

Digenean parasites of the Family Lecithodendriidae (Luhe, 1901) Odhner, 1901 infecting the frog *Hoplobatrachus tigerinus* (Anura: Dicroglossidae) of the Western Ghats, Wayanad Region, India

Keloth Shinad and Puthanpurayil Kandambeth Prasadana*

Ecological Parasitology and Tropical Biodiversity Laboratory. Department of Zoology. Kannur University. Mananthavady Campus. Wayanad - 670645. Kerala. India. Email: prasadank@kannuruniv.ac.in.

Abstract. Three species of digenean parasites, *Pleurogenoides euphlycti*, *P. wayanadensis* and *Meharorchis ranarum* of the frog *Hoplobatrachus tigerinus* (Anura, Dicroglossidae) from the Wayanad Region of the South Western Ghats are reported in this paper. Multiple infections were also recorded during the study. Prevalence of infection of *P. euphlycti*, *P. wayanadensis* and *M. ranarum* were 15.15%, 9.09% and 12.12%, respectively, and the intensity of infection were 5.2, 7.3 and 8, and the mean abundance were 0.78, 0.66 and 0.96, respectively. Mean abundance is an indication of the dispersion of parasites among hosts. The mean abundance and the intensity were at the maximum level in *M. ranarum* infection and that of prevalence of infection was at the maximum level in of *P. euphlycti* infection.

Keywords: Digenean parasites; Frog; *Hoplobatrachus tigerinus*; Western Ghats; Prevalence.

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ORCID

0000-0002-3876-9697
Keloth Shinad

0000-0001-7228-8435
Puthanpurayil
Kandambeth Prasadana

Introduction

Amphibians, particularly anurans, harbour a variety of adult and larval trematodes involving freshwater gastropods as first intermediate hosts in their life-cycles (Duda and Verma, 1996). The trematode fauna of amphibians of India has been the subject of numerous investigations, including those by Mehra and Negi (1926a, b; 1928), Bhalerao (1926, 1936a, b, c; 1937), Srivastava (1933a, b; 1934a, b), Pande (1937), Chauhan (1954), Gupta and Agrawal (1966, 1967), Pandey (1968, 1969a, b, 1981), Gupta, (1970, 1977), Mukherjee and Ghosh (1970, 1972), Singh (1977) Janardanan et al. (1987), Janardanan and Prasadani (1991), Brinesh and Janardanan (2014) and Shinad and Prasadani (2017a, b, 2018a, b, c, d). Singh (1977) made the first contribution to the trematode fauna of amphibians in Kerala. Later Janardanan et al. (1987) recorded *Pleurogenoides ovatus*, Rao, 1977 from *Rana tigrina* (*Hoplobatrachus tigerinus*) and later Janardanan and Prasadani (1991) elucidated its life cycle. The life cycle of a new species, *P. malampuzhaensis* was established by Brinesh and Janardanan (2014). Recently four new species of digenetic trematodes from amphibian hosts were reported from the Western Ghats by Shinad and Prasadani (2017a, b; 2018a).

H. tigerinus (Daudin, 1803) is a large frog, generally semi-aquatic in habit and probably the largest frog seen in India. They live in the fringes along rivers, reservoirs and marshes, inundated paddy fields and large ponds with floating vegetation. It is widely distributed throughout the low to moderate elevations in Nepal, Bhutan, western and central Myanmar through Bangladesh and India to northern Pakistan and south to the Western Ghats (Frost, 2014). Despite its common occurrence in different types of freshwater bodies, the parasite fauna had not been a subject of any in-depth study.

During our studies on the digenetic parasites of frogs of the Western Ghats, Wayanad region an analysis was made on the species composition of digenetic parasites of *H. tigerinus*.

This paper deals with the digenetic parasites found in *H. tigerinus* of the Western Ghats, Wayanad region with their prevalence, intensity of infection and mean abundance.

Materials and methods

The study was carried out in the Wayanad region of the Western Ghats (latitudes 11° 27' and 15° 58' North and 75° 47' and 70° 27' East longitude). Western Ghats is a treasure trove of biological diversity in India and is considered one of the "hottest hotspots" of biodiversity because of its very rich fauna & flora and the highest level of endemism. Prevalence, intensity of infection and mean abundance were measured following Bush et al. (1997).

Thirty three specimens of *H. tigerinus* collected during the period from January 2016 to January 2019 from various water bodies using sweep hand net were brought to the laboratory, maintained in cement cisterns and fed them occasionally with insects.

