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Prevalence, intensity and mean abundance of Digenean parasites of the water skipper, *Euphlyctis cyanophlyctis* (Anura, Dicroglossidae) of the south Western Ghats

K Shinad and PK Prasad

Abstract

Five species of Digenean parasites, *Diplodiscus cyanophlycti*, *Pleurogenoides cyanophlyctis*, *Pleurogenoides euphlyctis*, *Pleurogenoides wayanadensis* and *Pleurogenoides orientalis* of the frog, *Euphlyctis cyanophlyctis* (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 *D. cyanophlycti*, *P. cyanophlycti*, *P. euphlycti*, *P. wayanadensis* and *P. orientalis* were 8%, 16%, 10%, 6% and 2% respectively, the intensity of infection were 1.5, 5.2, 6.1, 9.5 and 4.5 respectively and the mean abundance were 0.12, 0.83, 0.61, 0.57 and 0.09 respectively. Mean abundance is an indication of the dispersion of parasites among hosts. Mean abundance and prevalence are at maximum level in the case of *P. cyanophlycti* infection.

Keywords: Digenean parasites, frog, *euphlyctis cyanophlyctis*

1. Introduction

Amphibians, particularly anurans, harbour a variety of adult and larval trematodes involving freshwater gastropods as first intermediate hosts in their life-cycles^[1]. The trematode fauna of amphibians of India has been the subject of numerous investigations, including those by Mehra and Negi^[2, 3, 4], Bhalerao^[5, 6, 7, 8, 9], Srivastava^[10, 11, 12, 13], Pande^[14], Chauhan^[15], Gupta and Agrawal^[16, 17], Pandey^[18, 19, 20, 21], Gupta^[22, 23], Mukherjee and Ghosh^[24, 25], Singh^[26], Janardanan *et al.*,^[27], Janardanan and Prasad^[28], Brinesh and Janardanan^[29] and Shinad and Prasad^[30, 31, 32, 33]. Singh^[26] made the first contribution to the trematode fauna of amphibians in Kerala. Later Janardanan *et al.*,^[27] recorded *Pleurogenoides ovatus*, Rao, 1977 from *Rana tigrina* (*Hoplobatrachus tigerinus*) and later Janardanan and Prasad^[28] elucidated its life cycle. The life cycle of a new species, *P. Malampuzhaensis* was established by Brinesh and Janardanan^[29]. Recently four new species of digenetic trematodes from amphibian hosts were reported from Western Ghats by Shinad and Prasad^[30, 31, 32, 33].

The water skipper, *E. cyanophlyctis*^[34] inhabits the pools/standing waters in the plains and sub-mountainous areas of the Western Ghats. It is widely distributed throughout South Asia^[35]. 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 Digenean parasites of frogs of the Western Ghats, Wayanad region an analysis was made on the species composition of Digenean parasites of *E. cyanophlyctis*. This paper deals with the Digenean parasites found in *E. cyanophlyctis* of the Western Ghats, Wayanad region with their prevalence, intensity of infection and mean abundance.

2. Materials and Methods

2.1 Study area: 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 and mean abundance of Infection were measured following Bush *et al.*,^[36]. Prevalence is the number (%) of hosts infected with one or more individuals of a particular parasite species (or taxonomic group) divided by the number of hosts examined for that parasite species. Intensity (of infection) is the number of individuals of a particular

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Parasite species in a single infected host, i.e., the number of individuals in an infra population. Intensity is a form of density with the sampling unit specifically defined as an individual infected host. Therefore, intensity is a convenient measure for Parasitologists because hosts are discrete and natural sampling units. Mean abundance is the total number of individuals of a particular parasite species in a sample of a particular host species divided by the total number of hosts of that species examined (including both infected and uninfected hosts). It is thus the average abundance of a parasite species among all members of a particular host population.

One hundred specimens of *E. cyanophlyctis* collected during the period from January 2016 to October 2017 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 skins 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 stereo zoom 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 supra vital 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 ace to car mine, following the procedure outlined by Cantwell [37]. Specimens were measured using the Nikon NIS Elements Imaging software. All measurements are in micro meters (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.

