E-ISSN: 2347-5129
P-ISSN: 2394-0506
(ICV-Poland) Impact Value: 5.62
(GIF) Impact Factor: 0.549
IJFAS 2018; 6(2): 135-139

www.fisheriesjournal.com
Received: 20-01-2018
Accepted: 21-02-2018

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First record of *Encyclometra colubrimurorum* (Rudolphi, 1819) Dollfus, 1929 (Digenea: Plagiorchiidae) metacercariae from a tadpole and an adult frog of the Western Ghats, India

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Abstract
In an attempt to map the digenetic trematode fauna of the frogs of Western Ghats, India as a part of a major research project, we came across the metacercaria of a trematode parasite, *Encyclometra colubrimurorum* (Rudolphi, 1819) Dollfus, 1929 infecting the liver, obliquus externus muscle and head of an adult frog, *Fejervaria sp*. (Anura, Dicroglossidae) and liver of a tadpole of the bicoloured frog, *Clinotarsus curtipes* (Jerdon, 1853) (Anura, Ranidae), collected during January 2016 to October 2017. Metacercaria has oval to round cyst with thin, transparent wall. Excysted metacercaria has fusiform, aspinose body with oval, subterminal oral sucker and equatorial acetabulam. Caeca was long, asymmetrical, unequal and extend almost up to posterior end of body. This paper is the first report of *E. colubrimurorum* metacercaria from the frog hosts, *C. curtipes* and *Fejervaria sp*. The prevalence of infection in *C. curtipes* was 25% and the intensity of infection was six. In *Fejervaria sp*. the prevalence of infection was 2.2% and the intensity of infection was 1.5. Although the metacercaria of *E. colubrimorarum* was reported earlier their morphological descriptions were not properly recorded. In this paper the morphology of the trematode metacercaria is described.

Keywords: *E. colubrimurorum*, digenean, frog, prevalence, *C. curtipes*, *Fejervaria sp*. Western Ghats, Wayanad

1. Introduction
The bicoloured frog (*Clinotarsus curtipes*) is a medium-sized frog seen in the tropical forests of the Western Ghats including the evergreen, semi-evergreen, moist and dry deciduous forests. They move about the forest floor and are well camouflaged amongst the leaf litter [1]. Their cryptic colouration and ungainly movement are typical to this frog. They can be seen from elevations of 1600 to nearly 6000 ft. above sea level. The frog breeds in natural and artificial ponds and large lakes [1]. This is an adaptable species which can tolerate not only changing forest types but also habitat degradation [1]. Although it is locally abundant, population is thought to be declining mainly due to habitat loss. Forest habitats of this species are being converted into tea, coffee, and eucalyptus plantations, and/or clear fell for timber and wood. Its tadpoles are large, black, wriggly creatures with tails. They are found at the base of freshwater streams and ponds, all year round. These tadpoles are a common in the freshwater bodies of the Western Ghats [2]. The bicolored frog tadpoles are the largest known tadpoles in India; they can grow up to 7-10 centimeters whereas an adult frog is only about 7 centimeters. The tadpoles usually live in small tanks or slow moving streams and swim from their birthplace to other micro habitats and keep wandering till they metamorphose into frogs. *Fejervaria sp.* belongs to the family Dicroglossidae and distributed in the southern Asia (Sri Lanka, India, Pakistan, Bangladesh, Nepal and Bhutan). It is also known as the South Asian cricket frog [2]. Two species of *Fejervaria* were documented from the Western Ghats, Wayanad region, *F. brevipalmata* (Peters, 1871) and *F. keralensis* (Dubois, 1980) [3]. While studying the digenetic trematodes infecting frogs of the Wayanad region of the Western Ghats, metacercaria of the digenetic trematode, *E. colubrimurorum* infecting the tadpole of *C. curtipes* and the adult of *Fejervaria sp*. was encountered. As of now, only three species of the genus *Encyclometra* have been reported - *E. colubrimurorum* (Rudolphi, 1819) Dollfus, 1929; *E. asymmetrica* Wallace, 1936 and *E. bungara* Srivastava and Ghosh, 1968. Nature of the

intestinal caeca is shown to be a constant and reliable character to differentiate the various species in the genus. Adults of all the three species have been reported from Indian snakes [4-10]. The life-cycle of the genus has not been elucidated so far. Metacercariae of *E. colubrimurorum* and *E. asymmetrica* have been reported by Yamaguti [11] and Chiang [12] respectively. Even though the metacercariae were reported earlier, the morphological characters were not described. So far, there is no report of *E. colubrimurorum* metacercaria from India. The present paper describes the morphology of *E. colubrimurorum* metacercaria and the paper forms the first report of this metacercaria infecting the tadpole of *C. curtipes* and the adult of *Fejervaria sp*.

