



STUDIES ON LARVAL TREMATODES OF GANGAPUR PROJECT (WATER RESERVOIR), GODAVARI RIVER : FURCOCERCUS CERCARIAE - I

Dr. Vikram R. Kakulte

HEAD, DEPARTMENT OF ZOOLOGY, K.T.H.M.

COLLEGE NASHIK 422402, MAHARASHTRA, INDIA

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, *Viviparous bengalensis*, *Melanoids tuberculatus*, *Melanus scabra*, *Lymnea acumulata*, *Lymnea luteola*, *Lymnea auricularia*, *Indoplanorbis exustus*, *Anisus (Gyrulus) convexiusculus*. Out of these 8 species, they reported cercarial infection in 6 species of snails and 2 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. Present paper deals with two species of freshwater cercariae which belong to Furcocercus cercariae of Baiswan Group. Out of these two species, one is reported from the host *Lymnea acuminata* and the other is from *Lymnea luteola*. The collection were carried out at Gangapur project (Water reservoir) Godavari river, Dharha river, Waldevi river, Girnare, ponds and ditches around Godavari river.

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,),Karkaykarte & Yadav 1981)

Present paper deals with two species of freshwater cercariae which belong to Furcocercus cercariae . 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.

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 (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 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.

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 "BAISWAN" GROUP

1) *CERCARIAE MICROCAECA n.sp.*

In the present work Furcocercus cercaria are represented by two species . The present cercariae belonging to Baiswan group was collected from the digestive gland of the snail *Lymnea acuminata*. When the infection was heavy the larval forms were observed in the gonads of the snail . The snails occur throughout this region during December to May. The present collection was from the (Freshwater Reservoir) the Gangapur Project, Godavari River, Dharna River, Waldevi river, and nearby places. The Baiswan group is represented here with two new species of cercaria, *Cercaria microcaeca n.sp.* and *Cercaria microacetabula n.sp.*

Collection data

Percentage of infection during the years 2014 and 2015

sr. NO.	Month	Locality	No.of snails examined	No,of snails infected	% of infection
1	January 2014	Gangapur Project Girnare Godavari River Waldevi River	192	15	7.81
2	February 2014	-do-	212	21	9.90
3	March 2014	-do-	240	24	10.00
4	April 2014	-do-	260	31	11.92
5	May 2014	-do-	290	35	12.06
6	June 2014	-do-	-	-	-
7	July 2014	-do-	-	-	-
8	August 2014	-do-	-	-	-
9	September 2014	-do-	-	-	-
10	October 2014	-do-	-	-	-
11	November 2014	-do-	62	-	-
12	December 2014	-do-	192	1	0.52
	Annual Percentage of infection 2014	Total	1448	127	4.35
13	January 2015	-do-	172	25	9.19
14	February 2015	-do-	253	23	9.09
15	March 2015	-do-	292	30	10.27
16	April 2015	-do-	319	33	10.34
17	May 2015	-do-	298	35	11.74
18	June 2015	-do-	-	-	-
19	July 2015	-do-	-	-	-
20	August 2015	-do-	-	-	-
21	September 2015	-do-	-	-	-
22	October 2015	-do-	-	-	-
23	November 2015	-do-	225	-	-
24	December 2015	-do-	200	2	1.00
	Annual Percentage of infection 2015	Total	1759	148	4.30

Average percentage of Infection (Mean) m 4.325

The cercaria is transparent. The entire cuticle has backwardly directed spines. The eye spots are absent.

The main body of the cercaria and the tail have more or less the same width when the main body is extended fully, otherwise the main body is slightly broader than the width of the tail. It measures 0.88 (0.86 to 0.90) in total length including tail and rami. The main body measures 0.30 to 0.32 in length with an average length of 0.31. The tail is slightly longer than the main body. It is 0.47 (0.42 to 0.52) long. The rami are continuous with the tail stem. The rami measure 0.12 (0.11 to 0.13) in length. The anterior organ which may represent the oral sucker measures 0.08 (0.07 to 0.09) by 0.05 (0.04 to 0.06) in dimensions. The ventral sucker, much smaller than the anterior organ, is located in the posterior part of the body. It is rounded and has a diameter of 0.03 (0.02 to 0.04).