3. Results

Five species of digenetic trematodes, *D. cyanophlycti*, *P. cyanophlycti*, *P. euphlycti*, *P. wayanadensis* and *P. orientalis* were found infecting the duodenum of the frog *E. cyanophlyctis*.

Collection localities: Birakuppa, Chandhanathodu, Chennaiode, Chooral mala, Chundel, Kammana, Karappuzha Dam, Kavummandham, Makkimala, Ondayangadi, Pakkam, Panamaram, Payyampally, Peechangode, Pulpally, Thalappuzha Wayanad District.

Period of collection: January 2016 to October 2017

Table 1: Digenean parasites with their prevalence and intensity of infection

Name of the parasite	Prevalence of infection	Intensity of infection	Mean abundance
<i>Diplodiscus cyanophlycti</i>	8 out of 100 frogs (8%)	12 from 8 infected frogs (1.5)	12 in 100 frogs (0.12)
<i>Pleurogenoides cyanophlycti</i>	16 out of 100 frogs (16%)	83 from 16 infected frogs (5.2)	83 in 100 frogs (0.83)
<i>Pleurogenoides euphlycti</i>	10 out of 100 frogs (10%)	61 from 10 infected frogs (6.1)	61 in 100 frogs (0.61)
<i>Pleurogenoides wayanadensis</i>	6 out of 100 frogs (6%)	57 from 6 infected frogs (9.5)	57 in 100 frogs (0.57)
<i>Pleurogenoides orientalis</i>	2 out of 100 frogs (2%)	9 from 2 infected frogs (4.5)	9 in 100 frogs (0.09)

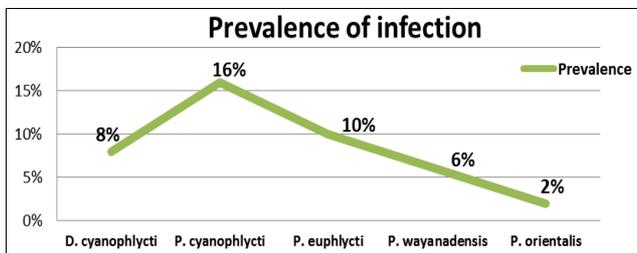


Fig 1: Digenean parasites with their prevalence of infection

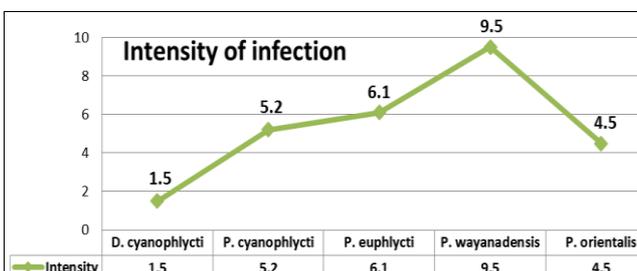


Fig 2: Digenean parasites with their intensity of infection

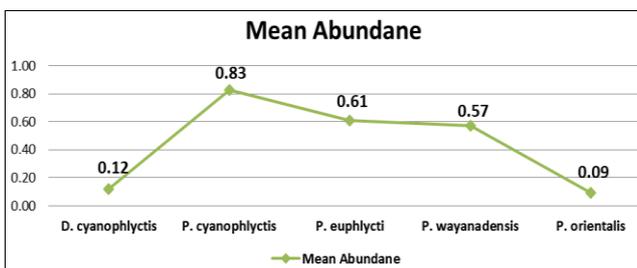


Fig 3: Digenean parasites with their mean abundance

3.1 Family: Paramphistomidae Fiscoeder, 1901

Genus: Diplodiscus Diesing, 1836

***Diplodiscus cyanophlycti* Shinad and Prasad, 2017 (Fig 4)**

The genus *Diplodiscus* of the family Diplo dissuade Cohn, 1904 was erected by Diesing [38] and ascribed *D. Subclavatus* (Pallas, 1760) as its type species. So far 17 species have been added to this genus from amphibians. Of these, six valid species have been reported from Indian amphibians by Srivastava [13], Pande [14], Bhalerao [9], Kaw [39], Pandey [18, 19], Mukherjee and Ghosh [25], Dwivedi [40], Singh [26] and Shinad and Prasad [30]. They are *Diplodiscus amphichrus* Tubangui, 1933, *D. amphichrus magnus* Srivastava, 1934, *D. mehrai* Pande, 1937, *D. lali* Pandey and Chakrabarty, 1968, *D. chauhani* Pandey, 1969 and *D. cyanophlycti* Shinad and Prasad, 2017.

