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## PROCEEDINGS OF RESEARCH PAPERS & ABSTRACTS

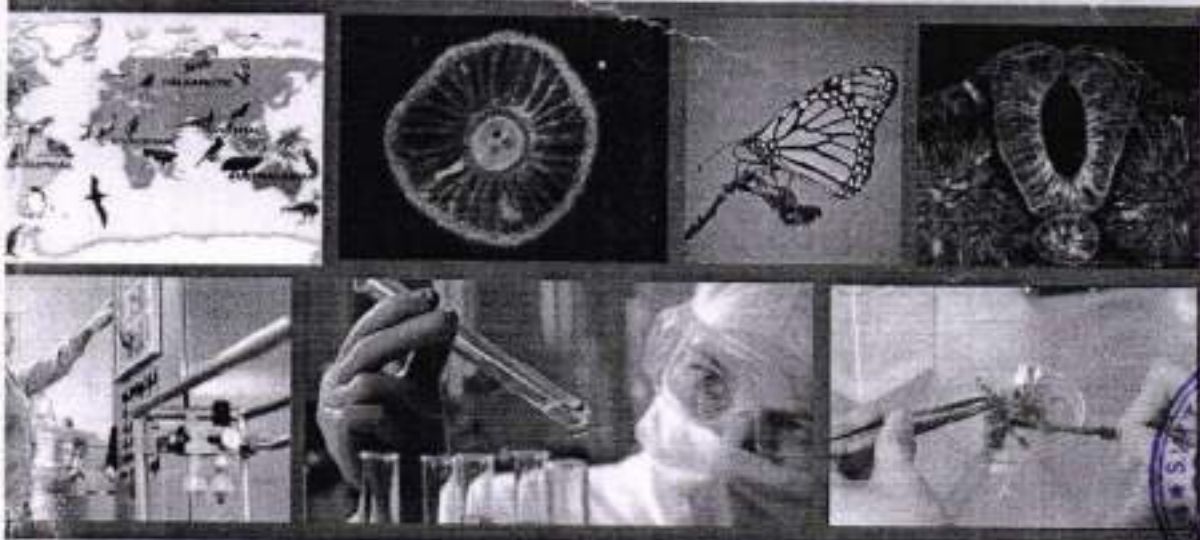


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**SOUVENIR**



(24)

**STUDIES ON LARVAL DIGENETIC TREMATODES INFECTED WITH  
FRESHWATER MOLLUSCAN SNAIL, *LYMNEA AURICULARIA* OF GANGAPUR  
PROJECT-GODAVARI RIVER: *FURCOCERCUS CERCARIAE* IV, NASHIK,  
MAHARASHTRA, INDIA**

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**ABSTRACT**

The earlier work on the snails and cercariae of this region was carried out by Karykarte 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 finding was published in the year 1981. They examined 8 species of snails viz, *Viviparus bengalensis*, *Melanoidys tuberculatus*, *Melanus scabra*, *Lymnea acumulata*, *Lymnea luteola*, *Lymnea auricularia*, *Indoplanorbis exustus*, *Anisus (Gyridus) convexiusculus*. Out of these 8 species, they reported cercarial infection in 6 species of snails and two species, *M. scabra* and *V. bengalensis* were free from larval infection. Their work included description of 11 species of fresh water 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 19 cercariae were collected and described. Out of these cercariae, two cercariae belonging to Monostome and one belonging to Echinostome are reported from the same host *Melania tuberculata* (1986). Present paper deals with freshwater cercariae which belongs to *Furcocercus cercariae* of Mirabilis group. The collection was carried out at Gangapur Project, Godavari river, Sangavi, Darna river, Girnare, ponds and ditches around Godavari river. The study is further extended to various responses of cercariae to various stimuli such as phototaxis, geotaxis, emergence and survival of cercariae in various percentage of artificial media. The collection constituted a new species of "Mirabilis" group. These cercariae were collected from the hepatopancreas and gonads of Molluscan snail *L. auricularia*.

**KEYWORDS:** Helminth parasites, Monostome, Amphistome, Distome

**INTRODUCTION**

Luhe (1990) made the first attempt to classify the cercariae in a comprehensive 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 survey 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 cercariae* and in 1936 he studied the North American cercariae. Sewell (1922) studied the freshwater cercariae from India and he modified the Luhe's classification and divided major groups into number of smaller groups. Porter (1938) studied the freshwater Larval trematodes 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 *Furcocercus cercariae* from Bombay. Chandler (1953) gave a key to the *Furcocercus 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 Xiphidocercarial 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, ), Karkayarte & Yadav 1981, Gautam, 1986 ). Present paper deals with freshwater cercariae which belongs to *Furcocercus cercariae* of "Mirabilis" group infected with molluscan snail *Lymnaea auricularia*. The classification followed in the paper is of Luhe (1909), Sewell (1922) and Porter (1938).

