

Research Paper

Cite this article: Prasadana PK, Shinad K, Sherin C, Arusha K (2020). Studies on the life cycle of *Pleurogenoides wayanadensis* Shinad & Prasadana, 2018 (Digenea: Pleurogenidae) from the Western Ghats, India. *Journal of Helminthology* **94**, e196, 1–10. <https://doi.org/10.1017/S0022149X20000772>

Received: 14 July 2020
Accepted: 23 August 2020

Key words:

Pleurogenoides wayanadensis; *Hoplobatrachus tigerinus*; *Euphlyctis cyanophlyctis*; *Bithynia (Digoniostoma) pulchella*; *Orthetrum* sp; life cycle studies; progenesis

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Studies on the life cycle of *Pleurogenoides wayanadensis* Shinad & Prasadana, 2018 (Digenea: Pleurogenidae) from the Western Ghats, India

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Abstract

The life cycle of *Pleurogenoides wayanadensis* Shinad & Prasadana, 2018, infecting the frogs *Hoplobatrachus tigerinus* and *Euphlyctis cyanophlyctis*, is elucidated in this study. All the life cycle stages from egg to egg-producing adults were elucidated under natural conditions and successfully established in the laboratory. The life cycle took about 58 to 65 days for completion. Miracidia were released by teasing the eggs with fine needles. Sporocysts were found in the freshwater snail, *Bithynia (Digoniostoma) pulchella*, collected from paddy fields at Payode, Western Ghats, Wayanad region, in the months of October and November 2019. Cercariae were of the virgulate xiphidiocercous type. Metacercariae were recovered from the eyes of the damselfly naiads of the species *Ischnura* sp. and *Copera* sp., and the thorax and abdomen of the dragonfly naiads, *Orthetrum* sp. The metacercariae showed progenetic development. The growth and development of the metacercariae in the naiads that were exposed to cercariae, and development of the trematode in frogs that were force-fed with encysted metacercariae, have been studied at regular intervals. The prepatent period is 14–19 days. The present life cycle study of a *Pleurogenoides* spp. forms the seventh report from the world, fourth report from India and the third from Kerala.

Introduction

Morphological characterization of life cycle stages with the establishment of the identity of the intermediate hosts provides an important complement to parasite taxonomy (Blasco-Costa & Poulin, 2017). This is an integrative approach in taxonomy (Dayrat, 2005). In order to understand the ecology and evolution of any parasite, the full characterization of its developing stages is essential. Although the biodiversity of parasites is being discovered at a high rate, very little efforts are being taken by researchers to elucidate their life cycles. We have elucidated the life cycle of *Pleurogenoides wayanadensis* in nature and established the same through experimental infection studies in the laboratory.

The genus *Pleurogenoides* of the family Pleurogenidae Looss, 1899 was established by Travassos (1921) to accommodate those species of the genus *Pleurogenes* Looss, 1896, and considered *Pleurogenoides tener* as its type species. On the basis of the length of intestinal caeca and position of genital pore, Mehra & Negi (1928) divided the genus *Pleurogenoides* into two sub-genera, *P. (Pleurogenes)* and *P. (Telogonella)* and, later, Srivastava (1934) dropped the genus *Pleurogenoides* and transferred its species to *Pleurogenes*. Macy (1936) retained the genus *Pleurogenoides* and the retention was accepted by Kaw (1943) and Mukherjee & Ghosh (1970). Of the 32 species of *Pleurogenoides* recorded worldwide from amphibians, 16 species were from India. Singh (1977) recorded *Pleurogenoides gastroporus* from *Hoplobatrachus tigerinus* in Kerala. Recently, three new species of *Pleurogenoides* – *Pleurogenoides cyanophlyctis*, *Pleurogenoides euphlyctis* and *Pleurogenoides wayanadensis* – were described by Shinad & Prasadana (2017, 2018a) from *Euphlyctis cyanophlyctis* (Schneider, 1799) and *H. tigerinus* (Daudin, 1803) of the Wayanad region of the Western Ghats, Kerala, India. Shinad & Prasadana (2018b, 2019) recorded the prevalence, intensity of infection and the mean abundance of *P. wayanadensis* from *E. cyanophlyctis* and *H. tigerinus*, respectively.

The life cycles of six species of *Pleurogenoides* have been studied and established in the world (Mathias, 1924; Neuhaus, 1940; Buttner, 1951; Okabe & Shibue, 1951; Shibue, 1953; Macy, 1964; Madhavi *et al.*, 1987; Janardanan & Prasadana, 1991; Brinesh & Janardanan, 2014). Reports are available on metacercariae from crabs, imago and naiads of both dragonflies and damselflies and larvae of other insects (Dissanaike & Fernando, 1960; Muraleedharan & Pande, 1967; Krasnolobova, 1970; Prakash & Pande, 1970; Vojtkova, 1970; Janardanan *et al.*, 1987; Dhanumkumari, 2000; Brinesh & Janardanan, 2014; Bolek *et al.*, 2019).