2. Materials and Methods

2.1 Study area

The study was carried out in the Wayanad region of the Western Ghats (latitudes 11^0 27'and 15^0 58' North and 75^0 47' and 70^0 27' East longitude). The Western Ghats is considered as one of the hottest biodiversity hotspots in the world because of its very rich fauna & flora and the highest level of endemism. The map of the study area (Fig. 1) was prepared using QGIS 2.16.1 software.

Prevalence and intensity of Infection were measured following Bush *et al.* [13]

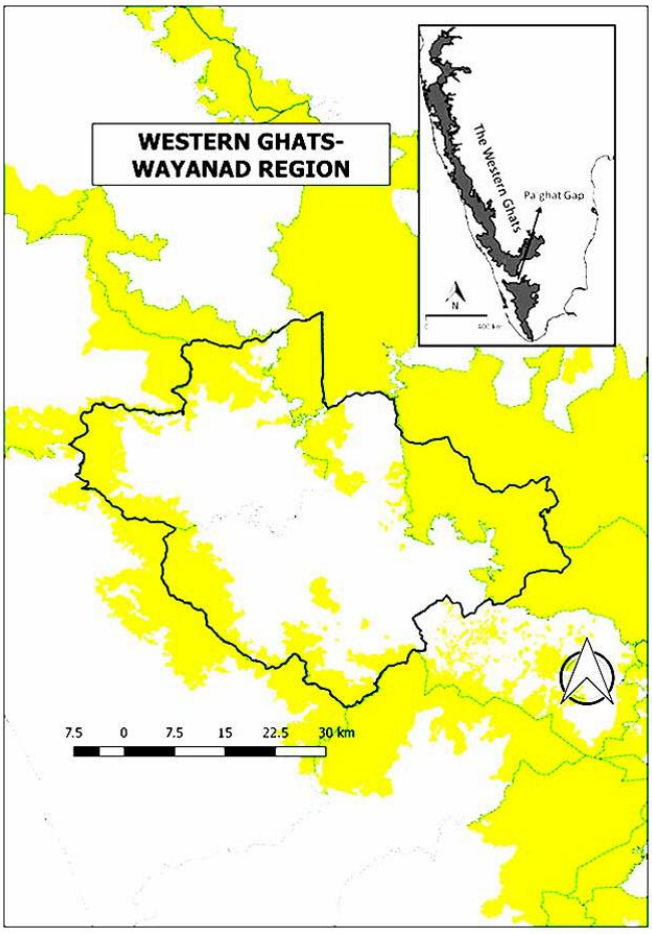

Fig 1: Study area – Western Ghats – Wayanad region

Four tadpoles of *C. curtipes* and 92 adults of *Fejervaria sp.* were collected during January 2016 to October 2017 from the water bodies using sweep hand net. Live specimens were brought to the laboratory, maintained in clean glass jars/aquariums and fed occasionally with insects. The frogs were narcotized with chloroform, dissected and examined under Labomed Luxeo4Z stereo zoom microscope for digenetic trematodes. The skin was removed and muscle tissues were macerated to detect the presence of metacercariae, if any. Internal organs like heart, liver, gallbladder, lungs, pancreas, intestine, kidney and urinary bladder were also dissected out from each frog, placed in separate Petri dishes containing 0.75% saline and the parasites isolated were examined under Nikon ECLIPSE Ni-U phase contrast research microscope without supravital staining or after staining with neutral red [14]. Permanent whole mounts of metacercariae were prepared after fixing them in 5% formalin under slight cover glass pressure and staining with acetocarmine, following the procedure outlined by Cantwell [15]. Measurements were taken with the support of the Nikon NIS Elements Imaging software. All measurements are in micrometers (µm), as range followed by mean in parentheses. Figures were drawn with the Nikon Y-IDT drawing tube attached to the Nikon ECLIPSE Ni-U microscope and details were added free hand from observations made on live specimens. Photographs were taken with a Nikon Y-TV55 camera.