#### MATERIAL AND METHODS

##### (1) Collection and maintenance of snails:

Studies on cercariae commenced with collection of first intermediate host ( snails). They were collected either hand picked or dragging a net through water and were transported to the laboratory. The snails were then transferred to glass water bowls and well aerated aquaria already provided with a rich water plants such as Vallisneria, Hydrilla, Chara, Spirogyra 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.

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For the study of cercariae heavily infected snails were selected. Two methods were followed for the morphological observations.

1) Natural emerging method

2) Crushing method.

1) In natural emerging method the snails ( 2 to 3 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 cercariae emerge only in darkness.

2) 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 infection of cercariae.

The cercariae 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, 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.

For 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 in Delafield's haematoxylin, cleared in clove oil and mounted in D.P.X.

5) Measurements :

Most of the specimens were measured in live state. In the present 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 objective if necessary. This method gave the most uniform results. All the measurements are in millimeters.

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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 to move 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 sugar 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.

### FURCOCERCUS CERCARIAE OF "MIRABILIS" GROUP

#### *CERCARIA ELONGORAMI* n.sps.

In the present work *Furcocercus* cercariae is described. These cercariae were collected from the hepatopancreas and gonads of molluscan snail *L. auricularia* belongs to "Mirabilis" group of *Furcocercus* cercariae. The snails were collected from the ponds near Gangapur Project-Godavari river, Darna river, Girnare, ponds and ditches around Godavari river. The larval forms were abundant during the months from December to May. The collection were constituted a new species of "Mirabilis" group.

#### Collection data

#### Percentage of infection during the years 2013-2014

| Sr. No. | Month       | Locality   | No. of Snails examined | No. of infested | % of Infection |
|---------|-------------|--|------------------------|-----------------|----------------|
| 1       | July 2013   | Gangapur Project, Godavari river, Nashik District, Maharashtra | --                     | --              | --             |
| 2       | August 2013 |  | --                     | --              | --             |

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|    |            |  |      |     |       |
|----|------------|--|------|-----|-------|
| 3  | Sept. 2013 |  | --   | --  | --    |
| 4  | Oct. 2013  |  | --   | --  | --    |
| 5  | Nov. 2013  |  | 219  | --  | --    |
| 6  | Dec. 2013  |  | 100  | 06  | 06.67 |
| 7  | Jan. 2014  |  | 298  | 65  | 21.81 |
| 8  | Feb. 2014  |  | 285  | 68  | 23.85 |
| 9  | Mar. 2014  |  | 217  | 60  | 27.64 |
| 10 | Apr. 2014  |  | 250  | 75  | 30.00 |
| 11 | May 2014   |  | 263  | 81  | 30.79 |
| 12 | June 2014  |  | --   | --  | --    |
|    |            | Annual percentage of Infection 2013-2014 | 1632 | 355 | 11.67 |

Percentage of infection during the years 2014-2015

| Sr. No. | Month       | Locality  | No. of Snails examined | No. of Infested | % of Infection |
|---------|-------------|---|------------------------|-----------------|----------------|
| 1       | July 2014   | Gangapur Project, Godavari river, Nashik District, Maharashtra, India | --                     | --              | --             |
| 2       | August 2014 |   | --                     | --              | --             |
| 3       | Sept. 2014  |   | --                     | --              | --             |
| 4       | Oct. 2014   |   | --                     | --              | --             |
| 5       | Nov. 2014   |   | 219                    | --              | --             |
| 6       | Dec. 2014   |   | 115                    | 07              | 06.08          |

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|    |           |  |             |            |              |
|----|-----------|--|-------------|------------|--------------|
| 7  | Jan. 2015 |  | 313         | 72         | 23.00        |
| 8  | Feb. 2015 |  | 290         | 73         | 25.17        |
| 9  | Mar. 2015 |  | 220         | 64         | 29.00        |
| 10 | Apr. 2015 |  | 240         | 77         | 32.08        |
| 11 | May 2015  |  | 273         | 90         | 32.96        |
| 12 | June 2015 |  | --          | --         | --           |
|    |           | <b>Annual percentage<br/>of Infection 2014-<br/>2015</b> | <b>1451</b> | <b>383</b> | <b>12.36</b> |

**Average percentage of infection (Mean) =12.015**

The cercaria is yellowish in colour. The cuticle is beset with backwardly directed spines. The eye spots are absent. The cercaria shows vibrating movements. Cystogenous gland cells are not observed.