The mouth directly leads into a tubular prepharynx, which opens into a long oesophagus, pharynx being absent. The intestinal caeca are extremely short and terminate in the pre-equatorial region. The penetration glands are long with densely stained nuclei and narrow necks. Their extension is variable. One pair is pre acetabular, other paracetabular and the third having their tips extended posterior to the acetabulum. Three pairs of opening are observed around the mouth.

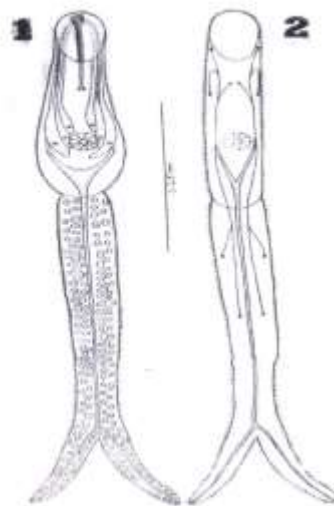


PLATE : 1

1) *CERCARIA MICROCAECA* n. sp.

2) *CERCARIA MICROCAECA* n. sp. Showing the excretory bladder, caudal excretory canal and flame cells.

Minimum survival time in sugar solution	02.40 hrs. in 2.2%
Maximum survival time in sugar solution	30.00 hrs. in 1.8%
Minimum survival time in salt solution	00.20 hrs. in 1.2%
Maximum survival time in salt solution	31.40 hrs. in 0.7%

DISCUSSION

The present larval form belongs to Furcocercous cercariae group as revealed by its bifid tail. Cort (1917) distinguished three subgroups of Furcocercous cercariae.

- Group 1 - Characterized by the absence of a pharynx, by the furcal rami being short, less than half the length of the main stem and definitely constricted off from it and by the presence of eye spots.
- Group 2 - Characterized by the absence of pharynx, by the furcal rami being short as in Group-1, but separated from that group by the absence of eye spots.
- Group 3 - Characterized by the presence of a pharynx, by the fact that the lobes of the tail are long, almost as long as the stem and are not constricted off from it and by the absence of eye spots

- Sewell, 1922

The present furcocercous cercaria belongs to group 2 as classified by Cort (1917). The group is characterized by absence of pharynx, furcal rami shorter than the tail stem and absence of eye spots. As the pharynx spincter is entirely wanting it belongs to series 2. The characters being rounded snout, anterior organ of penetrating nature than true sucker and well developed acetabulum. In this series it shows affinities to the Baiswan, the only group. In Baiswan group the following species are reported :

- (1) Cercariae indicae XXII Sewell, 1922;

(2) Cercaria shebae Porter, 1938

(3) Cercaria isendlwesii Porter, 1938;

(4) Cercariae indlcae XXII Sewell, 1922;

(redescribed by Karyakarte and Yadav, 1981)

The present form shows some resemblances with Cercariae indicae XXII Sewell, 1922 as redescribed by Karyakarte and Yadav 1981. However, the present form possesses certain specific characters and hence distinguished from Cercariae indicae XXII. The distinguished characters are tabulated here.

As the characters are quite specific the present form is considered as new to the science and named as *Cercaria microcaeca* n.sp.

Host : Lymnea acuminata

Habitat : Digestive gland and gonads

Locality : Waldevi River , Dharna River, Gangapur

(Water Reservoir) Project, Godavari River ponds and ditches ground Godavari River District Nashik , Maharashtra State, India.

II) Cerearia microacetabula n.sp.

The present cercaria belonging to Baiswan group was collected from the digestive glands of the snail Lymnea luteola. The digestive gland of the infected L. luteola was brownish and pulpy. burst easily and were mostly destroyed by the larval forms. The shell of the snails showed thinning and ballooning. The snail occur in Nashik region during the period between December and May. The snails were collected at Gangapur Project, Godavari River, Dharna River, Waldevi river, and nearby places around Nashik region.