3. Results

3.1 *Encyclometra colubrimurorum* (Rudolphi, 1819) Dollfus, 1929 (Fig. 2)

Description is based on the holotype and eight paratypes.

3.2 Encysted metacercaria

Cysts oval to round with thin, transparent wall; 1425.2 x 1083.2 in size. The larva remains curled up inside the cyst wall (Table 1).

3.3 Excysted metacercaria (Fig. 2, Table 1)

Body fusiform, aspinose, 1643.4 – 2855.2 x 752.5 – 868.4 (2240.4 x 783.3) in size. Oral sucker oval, sub-terminal, 215.3 – 343.3 x 256.1 – 409.3 (296.2 x 335.7) in size. Acetabulum equatorial, oval to round, larger than oral sucker, and measuring 276.9 – 517.2 x 248.2 – 532.7 (385.9 x 370.4). Distance between suckers 376.5 – 698.2 (532.9). Mouth sub-terminal; pharynx oval to round, muscular, measuring 95.6 – 165.3 x 116.7 – 217.7 (130.1 x 178.8). Oesophagus short, 25.7 – 68.2 x 24.5 – 27.5 (45.5 x 25). Pharynx and oesophagus surrounded by gland cells. Caeca long, asymmetrical, unequal, extend almost up to posterior end of body. Left caecum 1337.9 – 2120.7 x 60.2 – 122.9 (1723.9 x 86.9) long; right caecum 1116.6 – 1868 x 60.1 – 145.7 (1481.6 x 90.8) long. Testes fairly developed, round to oval, tandem, in the posterior half of body. Anterior testis, measured 53.1 – 80 x 52.4 – 75.7 (66.6 x 64.05); posterior testis 61.9 – 99.8 x 79.6 – 90.3 (80.9 x 85) in size. Distance between testes 48.6 – 120.0 (105.8). Cirrus sac poorly developed, immediately above anterior border of acetabulum and measured 158.2 – 215.2 x 50.2 – 59.8 (176.7 x 54). Ovary small, oval, median, just behind acetabulum and measured 70.1 x 69.4 in size. Excretory bladder large, saccular, filled with concretions; extending from posterior end to just below acetabulum; measured 629.6 – 1211.0 x 261.7 – 691.2 (813.8 x 540.7). Excretory pore terminal.

Table 1: Measurements (µm) of metacercariae of *Encyclometra colubrimurorum* (Rudolphi, 1819) Dollfus, 1929

Character	Range	Mean
Encysted metacercaria	-	1425.2 x 1083.2
Excysted metacercaria	1643.4 – 2855.2 x 752.5 – 868.4	2240.4 x 783.3
Oral sucker	215.3 – 343.3 x 256.1 – 409.3	296.2 x 335.7
Ventral sucker	276.9 – 517.2 x 248.2 – 532.7	385.9 x 370.4
Distance between suckers	376.5 – 698.2	532.9
Pharynx	95.6 – 165.3 x 116.7 – 217.7	130.1 x 178.8
Oesophagus	25.7 – 68.2 x 24.5 – 27.5	45.5 x 25
Left caecum	1337.9 – 2120.7 x 60.2 – 122.9	1723.9 x 86.9
Right caecum	1116.6 – 1868 x 60.1 – 145.7	1481.6 x 90.8
Anterior testis	53.1 – 80 x 52.4 – 75.7	66.6 x 64.05
Posterior testis	61.9 – 99.8 x 79.6 – 90.3	80.9 x 85
Distance between testes	48.6 – 120.0	105.8
Developing cirrus sac	158.2 – 215.2 x 50.2 – 59.8	176.7 x 54
Ovary	-	70.1 x 69.4
Excretory bladder	629.6 – 1211.0 x 261.7 – 691.2	813.8 x 540.7