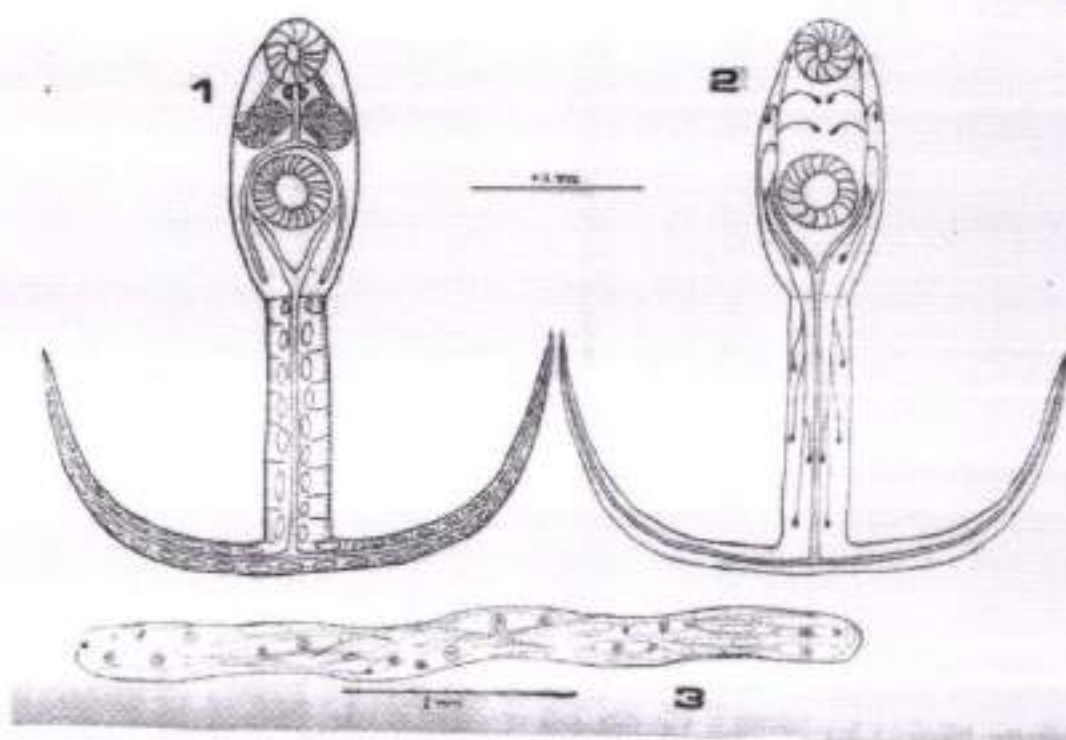
The main body is oval in shape and has 0.16 (0.16 to 0.18) by 0.07 (0.05 to 0.09) dimensions. The tail stem is more or less cylindrical and measures 0.15 (0.13 to 0.17) in length and 0.04 (0.03 to 0.05) in width. The tail rami are longer than the tail stem and measure 0.22 (0.21 to 0.23) in length. The rami are narrower than the stem (nearly 1/3). These structures are pointed at the tip. The oral sucker is subterminal in position and has a diameter of 0.04 (0.03 to 0.05). The ventral sucker is located in the posterior half of the body but the equatorial line passes through the anterior one fourth of it. It has a diameter of 0.05 (0.04 to 0.06). The mouth is ventral in position and leads into a prepharynx which in turn opens into a very short pharynx measuring 0.005 (0.004 to 0.006) in length and 0.014 (0.012 to 0.016) in width. The oesophagus is tubular and measures 0.03 (0.02 to 0.04) in length. The caeca are also tubular, arcuate in the region of ventral sucker and reach to posterior end where they terminate blindly at an average distance of 0.005. There are two pairs of penetration glands. These are rounded in shape with large necks opening in the region around the mouth. The penetration glands are confined in the pre-equatorial region of the body.

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**PLATE :**

- 1) *Cercaria elongorami* n.sp.
- 2) *Cercaria elongorami* n. sp. Showing excretory bladder, caudal excretory canal and flame cells
- 3) Sporocyst

The excretory bladder is Y-shaped. There are five pairs of flame cells in the main body and four pairs in the tail region.

The sporocyst is long and measures 3.76 (3.51 to 4.01) in length and 0.29 (0.21 to 0.37) in width. Most of the sporocysts contain 10 to 12 mature cercariae and numerous germ balls. The sporocysts shows extension and contraction. The mature cercariae come out by bursting sporocyst wall anteriorly or posteriorly.