Tubangui [41] described *D. amphichrus* from *Rana* sp. in the Philippines. Srivastava [12] recorded a new variety, *D. amphichrus* var. *magnus* from *E. cyanophlyctis* from UP and Pande [14] added another species, *D. mehrai*, from the same host in Kumaon Hills. Singh [42] regarded *D. amphichrus* var. *magnus* and *D. mehrai* as synonyms of *D. amphichrus*. Mukherjee [43] further synonymised *D. japonicus* (Yamaguti, 1936) with *D. amphichrus*. Fischthal and Thomas [44] raised the variety *magnus* of Srivastava to species rank and considered that *D. amphichrus* of Agarwal as synonym of *magnus* and so were *D. amphichrus*, *D. japonicus* and *D. mehrai*. Pandey and Chakrabarty [45] and Pandey [19] described two new species, *D. lalli* and *D. chauhani* from *H. tigrina* and *E. cyanophlyctis*, respectively and Pandey and Jain [46] upheld the validity of *D. mehrai*. Nama and Khichi [47] described a

new sub species, *D. amphichrus brevis* from *E. cyanophlyctis* and disagreed to the synonymy of *D. mehrai* to *D. amphichrus*. Srivastava [48] suggested that *D. lali* and *D. chauhani* are synonyms of *D. amphichrus*. Srivastava [48] also considered that the genus is represented by two distinct species *D. amphichrus* and *D. mehrai* in India. Recently, *D. cyanophlycti* was added by Shinad and Prasad [30].

3.1.1 Remarks

The species is characterized by stout, conical body with smooth surface, blunt anterior end and round posterior end. Its mouth is sub-terminal, followed by fairly well developed, spherical or ovoid pharynx. It has a sub-terminal and ovoid oral sucker and a large, conspicuous, cup shaped ventral sucker, located at posterior extremity, provided with an additional sucker with depression in centre. Its intestinal caeca is wide, extends along the lateral margin of body up to the anterior border of the posterior sucker. It has a single, round or ovoid testis at the posterior third of body and a small cirrus sac. The genital pore is median and immediately behind the bifurcation. It has a large and lateral vitelline follicle extends from the level of intestinal bifurcation to posterior sucker and meets in the median line anteriorly and posteriorly. Its eggs are large, oval, operculate and 110.0 – 130.0 in number.

Eight out of 100 *E. cyanophlyctis* were infected with *D. cyanophlycti* and the prevalence of infection is 8%. Twelve *D. cyanophlycti* were recovered from eight infected frogs and, therefore, the intensity of infection is 1.5. Twelve *D. cyanophlyctis* were recovered from a total of 100 frogs examined and, therefore, the mean abundance is 0.12 (Table 1).

Collection localities: Birakuppa, Chooralmala, Chundel, Panamaram and Pulpally Wayanad District.

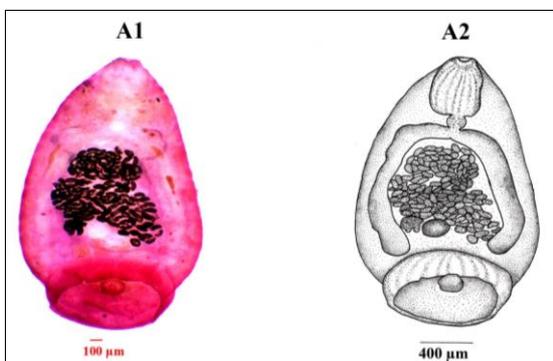


Fig 4: *Diplodiscus cyanophlycti* (A1 and A2)