The specimens were narcotized with chloroform, dissected, their skin were removed, and the muscle tissues were macerated to detect the presence of metacercariae. Internal organs were also dissected out from each frog, placed in separate Petri dishes containing 0.75% saline, macerated and examined under the stereozoom microscope. Adults, when present, were carefully removed, transferred to 0.75% saline in separate watch glasses and studied under Nikon ECLIPSE Ni-U phase contrast research microscope without supravital staining or after staining with neutral red. Permanent slides of adult parasites were prepared after fixing them in 5% formalin under slight cover glass pressure and staining with acetocarmine,

following the procedure outlined by Cantwell (1981). Specimens were measured using the Nikon NIS Elements Imaging software. All measurements are in micrometers (μm), as range followed by mean in parentheses.

Illustrations were made using the Nikon Y-IDT drawing tube attached to the Nikon ECLIPSE Ni-U microscope and the details were added free hand from observations made on live specimens. Photographs were taken with a Nikon Y-TV55 camera.

Results

Three species of digenetic trematodes, *P. euphlycti*, *P. wayanadensis* and *M. ranarum* were found infecting the duodenum of the frog *H. tigerinus* (Table 1; Figures 1 to 3)).

Collection localities: Ellumandam, Kannur University Campus Mananthavady, Karakkuni, Karappuzha, Panamaram, Peechangode, Pulpally, Pyngatri, Thalappuzha Engineering College and Valliyoorkavu Wayanad District. Period of collection: January 2016 to January 2019.

Table 1. Digenean parasites with their prevalence, intensity of infection and mean abundance.

Name of the parasite	Prevalence of infection	Intensity of infection	Mean abundance
<i>Pleurogenoides euphlycti</i>	5 out of 33 frogs (15.15%)	26 from 5 infected frogs (5.2)	26 in 33 frogs (0.78)
<i>Pleurogenoides wayanadensis</i>	3 out of 33 frogs (9.09%)	22 from 3 infected frogs (7.3)	22 in 33 frogs (0.66)
<i>Meharorchis ranarum</i>	4 out of 33 frogs (12.12%)	32 from 4 infected frogs (8)	32 in 33 frogs (0.96)

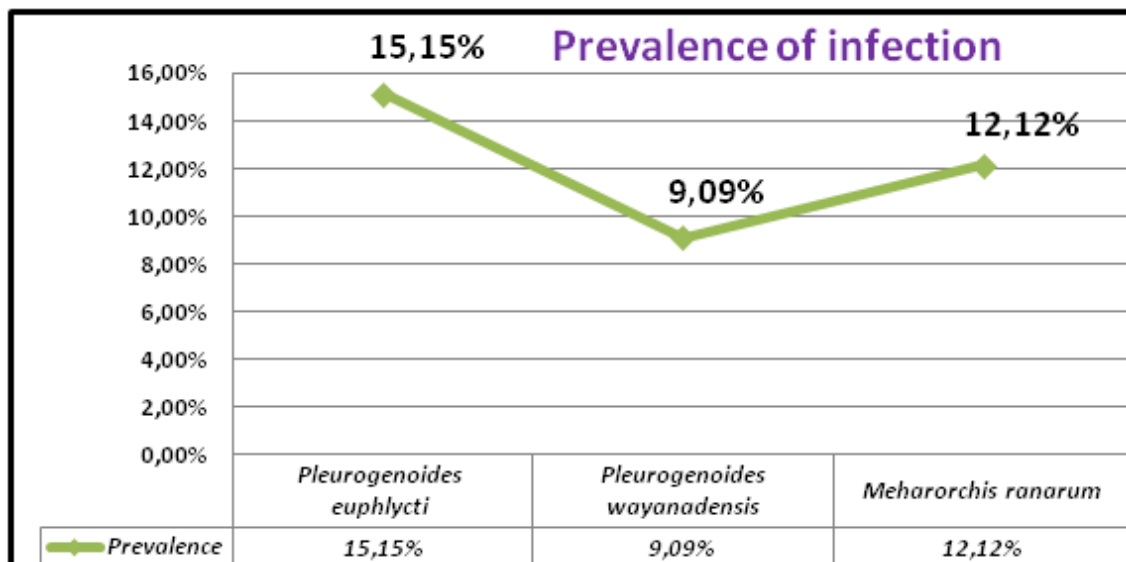


Figure 1. Digenean parasites with their prevalence of infection.