Collection data

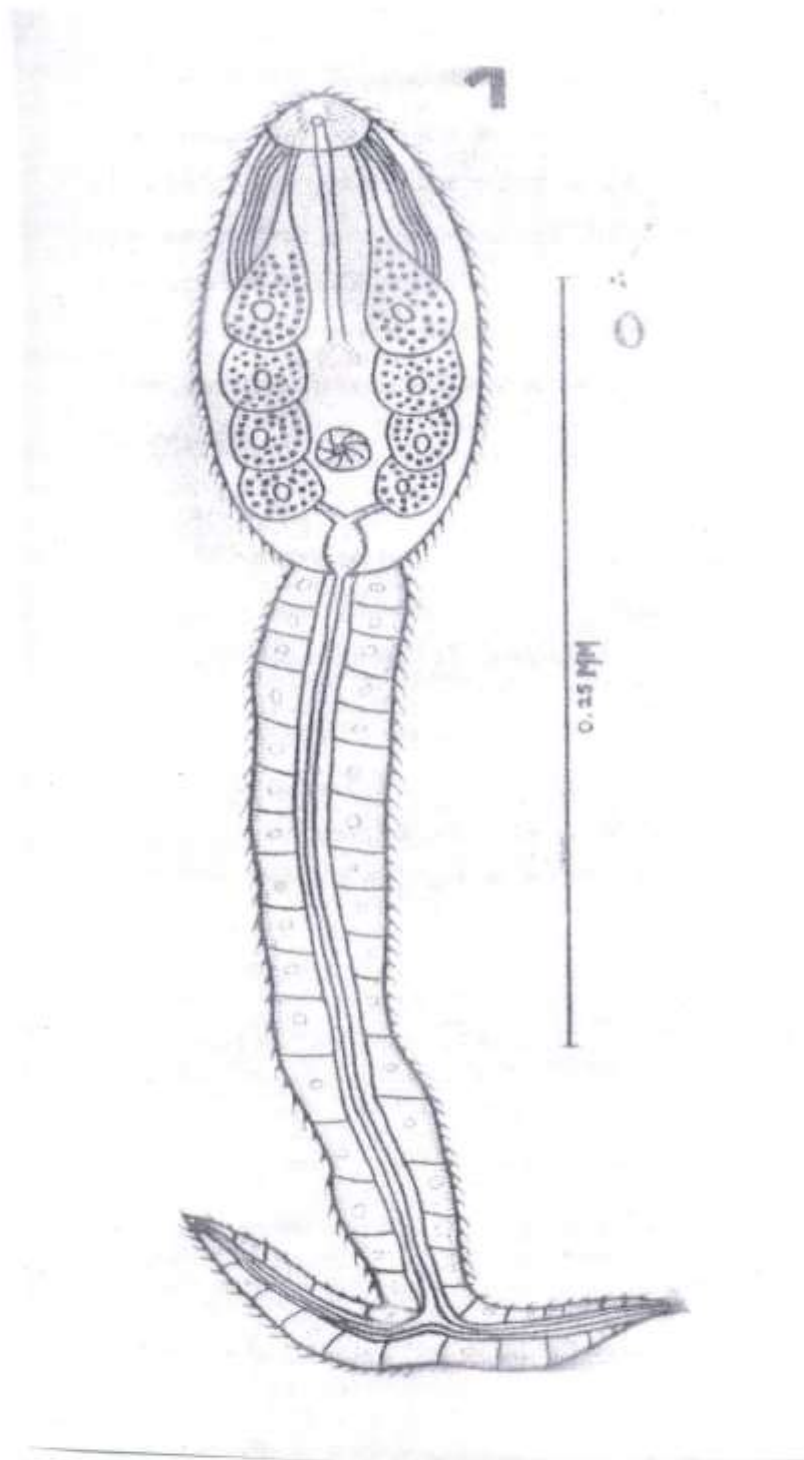
Percentage of infection during the years 2014 and 2015