3.2. Family: Lecithodendriidae (Luhe, 1901) Odhner, 1901 Genus *Pleurogenoides* Luhe, 1901

Pleurogenoides cyanophlycti Shinad and Prasad, 2017 (Fig. 5)

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 2 sub genera, *P. (Pleurogenes)* and *P. (Telogonella)*. Srivastava [12] also dropped the genus *Pleurogenoides* and transferred the species under it to *Pleurogenes*. But Macy [49] retained the genus *Pleurogenoides* and the same was accepted by Kaw [50] and Mukherjee and

Ghosh [24]. 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* Brinsh and Janardanan, 2014 were recorded from India. Life cycle of two species of *Pleurogenoides*, *P. ovatus* and *P. malampuzhaensis* have been elucidated from Kerala (Janardanan and Prasad [28]; Brinsh and Janardanan [29]). Recently three new species of *Pleurogenoides*, *P. cyanophlycti*, *P. euphlycti* and *P. wayanadensis* were described by Shinad and Prasad [31, 32] from *E. cyanophlyctis* of the Wayanad region of the Western Ghats.

3.2.1 Remarks

The species is characterized by an oval body with a blunt anterior and broadly round posterior ends and the body covered with minute, backwardly directed spines which are closely set at the anterior region but widely spaced at the posterior end. It has a sub-terminal and almost round oral sucker which is larger than the ventral sucker. Its ventral sucker is spherical and equatorial. Its mouth is sub-terminal with an ovoid and muscular pharynx and short oesophagus. Its intestinal caeca bifurcates anterior to ventral sucker into short, blunt, laterally inclined, unequal caecum and ends slightly behind testes. It has two slightly spherical or oval, symmetrical testes, situated at equatorial or post-equatorial and one on either side of the ventral sucker in the lateral body margin. Its cirrus sac is clavi form extending from the anterior margin of the ventral sucker 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-spinosad cirrus. It has a spherical ovary which is intercaecal and located anterior to right testis. Its uterus is post-acetabular and winding mostly in the hind body region. Its eggs are ovoid, operculate; vitellaria are well developed, extending from the mid-level of oral sucker to the anterior margin of testes, on each side, converging medially on either side of pharynx, but not meeting together.

Sixteen out of 100 *E. cyanophlyctis* were infected with *P. cyanophlycti* so the prevalence of infection was 16%. Eighty three *P. cyanophlycti* were recovered from 16 frogs and the intensity of infection was 5.2. Its mean abundance was 0.83 as 83 individual *P. cyanophlycti* were recovered from the 100 *E. cyanophlycti* examined (Table 1).

A few hosts infected with *P. cyanophlycti* exhibited multiple infections with *D. cyanophlycti*.

Collection localities: Chundel, Kammana, Makkimala, Panamaram, Payyampally, Peechangode, and Pulpally Wayanad District.

Other hosts documented with *P. cyanophlycti*:

1. *Hylarana aurantiaca*: One out of 19 *H. aurantiaca* was infected with *P. cyanophlycti* so the prevalence of infection was 5.3%. Three *P. cyanophlycti* were recovered from one frog and the intensity of infection was

three. Three *P. cyanophlycti* were recovered from a total of 19 frogs examined and, therefore, the mean abundance is 0.15. Collection locality: Makkimala Wayanad District.

2. *Fejervarya sp.*: Two out of 192 *Fejervarya sp.* were infected with *P. cyanophlycti* so the prevalence of infection was 1%. Two *P. cyanophlyctis* were recovered from two frogs and the intensity of infection was 1. Two *P. cyanophlycti* were recovered from a total of 192 frogs examined and, therefore, the mean abundance is 0.01.

Collection locality: Peechangode Wayanad District.