3.4 Taxonomic summary

Table 2: Taxonomic summary of the parasite obtained from two hosts

Holotype	***Encyclometra colubrimurorum* Accession No. Z-P/H-F 121. Deposited in the Helminth parasite collections, Ecological Parasitology and Tropical Biodiversity Laboratory, Department of Zoology, Kannur University.**	
Hosts	*C. curtipes*	*Fejervaria sp.*
Accession Nos.	Accession No. Z-F/C-03. Deposited in the Herpetology collections, Department of Zoology, Kannur University.	Accession No. Z-F/ F-33. Deposited in the Herpetology collections, Department of Zoology, Kannur University.
Type localities	Bavali in Wayanad District	Atturkunnu Pulpally in Wayanad District
Site of infection	Liver, obliquus externus and head	Liver
Period of collection	January 2016 to October 2017	January 2016 to October 2017
Prevalence	One of Four frogs (25%) screened were infected	Two of 92 frogs (2.2%) screened were infected
Intensity	Six *E. colubrimurorum* recovered from one infected frog (6)	Three *E. colubrimurorum* recovered from two infected frogs (1.5)

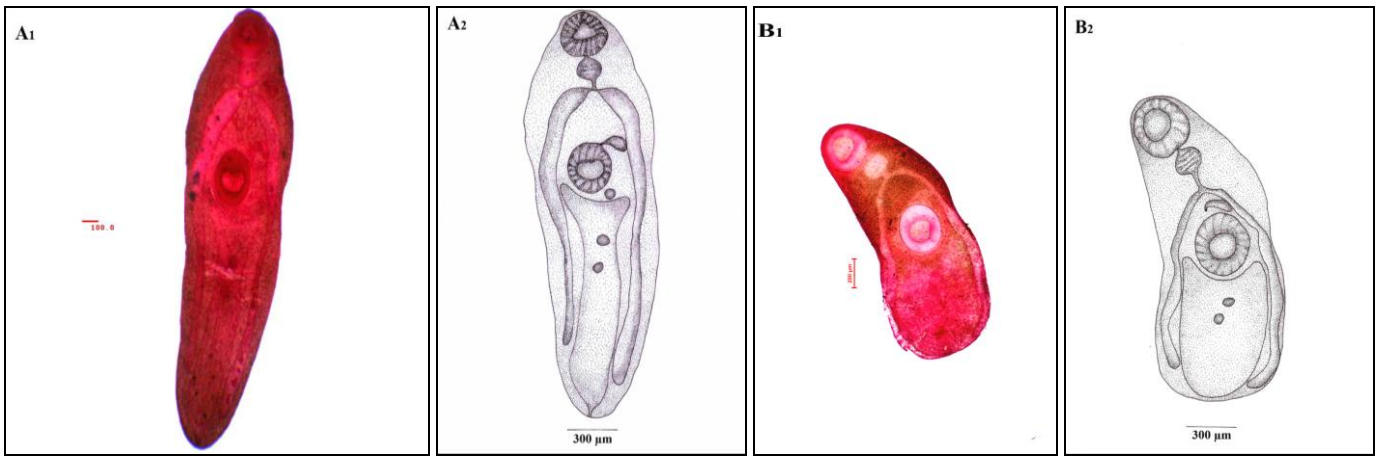

Fig 2: *E. colubrimurorum* metacercariae, a whole mount ventral view (A1 and A2); another specimen (B1 and B2) showing variation in size and shape of the body, position of the gonads and the caecal termination.

A total of nine individual trematode metacercariae (Table 3) were documented during the study, of which six were from *C. curtipes* and three from *Fejervaria sp.*

Table 3: Prevalence and intensity of infection of *E. colubrimurorum* in *C. curtipes* and *Fejervaria sp.*

Trematod (Digenea) metacercaria	Host	Number (N)	Prevalence (%)	Intensity
E. colubrimurorum	*C. curtipes*	6	1/4 (25)	6
	Fejervaria sp.	3	2/92 (2.2)	1.5

4. Discussion

The present metacercaria was recovered from liver, obliquus externus muscle and head of *Fejervaria sp.* and liver of *C. curtipes*. It has asymmetrical caeca, sac-like excretory bladder tandemly placed testes and crescentic cirrus sac situated above the anterior border of acetabulum. Based on these characters, the present form is included under the genus *Encyclometra* Baylis and Cannon, 1924 of the family Plagiorchiidae (Luhe, 1901) Ward, 1917. As far as is known, only three species of the genus *Encyclometra* have been reported: *E. colubrimurorum* (Rudolphi, 1819) Dollfus, 1929;

