader and shows typical serrated margins. There are two conspicuous eyespots located marginally just posterior to the pharyngeal level. The oral sucker is subterminal and smaller than the acetabulum. It has a diameter of 0.06 (0.04 to 0.08). The ventral sucker is located in the last third of the body. It has a diameter of 0.13 (0.09 to 0.17). The ratio between the diameters of oval and ventral sucker is 1:2.1. The mouth leads into a prepharynx, measuring 0.009 by 0.007 (0.006 to 0.012 by 0.005 to 0.009). The pharynx measures 0.045 by 0.325 (0.037 to 0.053 by 0.299 to 0.251) and in turn opens into a long oesophagus. The oesophagus bifurcates in the second third of the body into two intestinal caeca which run marginally and terminate at a distance of 0.06 (0.04 to 0.08) from the posterior end. The digestive glands are located in the oesophageal region. There are five flask shaped glands opening with their narrow necks near the mouth. The excretory bladder is V-shaped. The canal runs to the tip of the tail. The two cornua run upto acetabular region and fork into collecting tubules.

REDIA:-

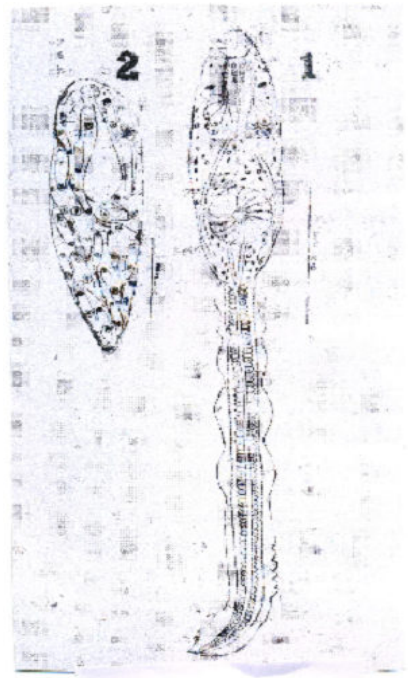

PLATE : 1
1)Cercariae darnâ n.sp.
2)Redia

The cercariae develop in redia. It has a length of 1.852 (1.611 to 2.083) and width of 0.49 (0.46 to 0.52) ,. It is yellowish in colour with a blunt anterior and pointed posterior ends. The pharynx measures 0.09 by 0.07 (0.06 to 0.12 by 0.05 toi 0.09) and opens into a saccular gut. Numerous cercariae are observed in the redia at a time.

RESPONSES:-

(1) Phototaxis Positive
(2) Geotaxis Negative
(3) Emergence At night or early in the morning
(4) Survival of cercarise in DTW (at labouratory Temperature 28^0 c) 53 hrs.
(5) Survival of cercariae in various percentage of sugar and salt solutions are as shown in the following table (at laboratory temperature e 28^0 c

Sr. No	Sugar solution %	Survival time Hrs.Min	Salt solution %	Survival time Mrs. Min.
1	0.5%	8.30	0.5%	41.30
2	0.7%	13.0	0.7%	45.00
3	0.9%	17.30	0.9%	49.00
4	1.0%	31.15	1.0%	02.30
5	1.2%	36.20	1.2%	00.20
6	1.4%	39.10	1.4%	00.10
7	1.6%	43.00	1.6%	00.00
8	1.8%	48.15	1.8%	00.00
9	2.0%	10.30	2.0%	00.00
10	2.2%	04.05	2.3%	00.00

Minimum survival time in sugar solution 4.05 hrs. in 2.2%
Maximum survival time in sugar solution 48.15 hrs. in 1.8%
Minimum survival time in solt solution 00.10 hrs. in 1.4%
Maximum survival time in salt solution 49.00 hrs. in 0.5%

DISCUSSION

In possessing well developed oral and ventral suckers, the latter away from posterior extremity, the present form belongs to a Distome group of cercariae. As the tail is narrower than the body it is included in Leptocerous cercariae group of cercariae. At the anterior extremity is lacking a stylet it is included in Gymocephalus group. As the cercaria has tail longer than the body and provided with paired lateral fin membranes throughout its length, eye spots present and development in rediae, it is included in Parapleurolophocerca group. In Parapleurolophocerca group following cercariae are reported so far Cercaria hymenocerca Villot, 1875, Cercarise indicae xxxI Sewell, 1922; Cercarise indicae L. Sewell, 1922; *Cercaria melanoides* Porter, 1938; Cercaris britsiae, porter, 1938; Cercaris plieguicauda Nasir and Diaz, 1973. The present form shows affinities with C. indicae xxxi, C. indicae l., *C. melanoides and C. britsiae.* C. indicae xxxI and C. indicae L. have a pyriform body. retractile oral sucker and a pair of salivary glands composed of several cells extending posterior to the ventral sucker. As compared to these characters, the present form has elongated main body, a simple oral sucker and a pair of salivary glands, each composed of five cells, extending to caecal bifurcation, far anterior to the ventral sucker. In addition to these characters the ventral sucker is located in the middle region of the body in the known and in the last third of the body in the new form. The present form has a well developed prepharynx, pharynx, long oesophagus and intestinal caeca. In contrast to this the digestive tract is represented by only a prepharynx and pharynx in the known forms. Porter (1938) described two forms in Parapleurolophocerca group namely *Cercaria melanoides* and *Cercaria britsiae*. In *C. melanoides* porter (1938) and the present form, the location of ventral sucker is the