sr. NO.	Month	Locality	No.of snails examined	No,of snails infected	% of infection
1	January 2014	Gangapur Project Girnare Godavari River Waldevi River	513	41	7.99
2	February 2014	-do-	215	19	8.83
3	March 2014	-do-	232	25	10.77
4	April 2014	-do-	292	33	11.30
5	May 2014	-do-	345	44	12.75
6	June 2014	-do-	-	-	-
7	July 2014	-do-	-	-	-
8	August 2014	-do-	-	-	-
9	September 2014	-do-	-	-	-
10	October 2014	-do-	-	-	-
11	November 2014	-do-	-	-	-
12	December 2014	-do-	212	4	1.88
	Annual Percentage of infection 2014	Total	1809	166	4.46
13	January 2015	-do-	425	25	5.88
14	February 2015	-do-	293	29	9.89
15	March 2015	-do-	410	45	10.97
16	April 2015	-do-	340	44	12.94
17	May 2015	-do-	312	43	13.78
18	June 2015	-do-	-	-	-
19	July 2015	-do-	-	-	-
20	August 2015	-do-	-	-	-
21	September 2015	-do-	-	-	-
22	October 2015	-do-	-	-	-
23	November 2015	-do-	-	-	-
24	December 2015	-do-	225	3	1.17
	Annual Percentage of infection 2015	Total	2035	189	4.55

Average percentage of Infection (Mean) = 4.505

The cercaria is brownish in colour. The main body is oval with a cylindrical long tail, longer than the body proper. There are two short rami pointed at the tips. The eye spots are absent. The cercaria shows active swimming movements. Due to active swimming the cercaria vary considerably in size and shape according to the degree of contraction at the time of measurements.

The living cercaria in extended state measures 0.49 (0.43 to 0.55) in length including tail and rami. The main body measures 0.15 (0.11 to 0.19) in length and 0.08 (0.06 to 0.10) in maximum to breadth measured at the preacetabular region- The length of the tail stem is 0.25 (0.23 to 0.27) and the rami measure 0.08 (0.06 to 0.10), The anterior organ has sucker like appearance and has the dimensions, 0.02 by 0.03 (0.01 to 0.03 by 0.02 to 0.04). The acetabulum is spherical in shape, smaller than the anterior organ, situated in the posterior third of the body and has a diameter of 0.02 (0.01 to 0.03). The mouth is surrounded by oral organ. There is no pharynx. The tubular oesophagus runs posteriorly to the middle of the main body and bifurcates into two caeca which are rudimentary. There are four pairs of penetration glands on each side of the body. Their cytoplasm is finely granular and nuclei are rounded and distinct. Anterior two pairs are preacetabular, third pair is parecetabular and reach to the posterior margin of the acetabulum, the fourth pair of penetration gland is partly acetabular and terminates at a distance 0.02 (0.01 to 0.03) from the posterior tip of the main body. The penetration glands are arranged one behind the other vertically. These are flask shaped with their narrow necks opening around the mouth.

**PLATE : 2****1) CERARIA MICROACETABULA n. sp.**

The excretory system is represented by an oval bladder and two excretory tubules. The tubules run up to the middle of the body and give out anterior and posterior branches. Caudal excretory canal runs the entire length of the tail stem, enters into the rami and opens at the tips by two nephridiophores.

RESPONSES :

- (1) Phototaxis Positive
- (2) Geotaxis Negative
- (3) Emergence During the whole day
- (4) Survival of cercariae in DTW 29 ½ hrs.
(at laboratory temperature 28 C)
- (5) Survival of cercariae in various percentages

of sugar and salt solutions is as shown in the following table (at laboratory temperature 28°C)

Sr. No.	Sugar solution %	Survival time Hrs. Min.	Salt solution %	Survival time Hrs. Min.
1	0.5%	21.30	0.5%	27.10
2	0.7%	22.10	0.7%	30.10
3	0.9%	23.50	0.9%	29.30
4	1.0%	24.10	1.0%	00.40
5	1.2%	28.00	1.2%	00.10
6	1.4%	28.50	1.4%	00.00
7	1.6%	29.00	1.6%	00.00
8	1.8%	30.00	1.8%	00.00
9	2.0%	06.50	2.0%	00.00
10	2.2%	4.30	2.2%	00.00