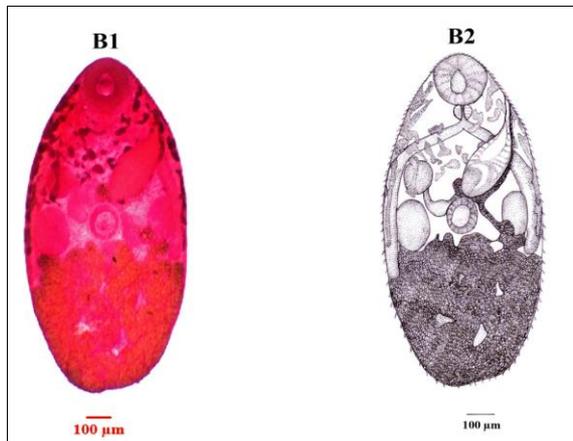


Fig 5: *Pleurogenoides cyanophlycti* (B1 and B2)

3.3 *Pleurogenoides euphlycti* Shinad and Prasad, 2018 (Fig. 6)

P. euphlycti was described from two frog hosts, *E. cyanophlyctis* and *H. tigerinus* from the Wayanad region of the Western Ghats by Shinad and Prasad [32].

3.3.1 Remarks

The species is characterized by slightly ovoid body with a slightly ovoid anterior end 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 and almost spherical 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 equally 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; also 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.

Ten out of 100 *E. cyanophlyctis* were infected with *P. euphlycti* and the prevalence of infection is 10%. Sixty one *P. euphlycti* were recovered from 10 infected frogs and the intensity of infection is 6.1. Its mean abundance was 0.61 as 61 individual *P. cyanophlycti* were recovered from the total 100 *E. cyanophlycti* examined (Table 1).

A few hosts infected with *P. euphlycti* exhibited multiple infections with *P. wayanadensis*.

Collection localities: Birakuppa, Chooralmala, Kavummandham and Panamaram Wayanad District.

Other recorded hosts infected with *P. euphlycti*:

1. *H. tigerinus*: Five out of 23 *H. tigerinus* were infected with *P. euphlycti* so the prevalence of infection was 21.7%. Twenty six *P. euphlycti* were recovered from five frogs and the intensity of infection was 5.2. Twenty six *P. euphlycti* were recovered from a total of 23 frogs examined and, therefore, the mean abundance is 1.13.

Collection locality: Panamaram Wayanad District.

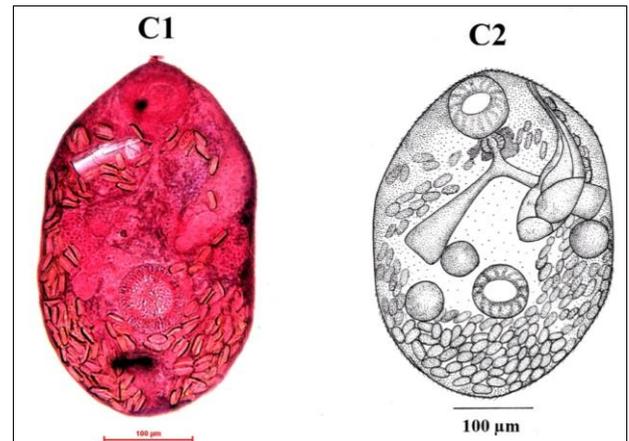


Fig 6: *Pleurogenoides euphlycti* (C1 and C2)

3.4. *Pleurogenoides wayanadensis* Shinad and Prasad, 2018 (Fig. 7)

P. wayanadensis was described from two frog hosts, *E. cyanophlyctis* and *H. tigerinus* from the Wayanad region of the Western Ghats by Shinad and Prasad [32].

3.4.1 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. It has equal 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 un-spinose 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.

Six out of 100 *E. cyanophlyctis* were infected with *P. wayanadensis* and the prevalence of infection is 6%. Fifty seven *P. wayanadensis* were recovered from 6 frogs and the intensity of infection is 9.5. Its mean abundance was 0.57 as 57 individual *P. cyanophlycti* were recovered from the 100 *E. cyanophlycti* examined (Table 1).

A few hosts infected with *P. wayanadensis* also harboured *P. euphlycti*.

Collection localities: Kavummandam and Panamaram Wayanad District.