E. asymmetrica Wallace, 1936 and *E. bungara* Srivastava and Ghosh, 1968. Adults of all the three species have been reported from India. Mehra [4] from *Natrix piscator* and *N. mucosus* at Allahabad (U.P.); Bhalerao [5, 6] reported it from *Ptyas* (=*Zamensis*) *mucosus* at Culcutta; Gupta [7] and Agarwal [8] from *N. piscator* Ludhiana (Punjab) and Lucknow (U.P.), respectively; Srivastava and Ghosh [9] from the snakes, namely *P. mucosus, N. piscator, N. stolata* and *Atretium schistosum* from Culcutta (West Bengal) and Patna (Bihar); Dwivedi and Chauhan [10]. The adults of *E. colubrimurorum* were recorded from Turkey from the following hosts: European green tree frog, *Hyla arborea* (Dusen and Oz [16]); laughing frog, *Rana ridibunda*; Balkan racer, *Hierophis gemonensis*; green whip snake, *Hierophis viridiflavus*; Oriental rat snake, *P. mucosus*; *N. natrix*; Asiatic water snake, *Xenochrophis piscator* (Capuse [17]); *N. natrix* (Biserkov [18]; Shimalov and Shimalov [19]); *N. tessellata*, (Biserkov [18]); nose- horned viper, *Vipera ammodytes*, (Biserkov [18]). *E. bungara* has been reported only from the banded krait (*Bungarus faciatus*) in India (Srivastava and Gosh [9]). The finding of this species in *Enhydris plumbea* in Laos represents a new host records and the first record of *E. bungara* from Southeast Asia. Fischthal and Kuntz [20, 21] reported *E. colubrimurorum*, a common parasites of reptiles in Europe and Asia, in the same host (*E. plumbea*) from North Borneo and Korea. The life-cycle of the genus has not so far been elucidated. Metacercariae of *E. colubrimurorum* and *E. asymmetrica* have been reported, by Yamaguti [11] and Chiang [12] respectively. Yamaguti [11] in Japan fed metacercariae from the muscle of *Rana nigromaculata* to *Elape climacophora* and *E. quadrivirgata* and obtained the adults. Chiang [12] in South China, fed metacercariae from muscles of the paradise fish, *Macropodus opercularis, Ooeidozygma lima*, *R. rugulosa* and *R. limnocharis* to *Takydrotnus sexlineatus meridionalis* and *Calotes versicolor* and recovered adults in three weeks.

The present metacercaria shows close resemblance to the adult of *E. colubrimurorum* in most respects and is, therefore, reported here as *E. colubrimurorum* metacercaria. So this is the first report of *E. colubrimurorum* metacercariae from India and also this forms the first report from the two frog hosts studied. Gupta and Mehrotra [22] pointed out that, intra-specific variation can be seen among adult *E. colubrimurorum*. In the present study metacercaria with variation in size and shape of the body, position of the gonads and the caecal termination as mentioned by Gupta and Mehrotra [22] are documented (Fig. 2).

5. Conclusion

The present study is a part of an ongoing research project to prepare a database on trematode parasites infecting frogs of Western Ghats Wayanad region. Trematode parasites have complex life cycles, requiring multiple hosts. If these parasites are present in an ecosystem, then one can infer that their respective hosts must also be present. Thus, these parasites may serve as reliable indicators of species diversity in an ecosystem. Diverse assemblages of larval trematode parasites are easily sampled in intermediate host snails. Through their life cycles these parasites are functionally coupled with the surrounding free-living diversity of vertebrate and invertebrate animals. After elucidating and establishing the life cycles of trematode parasites of frogs, the larval trematodes can be universally taken as indicators of frog diversity. The present report is the first step in the process.

6. Acknowledgements

The authors are grateful to the Kerala State Council for Science, Technology and Environment (KSCSTE), Government of Kerala for providing financial assistance in the form of a major research project (SRS/220/2015/KSCSTE) to carry out this study. The permission accorded by the Department of Forest and Wildlife, Government of Kerala (Order No WL10-63909/2016) for collecting frogs from the Wayanad forest region is also gratefully acknowledged. The authors are indebted to Mr Jithin M C Field Assistant for collecting the specimens for the study.

6.1 Author's contribution

Dr P. K. Prasadan designed and guided the study. Mr Shinad K. carried out the survey, collected and studied the adult trematodes in detail. The manuscript was written by both the authors.

6.2 Compliance with ethical standards

6.3 Conflict of interest

The authors declare that that there is no conflict of interest between them.

6.4 Ethical approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed in the study involving animals were in accordance with the ethical standards of the institution or practice at which the study was conducted.

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