Minimum survival time in sugar solution 04.30 hrs. in 2.2%

Maximum survival time in sugar solution 30.00 hrs. in 1.8%

Minimum survival time in salt solution 00.10 hrs. in 1.2%

Maximum survival time in

salt solution

30.10 hrs. in 0.7%

DISCUSSION

The **Furcocercus** cercaria under discussion shows apparent resemblance to Cercaria microcaeca n.sp. reported earlier. However it is distinguished on the strength of the following character.

The anterior organ is squarish in the earlier form and well defined and rounded in the latter.

The intestinal caeca terminate far anterior in *C. microcaeca* and very near to acetabulum in the new form.

The penetration glands differ in number and structure in the two forms. There are four pairs in the present form and three pairs in already described cercaria, *C. microcaeca*. The penetration glands are vertically arranged one behind the other in the former and grouped together in the latter. Structurally also they differ in two.

The excretory bladder also differ in the two. In the new form it has a bulged stem and narrow cornua whereas, in the known form the stem is narrow and the cornua emerge immediately.

Considering the nature of penetration glands, digestive system and the excretory system it is difficult to group the new form with *C. microcaeca*. It is considered new to science and named as Cercaria microacetabula n.sp.

Host : *Lymnea luteola*

Habitat : Hepatopancreas

Locality : Waldevi River , Dharna River, Gangapur

(Water Reservoir) Project, Godavari River pands and ditches groud Godavari River District Nashik , Maharashtra State, **India**.