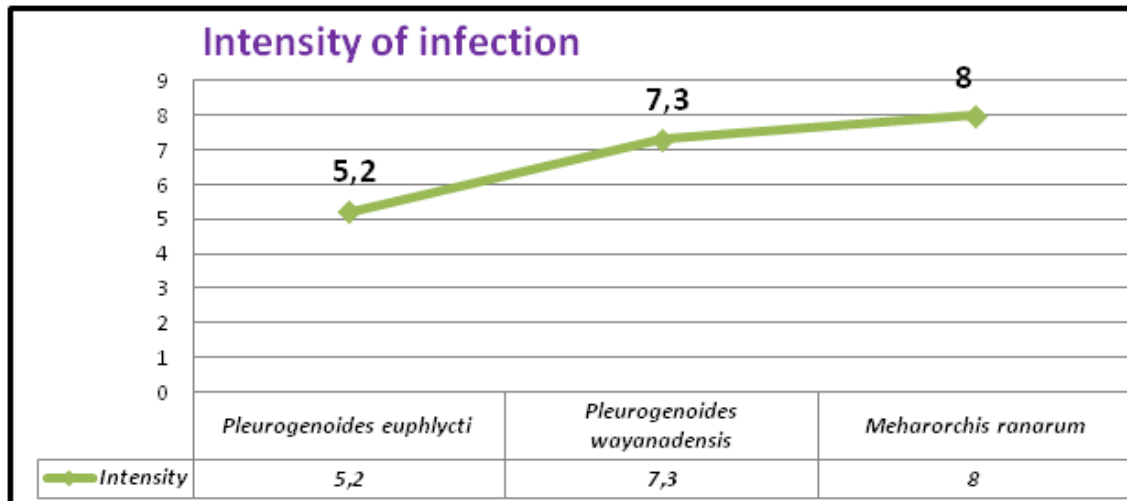


Figure 2. Digenean parasites with their intensity of infection.

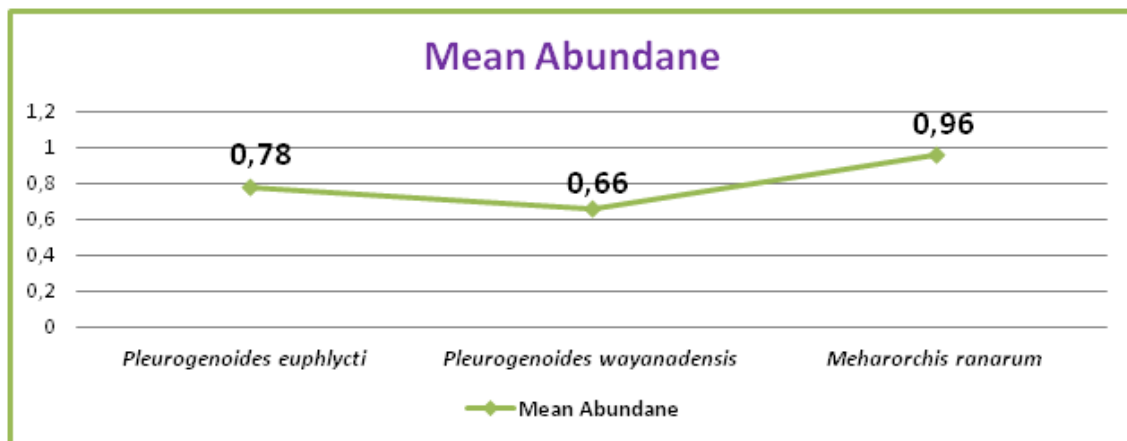


Figure 3. Digenean parasites with their mean abundance

Family: Lecithodendriidae
(Luhe, 1901) Odhner, 1901

Genus *Pleurogenoides* Luhe, 1901

***P. euphlycti* Shinad and Prasad, 2018 (Figure 4)**

Based on the length of the intestinal caeca Travassos (1921) established the genus *Pleurogenoides* of the Family Pleurogenidae Looss, 1899 to accommodate those species of the genus *Pleurogenes* Looss, 1896, and considered *P. tener* as its type species. On the basis of the length of intestinal caeca and position of genital pore, Mehra and Negi dropped the genus *Pleurogenoides* into two sub

genera, *P. (Pleurogenes)* and *P. (Telogonella)*. Srivastava (1934a) also dropped the genus *Pleurogenoides* and transferred the species under it to *Pleurogenes*. But Macy (1936) retained the genus *Pleurogenoides* and the same were accepted by Kaw (1943) and Mukherjee and Ghosh (1970). Of the 32 species of *Pleurogenoides* recorded worldwide from amphibians, 16 species, viz - *P. gastroporus* (Luhe, 1901) Travassos, 1921, *P. sphaericus* (Klein, 1905) Travassos, 1921, *P. gastroporus* var. *equalis* Mehra and Negi, 1926, *P. medians* var. *equalis* Mehra and Negi, 1926, *P. prayagi* Mehra and Negi, 1926,