same but the former differs from the latter in the absence of oesophagus and intestinal caeca. Further, the salivary glands extend to the posterior region of the body in C. melanoides whereas; these are restricted in the anterior region of the body in new species. The sucker sizes also differ in the two. The oral sucker is bigger than the ventral sucker in C. melanoides whereas; reverse in the case in the present form. C. britsiae Porter (1938) and the present form have well developed digestive system as represented by prepharynx, pharynx, cesophaqus and intestinal caeca. The two forms also have a common character of sucker sizes and their location. However, they differ considerably in the characters given in the following table.

Character	**C.britsiae Porter 1938**	**Present form**
Ratio between body length and tail length	1:1.07	1: 1.5
Ratio between the diameters of oral and ventral suckers	1 : 1.2	1 : 2.1
Position of the eyespots	Pharyngeal level	Posterior to pharyngeal level
Salivary glands	On each side of the body salivary glands form a linear series of seven cells extending throughout body length	On each side of the body salivery glands are grouped of five cells extending to oesophageal bifurcation
Excretory Eystem	Excretory bladder is shallow and is transversely elongated across the body. from each of it's cornu a main excretory tube passes anteriorly and forks at the level of the fourth salivary glands into anterior and posterior collecting tubules	
Host	***Melanoides tuberculata***	***Melania scabra***
Locality	Crocodile rigver limpopo, south Africa	Gangapur Project, Dharna river, Godavari river, Nashik District Maharashtra. India

Considering there characters a new species is established and named as *Cercaria dharna n.sp.*

Host : *Melania scabra*
Habitat : Digestive gland
Locality : Gangapur Project, Dharna river, Godavari river, Nashik District Maharashtra. India.

Cercaria dimorpha n.sp.

This cercaria was collected from the digestive glands of the snail host, *Lymnea luteola*. The snails were collected at Gangapur Project, Darana river and also from ditches and ponds around Godavari river. The snails occur in Nashik district Maharashtra State India during the period between December and May. As seen in the collection data the percentage of infection of this cercaria was not very heavy during both the years 2012 and 2013.

Collection data

Percentage of infection during the years 2012 and 2013

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Sr.	Mont	Locality	No.of snails examined	No. of snails infected	% of Infection
1	January 2012	Gangapur Project Dharna River Godavari River Nashik District Maharashtra state India	513	5	0.97
2	February 2012	-do-	215	4	1.86
3	March 2012	-do-	232	9	3.87
4	April 2012	-do-	292	17	5.82
5	May 2012	-do-	245	27	7.82
6	June 2012	-do	---	----	----
7	July 2012	-do-	-	-	-
8	August 2012	-do-	-	-	-
9	September 2012	-do-	-	-	-
10	October 2012	-do-	-	-	-
11	November 2012	-do-	-	-	-
12	December 2012	-do-	212	-	-
Infection 2012		Annual percentage of Total	1809	62	1.69
13	January 2013	-do-	425	8	1.88
14	February 2013	-do-	293	11	3.75
15	March 2013	-do-	410	24	5.85
16	April 2013	-do-	340	27	7.94
17	May 2013	-do-	312	28	8.97
18	June 2013	-do-	-	-	-
19	july2013	-do-	-	-	-
20	August 2013	-do-	-	-	-
21	September 2013	-do-	-	-	-
22	October 2013	-do-	-	-	-
23	November 2013	-do-	-	-	-
24	December 2013	-do-	255	-	-
Infection 2013		Annual percentage of Total :	2035	98	2.36

Percentage of infection (Mean) = 2.03

The cercaria is fairly large oval in shape and tapering at anterior end. The main body is spinose. It is yellowish in colour. The cystogenous cells are uniformly spread all over the body. It is an active swimmer and shows vibrating movements. The main body measures 0.45 (0.41 to 0.49) in length and 0.29 (0.27 to 0.31) in breadth. The tails is 0.58 (0.55 to 0.61) long and 0.08 (0.05 to 0.11) wide. It is provided with clear transparent finfold. The finfold extends along the lateral margins from the anterior end to the distal extremity which is sharply pointed. The oral sucker is rounded, sub-terminal and smaller than acetabulam. It has a diameter of 0.072(0.070 to 0.074). The ventral sucker is rounded and located in posterior half of the body. It has a diameter of 0.090 (0.088 to 0.092). The ratio between the diameters of oral sucker to ventral sucker is 1: 1.2. The mouth leads into a small prepharynx, measuring 0.11 (0.009 to 0.013) in length. The pharynx is small and muscular. It is 0.020 (0.018 to 0.022) in length and 0.029 (0.027 to 0.031) in breadth. The oesophagus is 0.10 (0.08 to 0.11) in length which in turn bifurcates