**RESPONSES :**

|                |                                  |
|----------------|----------------------------------|
| (1) Phototaxis | Positive                         |
| (2) Geotaxis   | Negative                         |
| (3) Emergence  | at day time only in the presence |

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of light

(4) Survival of cercariae

in DTW (at laboratory  $30\frac{1}{2}$  hrs.

temperature  $28^{\circ}\text{C}$ )

(5) Survival of cercariae in various percentages of sugar and salt solution is as shown in the following table (at laboratory temperature  $28^{\circ}\text{C}$ )

| Sr. No. | Sugar Solution % | Survival time Mrs. Min. | Salt Solution Hrs. Min | Survival Hrs Min. |
|---------|------------------|-------------------------|------------------------|-------------------|
| 1       | 0.5%             | 21.10                   | 0.5%                   | 26.00             |
| 2       | 0.7%             | 22.00                   | 0.7%                   | 24.00             |
| 3       | 0.9%             | 22.50                   | 0.9%                   | 00.40             |
| 4       | 1.0%             | 27.10                   | 1.0%                   | 00.15             |
| 5       | 1.2%             | 28.00                   | 1.2%                   | 00.00             |
| 6       | 1.4%             | 29.10                   | 1.4%                   | 00.00             |
| 7       | 1.6%             | 05.50                   | 1.6%                   | 00.00             |
| 8       | 1.8%             | 08.10                   | 1.8%                   | 00.00             |
| 9       | 2.0%             | 04.30                   | 2.0%                   | 00.00             |
| 10      | 2.2%             | 00.30                   | 2.2%                   | 00.00             |

Minimum survival time  
in sugar solution

00.30 hrs. in 2.2%

Maximum survival time  
in sugar solution

29.10 hrs. in 1.4%

Minimum survival time  
in salt solution

00.15 hrs. in 1.0%

Maximum survival time  
in salt solution

26.00 hrs. in 0.5%

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### DISCUSSION

The cercaria under discussion shows affinities to the group - 3 of Furcocerous cercariae as classified by Cort, 1917. The group 3 is characterized by the presence of pharynx, by the fact that the lobes of the tail are long, almost as long as stem and are not constricted from it and by the absence of eye spots. In the group 3 the present form shows affinities to Mirabilis group which includes following cercariae :

- 1 *Cercaria mirabilis* Braun, 1891
- 2 *Cercaria anchoroides* Ward, 1917
- 3 *Cercaria wrightii* Ward, 1918
- 4 *Cercaria brookaveri* Faust, 1918
- 5 *Cercaria macrostoma* Faust, 1918
- 6 *Cercaria fusca* Pratt, 1919

The present form shows apparent resemblance to *C. fusca* Pratt, 1919. However, there are numerous specific characters in the present form and hence showing marked differences from the larval form reported by Pratt (1919).

The preset cercaria is much smaller than the known form (*C. fusca* 3.00, Present form 0.53)

The oral sucker is elongated and measures 0.52 by 0.35 as against rounded and having a diameter of 0.04 (0.03 to 0.05)

The tail is having wart at the proximal end in the known form while such structures are entirely absent in the present form. In addition to this character, the tail is bifurcate in the former and longifurcate in the latter.

The sporocyst is tenticular in shape and possesses one to four cercariae at a time in the known form. As compared this sporocyst is elongated and contains 10 to 12 cercariae at a time.

Other minor charcters and measurements are shown in the following table

| Character   | <i>Cercaria fusca</i> Pratt (1919) | Present form        |
|-------------|------------------------------------|---------------------|
| Body length | 1                                  | 0.16 (0.14 to 0.18) |
| Tail length | 2                                  | 0.15 (0.13 to 0.17) |
| Rami length | 0.5                                | 0.22 (0.21 to 0.23) |







|                       |                                     |                                  |
|-----------------------|-------------------------------------|----------------------------------|
| Tail width            | 0.27                                | 0.04 (0.03 to 0.05)              |
| Oral sucker structure | Elongated                           | Rounded                          |
| Dimension             | 0.52 by 0.35                        | 0.04 (0.03 to 0.05)              |
| Position              | Ventral                             | Subterminal                      |
| Pharynx               | Opens into very short<br>oesophagus | Opens into tubular<br>Oesophagus |
|                       | Diameter 0.13                       | Diameter 0.014 (0.012 to 0.016)  |

The known form is reported from *Comobaxis livescens* Oneida River, New Oneida, New York and the new form from Gangapur Project Darna river, Godavari river, Nashik District Maharashtra India.

The larval form named as *Cercaria elongoxami* n.sp.

Host : *Lymnea auricularia*

Habitat: Digestive gland

Locality : Gangapur Project, Darna river,

Godavari river, Nashik District

Maharashtra, India.

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