P. solus (Johnston, 1912) Travassos, 1930, *P. orientalis* Srivastava, 1934, *P. bufonis* Kaw, 1943, *P. sitapurii* (Srivastava, 1934) Kaw, 1943, *P. sawanensis* Gupta, 1954, *P. amritsarensis* Gupta and Chopra, 1984, *P. ovatus* Rao, 1977, *P. jamshedpuransis* Husnain and Sahay, 1988, *P. ranchiensis* Dan and Sahay, 1991, *P. sahranensis* Chakrabarti et al., 2011 and *P. malampuzhensis* Brinesh and Janardanan, 2014 were recorded from India. Life cycles of two species of *Pleurogenoides*, *P. ovatus* and *P. malampuzhaensis* have been elucidated from Kerala (Janardanan and Prasad, 1991; Brinesh and Janardanan, 2014) respectively. Recently three new species of *Pleurogenoides*, *P. cyanophlycti*, *P. euphlycti* and *P. wayanadensis* were described by Shinad and Prasad (2017b, 2018a) from *Euphlyctis cyanophlyctis* of the Wayanad region of the Western Ghats.

Remarks: The species is characterized by slightly ovoid body with a slightly ovoid anterior and broadly round posterior ends. Its body is covered with minute, backwardly directed spines which are closely set at the anterior and posterior regions and sparsely distributed in the central region. Its oral sucker is sub-terminal, almost circular and almost equal to or slightly larger than ventral sucker. It has a circular and post-equatorial ventral sucker. Its mouth is sub-terminal with a muscular pharynx. Oesophagus is short or absent. It has a baseball racket shaped intestinal caeca ending at the level of ovary and left testis. Its two testes are equal sized, spherical, equatorial or post-equatorial, symmetrical and placed one on either side of ventral sucker in the lateral body margins. It has a claviform cirrus sac which extends from the equator to genital pore, at the left body margin near the level of oral sucker; it encloses bipartite seminal vesicle, well developed pars-prostatica, long and narrow ejaculatory duct and an un-spinose cirrus. Its ovary is small, spherical or

ovoid, lies close to the level of right caecum, above the level of ventral sucker and right testis. Uterus is post-acetabular and winding, mostly in the hind body. Its eggs are ovoid, operculate and have well developed vitellaria, present only at the posterior region of pharynx.

Five out of 33 *H. tigerinus* were infected with *P. euphlycti* and the prevalence of infection is 15.15%. Twenty six *P. euphlycti* were recovered from five frogs so the intensity of infection is 5.2 and 26 *P. euphlycti* were recovered from a total of 33 frogs so the mean abundance is 0.78 (Table 1).

Multiple infections were observed in few frogs with *P. wayanadensis* and *M. ranarum*.

Collection localities:
Panamaram, Wayanad District.

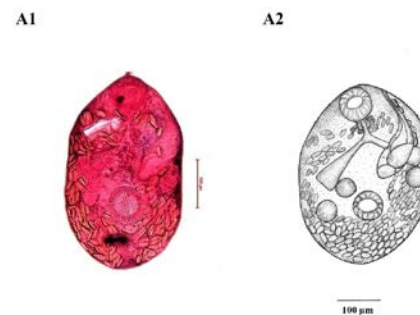


Figure 4. *P. euphlycti* (A1 and A2).

***P. wayanadensis* Shinad and Prasad, 2018 (Figure 5)**

Remarks: The species has an ovoid body, with a slightly ovoid anterior and broadly round posterior ends. Body is covered with minute spines which are backwardly directed and that are closely set throughout the body. It has a circular, sub-terminal oral sucker larger than ventral sucker. Its ventral sucker is circular and equatorially placed. Its mouth is sub-terminal and pharynx is muscular and ovoid. Its oesophagus is short or absent. Its caeca reach up to the

middle of testes and below ventral sucker. It has two, spherical testes, equatorial or post-equatorial, symmetrical and placed one on either side near the posterior margin of ventral sucker in the lateral body margins. It has claviform cirrus sac, extends from the posterior margin of ventral sucker to genital pore, at the left body margin near the mid level of oral sucker and it encloses bipartite seminal vesicle, well developed pars-prostatica, long and narrow ejaculatory duct and an unspinose cirrus. It has a spherical ovary, at the zone of ventral sucker, in between ventral sucker and right caecum. Uterus is post-acetabular and winding, mostly in the hind body. Eggs are ovoid, operculate and vitellaria are absent.