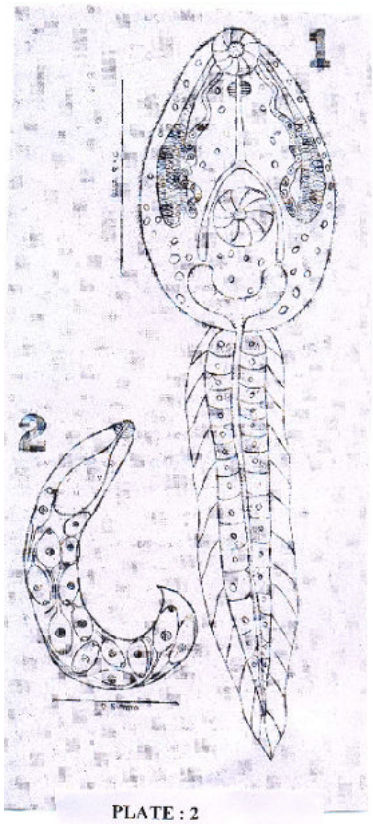

PLATE : 2
1)Cercariae dimorpha n.sp.
2)Redia

into intestinal caeca. The caeca terminate at a distance of 0.11 (0.10 to 0.12) from the posterior end of the main body. The salivary glands are dimorphic the outer pair is spindle shaped and consisting of numerous, oval shaped gland cell with prominent has nuclei. The inner pair adopts a wavey coarse and has rounded cells which are limited in number. This pair is refractile in nature. The ducts of salivary glands open at the base of the mouth. Both the glands never exceed the posterior margin of the ventral sucker. The excretory bladder is crescentric. The caudal canal runs to posterior end of the tail.

REDIA

The redia is large ranging from 1.97 to 2.01 in length and from 0.23 to 0.25 in width. It is elongated but curved posteriorly. It has a small pharynx, measuring 0.41 (0.37 to 0.45) in the length. The gut is saccular. The radia contains 12 to 14 cercariae at a time.

RESPONSES

(1) PhototaxisPositive
(2) Geotaxis Negative
(3) Emergence Day time only
(4) Survival of cercariae in DTW (at laboratory Temperature 28 C 51 hrs.

(5) Survival of cercariae in various percentage of sugar and salt solutions are as shown in the following table. (at laboratory temperature 28 C)

Sr. No	Sugar Solution %	Survival time Hrs.Min.%	Salt Solution	Sarvival time Hrs.Min.
1	0.5%	12.30	0.5%	42.00
2	0.7%	19.30	0.7%	42.30
3	0.9%	20.00	0.9%	10.00
4	1.0%	28.00	1.0%	00.30
5	1.2%	33.20	1.2%	00.15
6	1.4%	37.30	1.4%	00.09
7	1.6%	47.00	1.6%	00.00
8	1.8%	47.15	1.8%	00.00
9	2.0%	41.30	2.0%	00.00
10	2.2%	4.30	2.2%	00.00

Minimum survivaltime
In sugar solution 4.30 hrs. in 2.2%

Maximum survival time
In sugar solution 47.15 hrs. in 1.8%

Minimum survival time
In salt solution 00.09 hrs.in 1.4%

Maximum survival time
In salt solution 42.30 hrs.in 0.7%

DISCUSSION

The present cercaria belonging to Paraplelurolophocerca group showssome specific character different from Cercariae indicae XXXI Sewell,1922, *Cercariae indicae* L Sewell, 1922, *Cercaria melanoides* Porter, 1938 . *Cercariae bristiae* Porter, 1938 and *Cercaria dhanna n . sp*.described earlier in the present work.

The specific differentiating characters are:

1. Dimorphic salivary glands having two types of gland cells and the cells never extending in post-acetabular region.

2. Crescentric excretory bladder.
3. Absence of eye spots.
4. Host*, Lymnea luteola* and locality Gangapur project, Dharna River Girnare, Godavari River, Nashik District Maharashtra state India.

These characters are specific and cercaria is considered as new and named as *Cercaria dimorpha n.sp.*

1) Host : *Lymnea luteola*
2) Habitet : Digestive gland
3) Locality : Gangapur project, Dharna River Girnare, Godavari River, Nashik District Maharashtra State, India

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