Bibliography :-

- 1) **A Farahnak, R. Vafale-Darian, I Mobedi(2006)**: A Faunistic Survey of Cercariae from Fresh Water Snails, *Meleanopsis* spp. And their Role in Disease Transmission. *Iranian J. Publ. Health, Vol.35, No.4,2006*, pp 70-74.
- 2) **Cort W.W.(1914)**: Larval trematodes from North American Fresh water snails. *J.Parasitology. I.,65-84.(W.L.26643)*.
- 3) **Eric S. Loker(2005)** : Research on the Molluscan Intermediate Hosts for Schistosomiasis; Presented to the Scientific Working Group on Schistosomiasis *World Health Organization Geneva, Switzerland. 14-16 November 2005*.
- 4) **Faust ,E.C.(1919b,1921,1924,1926)**: Two new *Cystocercus* cercariae from North America, *J. of Parasitology. 4(4): 148-153, W.L.26643*.
- 5) **Ghulam Murtaza Arshad, Ashar Maqbool, Muhammad Fiaz Qamar, Syed Muhammad Hussan Bukhari, Haji Ahmad Hashmi and Muhmmad Ashraf(2011)**: Prevalence and Ecology of Fresh Water Snails in Some Selected Districts of Southern Punjab, Pakistan. *Pak.j. life soc, Sci.(2011):17-20*.
- 6) **Gupta, N.K. and Taneja, S.K.(1970)**: Two monostome cercariae from the snail *Melanoides tuberculatus* of Chandigarh. *Research Bulletein of Punjab University(Sciecne). 20(1/2):33-38*.
- 7) **Ito, J. , Yasuraoka, Santos, A. T.Jr.and Blas, B.L.(1977)**: Studies on the freshwater cercariae in Leyte Island, Philippines. 2. Cercariae from *Oncomelania quadrasi*. *Jap. J. Exp. Medicine. 47(3): 151-162. W.L 25230*.
- 8) **Karyakarte, P.P. and Yadav, B.B.(1981)**: Studies of freshwater larval trematodes of Marathwada. *Revista di Parasitologica. 42(1):79-107. W.L.(47948)*.
- 9) **Khan, A.K.(1978)**: Studies on trematodes parasites of fishes of Marathwada. Ph.D. thesis, Marathwada University, Aurangabad.
- 10) **Labour, M.U.(1911)**: A review of British marine cercariae. *Parasitology, 416-456, (W.L. 37153)*.
- 11) **Lohe, M.(1990)**: Parasitische Plattwürmer. I. Trematoda. *Dis Sqsswasser Fauna Deutschlands. Heft. 17:217 p*.
- 12) **Malaki, A., and Singh, K.s.(1963)**: Parasitological Survey of Kauman region. Part XVI. Three cercariae from Kauman. *India. J. of Helminthology 14:133-153.(W.L.26086)*.
- 13) **Miller, H.M., and Northup, F.(1926)**: The seasonal infection of *Nassa obsolete*(Say)with larval trematodes. *Biol. Bull. Woods Hole, 5:p490*.
- 14) **Mohandas, A. (1977)**: Studies on two new species of cercariae and the histopathology of the digestive gland of their host. *Diogoniostoma pulchella*(Benson). *Acta Parasit. 25(1/10):17-24*.
- 15) **Nasir, P.(1971)**: Freshwater larval trematodes XXVIII. Three new species of cercariae. *Proc.helminth. Soc.Wash. 38(2):206-210. (W.L.39234)*.
- 16) **Nkwengulia, G.and ESP Kigadye(2012)**: Occurance of digenean larvae in freshwater snails in the Ruvu basin, Tanzania.
- 17) **Oleg Ditrich, Tomas Schoiz, Leopoldina Aguiree-Macedo and Josquin Vargas-Vazquez(1997)**: Larval stages of trematodes from freshwater molluscs of the Yucatan Peninsula, Mexico. *Folia Parasitologica 44: 109-127,1997*.
- 18) **Porter,C.A.(1938)**: The larval trematodes found in certain South African mollusks. *Publ. S. Afr. Inst. Med. Res. 8:1-492*.
- 19) **Sami Bdir, Ghaleb Adwan(2011)**: Larval stages of digenetic trematodes in *Melanopsis praemorsa* snails from freshwater bodies in Palestine. *Asian Pacific Journal of Tropical Biomedicine(2011),200-204*.
- 20) **Sey,O, Nahhas,F.M.Uch,S and Vano,C.(2003)**: Dignetic trematodes from marine fishes off the coast of Kuwait, Arabian gulf: *Fellodistomidae* and some smaller families, New host and Geographic Records. *Acta Zoologica Academiae Scientiarum Hungaricae 49(3), pp 179-200, 2003*.
- 21) **Shrivastava, H.D.(1933)**: On new trematodes of frog and fishes of United Provinces, India *Bull. Acad. Sci. Allah. B.11:41-60*.
- 22) **Soparkar, M.B.(1917)**: A trematode parasite of Anopheline Mosquito. *Ind. J. of Med. Res. 512-515. (W.L.23005)*.
- 23) **Sewell, R.B.S.(1922)**: Cercariae Indicae. *Indian Journ. Med. Res. 10:Special suppl. 1-370. (W.L.23005)*.
- 24) **Todd C, Huspeni and Devin D. Lafferty(2004)**: Using larval trematodes that parasitize snails to evaluate a saltmarsh restoration project. *Ecological Applications14(3), 2004pp795-804*.
- 25) **Thapana Chontanarth, Chalobal Wongsawad(2012)**: Epidemiology of cercarial stage of trematodes in freshwater snails from Chiang Mai Province, Thailand. *Asian Pacific Journal of Tropical Biomedicine(2012), 1-6*.

- 26) **Uthpala A. Jayawardena , Rupika S. Rajakaruna and Priyanie H. Amerasinghe(2010):** Cercariae of trematodes in freshwater snails in three climatic zones in Sri lanka. Cey. J. Sci. (Bio.Sci.) 39(2), 95-108, 2010.
- 27) **Villot, A.(1875):** Recherches sur les Helminthes Librus on parasites des cotes de la Bretagne. Archiv. Zool. Exp.gen. 4:451-462. (W.L.4379).