Other documented hosts infected with *P. wayanadensis*:

1. *H. tigerinus*: Three out of 23 *H. tigerinus* were infected with *P. wayanadensis* so the prevalence of infection was 13%. Twenty two *P. wayanadensis* were recovered from three frogs and the intensity of infection was 7.3. Twenty two *P. wayanadensis* were recovered from a total of 23 frogs examined and, therefore, the mean abundance is 0.95. Collection locality: Panamaram Wayanad District.

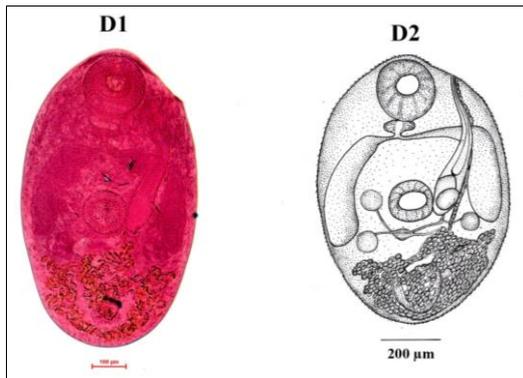


Fig 7: *Pleurogenoides wayanadensis* (D1 and D2)

3.5. *Pleurogenoides orientalis* Srivastava, 1934 (Fig. 8)

P. orientalis was described by Srivastava [12]. The study on the life history of *P. orientalis* was established by Madhavi *et al.*, [51]. Madhavi and Ratna Kumari [52] studied the growth and development of *P. orientalis* in the intermediate and definitive hosts.

3.5.1 Remarks

The species is characterised by oval body with blunt anterior end and broadly round posterior end. Body is covered with minute, backwardly directed spines which are closely set at the anterior region but widely spaced at the posterior region. Its oral sucker is almost oval, sub terminal, larger than ventral sucker. It has a spherical and equatorially spaced ventral sucker. Mouth is sub terminal, pharynx is ovoid or rectangular and oesophagus is short, bifurcated anterior to ventral sucker into short, blunt, laterally disposed intestinal caeca, ending to the level or slightly behind testes. Its spherical or oval testes are placed equatorial or post-equatorial and symmetrical at the lateral body margins. It has clavi form shaped cirrus sac, extends from the posterior margin of ventral sucker to the genital pore, at the left body margin near the mid-level of oral sucker and it encloses S-shaped seminal vesicle, narrow pars-prostatica, long and narrow ejaculatory duct and an unspinose cirrus. It has a oval or spherical shaped ovary placed intercaecal in the ventral sucker zone between ventral sucker and right testis. Its uterus is post-acetabular and winding mostly in the hind body. Eggs are ovoid and operculate. Its vitellaria are well developed, extending from the mid-level of oral sucker to the anterior margin of testes, on each side.

Two out of 100 *E. cyanophlyctis* were infected with *P. orientalis* and the prevalence of infection is 2%. Nine *P. orientalis* were recovered from two frogs and the intensity of infection is 4.5. Its mean abundance was 0.09 as 9 individual *P. cyanophlycti* were recovered from the 100 *E. cyanophlycti* examined (Table 1).

Collection locality: Chooralmala Wayanad District.

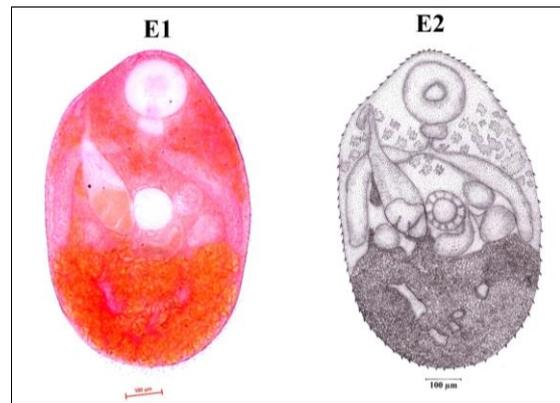


Fig 8: *Pleurogenoides orientalis* (E1 and E2)

4. Acknowledgements

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4.1 Author's contribution

Dr P. K. Prasad 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.

4.2 Compliance with ethical standards

All the required ethical standards were complied with.

4.3 Conflict of interest

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

4.4 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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