Three out of 33 *H. tigerinus* were infected with *P. wayanadensis* and the prevalence of infection is 9.09%. Twenty two *P. wayanadensis* were recovered from three frogs and the intensity of infection is 7.3 and 22 *P. wayanadensis* were recovered from a total of 33 frogs so the mean abundance is 0.66 (Table 1).

Multiple infection was observed in a few frogs with *P. euphlycti* and *M. ranarum*.

Collection localities:
Panamaram, Wayanad District.

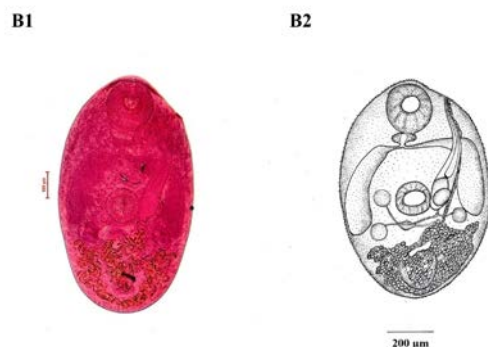


Figure 5. *P. wayanadensis* (B1 and B2).

Family: Lecithodendriidae
(Luhe, 1901) Odhner, 1901

Genus *Mehraorchis* Srivastava, 1934

Mehraorchis ranarum
Srivastava, 1934 (Figure 6)

The genus *Mehraorchis* was erected by Srivastava (1934) with *M. ranarum* from *Rana cyanophlyctis* as its type species. So far three species, *M. tigrinarum* Gupta, 1954, *M. jainiformis* Bharadwaj, 1961 and *M. cyanophlycticus* Karyakarte, 1973 have been added to the genus from Indian amphibians. They were encysted in hepatic system or free in intestine of amphibians and reptiles. Gupta and Agarwal (1966) synonymised *M. tigrinarum* with *M. ranarum*. The first report of *M. ranarum* from Kerala was made by Singh from the intestine of *Rana tigrina* in Punalur in 1977. The second report of this parasite was made by Muraleedharan, 1989 from the body cavity and ileum of the two frog hosts *R. Limnocharis* and *R. tigrina* in Chundale, Wayanad district.

Remarks: They are large virgulate digeneans. The species is characterized by ovoid body covered with prominent spines which are numerous at the anterior region and gradually decrease posteriorly. It has a terminal to sub terminal oral sucker and an equatorial to pre-equatorial, ventral sucker at. It has sub terminal mouth, globular pharynx, long oesophagus and intestinal caeca that, terminates near the posterior end of body. It has two symmetrical testes at the anterior third. It has a muscular and claviform cirrus sac, which extends from the intestinal bifurcation to the genital pore, at the left body margin near the mid level of oral sucker and it encloses well developed seminal vesicle, pars-prostatica and ejaculatory duct. It has an oval shaped ovary placed intercaecal in the ventral sucker zone between ventral sucker and right testis. Its uterus is post ovarian and filling mostly in the posterior half of the body. Eggs are ovoid and non operculate. Its vitellaria are well developed, lateral

and flower like, extending from the level of pharynx to the posterior margin of ovary, on each side.

Four out of 33 *H. tigerinus* were infected with *M. ranarum* so the prevalence of infection is 12.12%. Thirty two *M. ranarum* were recovered from four frogs and the intensity of infection is 8 and 32 *M. ranarum* were recovered from a total of 33 frogs so the mean abundance is 0.96 (Table 1).

Multiple infections were observed in a few frogs with *P. wayanadensis* and *P. euphlycti*.

Collection localities:
Ellumandam, Karappuzha and Panamaram Wayanad District.

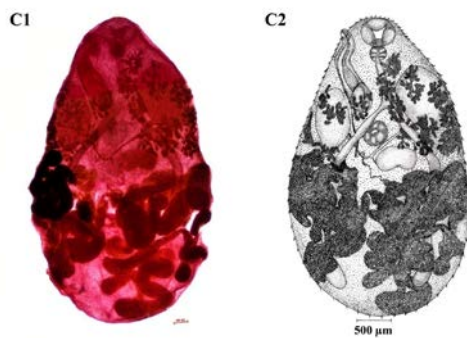


Figure 6. *M. ranarum* (C1 and C2).

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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.

Conflict of interest

Authors declare that there is no conflict of interest.

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