The objectives of the present study included: (1) the morphological characterization of the developing stages of the parasite and to trace the intermediate hosts so as to elucidate the complete life cycle in nature; and (2) the establishment of the life cycle in the laboratory through experimental infection studies. The metacercariae exhibited progenetic development – the sexual maturation in an organism still in a morphologically juvenile stage (Gould, 1977). The present paper describes the morphology of the developing stages of *P. wayanadensis* obtained from natural infection from the definitive and intermediate hosts and the life cycle stages from egg to egg-producing adult elucidated and experimentally established in the laboratory.

Materials and methods

Study area

The study was carried out and the specimens were collected from different paddy fields and freshwater bodies of the Thirunelly and Payode villages of the Western Ghats, Wayanad region, Kerala. The Western Ghats is considered one of the 'hottest hotspots of biodiversity due to its very rich biodiversity and high endemism' UNESCO (2012).

Collection and examination of frogs, dragonfly naiads and freshwater snails

The common Indian skittering frogs *E. cyanophlyctis* (Schneider, 1799) were collected from the paddy fields and the nearby streams of Thirunelly and Payode villages of the Western Ghats, Wayanad region, from October and November 2019, and were subsequently brought to the laboratory and examined for digenetic trematodes. After narcotization with chloroform, the frogs were dissected and the intestines were transferred to physiological saline for the detection of *P. wayanadensis* under a stereo zoom microscope (binocular LABOMED Luxeo 4Z, LABO AMERICA INC). The bithynid snail *Bithynia (Digoniostoma) pulchella* (Benson, 1836) was collected from the same habitat during the same period. Live snails were transferred to the laboratory and maintained in groups of 10–20 in glass beakers containing tap water. Snails that shed cercariae were isolated and kept individually in separate beakers. Water in the beakers was checked regularly for cercariae. The collected dragonfly naiads of the family Libellulidae were brought alive to the laboratory, maintained in clean aquariums/glass containers and occasionally fed with small aquatic insects. Naiads were sacrificed and carefully observed under the stereo zoom microscope for metacercariae. All the three host specimens were deposited in the Parasite Host Collections, Department of Zoology, Kannur University.

Studies on developing stages of the trematode

The recovered *P. wayanadensis* (metacercariae from the abdomen and thorax of naiads and adults from the duodenum of frogs) were transferred to 0.75% saline. The metacercariae were excysted by rupturing the cyst wall with a fine needle. Isolated *P. wayanadensis* were examined under the Nikon phase contrast research microscope (ECLIPSE Ni-U, NIKON, JAPAN) without supravital staining or after staining with neutral red. Permanent whole mounts were prepared after fixing them in 5% formalin under slight cover glass pressure and staining with acetocarmine, following the procedure outlined by Cantwell (1981). Eggs recovered from the adult

P. wayanadensis were isolated and the miracidia were released from these eggs with a pair of fine-pointed needles. Both these eggs and miracidia were studied with vital staining under a Nikon phase contrast research microscope. A few infected snails were later crushed and examined for intra-molluscan stages. Sporocysts recovered from the hepatopancreas of infected snails were studied after supravital staining with neutral red. Cercariae emerged from the infected snails were studied live with or without vital staining using the Nikon phase contrast research microscope. Genital primordia were observed by using lacto-acetic carmine stain.

Measurements, sketches and photographs

A Nikon Y-TV55 camera, NIKON, JAPAN attached to a Nikon ECLIPSE Ni-U, NIKON, JAPAN phase contrast research microscope was used to take photographs. The trematodes were measured using the Nikon NIS-Elements, NIKON, JAPAN imaging software. Measurements (in μm) were taken on heat-killed specimens. All measurements are in micrometers (μm), as range followed by mean in parentheses. Descriptions are based on the measurements of a minimum of ten specimens. 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.

Experimental infections/lifecycle study

Eggs recovered from the adult *P. wayanadensis* from *E. cyanophlyctis* were isolated. For the release of miracidia, slight pressure was applied on the cover slip and/or teased with a pair of fine, sharp needles. Viable eggs and miracidia were fed to the helminth/infection-free *B. (D.) pulchella* (five in number) maintained in the laboratory. The snails were examined periodically to observe the development of sporocysts and cercariae. Fed snails were crushed within 5–9 days of post-feeding; their hepatopancreas were smeared on slides and observed for sporocysts. Helminth-free odonate naiads were used for experimental infection studies. Fifteen dragonfly naiads were individually exposed to fresh cercariae emerged from snails and observed periodically (3–5 days after post infection) to determine the course of metacercarial development. Ten helminth-free frogs were then force-fed with 33-day-old metacercariae. The specimens were then dissected and observed at three days of periodic intervals (three days post infection) for developing flukes. Observations were made on fresh specimens.

Infection-free specimens

The naiads and frogs used for the experimental infection studies were collected from infection-free areas (specific areas in the neighbouring district from where the collected specimens did not show *P. wayanadensis* infection in the regular parasitic survey for the past two years). Such frog specimens were maintained in the laboratory and their faecal material was carefully analysed for any eggs of *P. wayanadensis* continuously for a period of three months. Five laboratory-reared frogs of the same species were used to confirm the results obtained in the experimental infection studies. The frogs that were free from *P. wayanadensis* infection were used for the study. The specimens were maintained separately in clean aquariums/glass containers in the laboratory. Laboratory-born *B. (D.) pulchella* were used as infection-free specimens for the experimental infection studies.

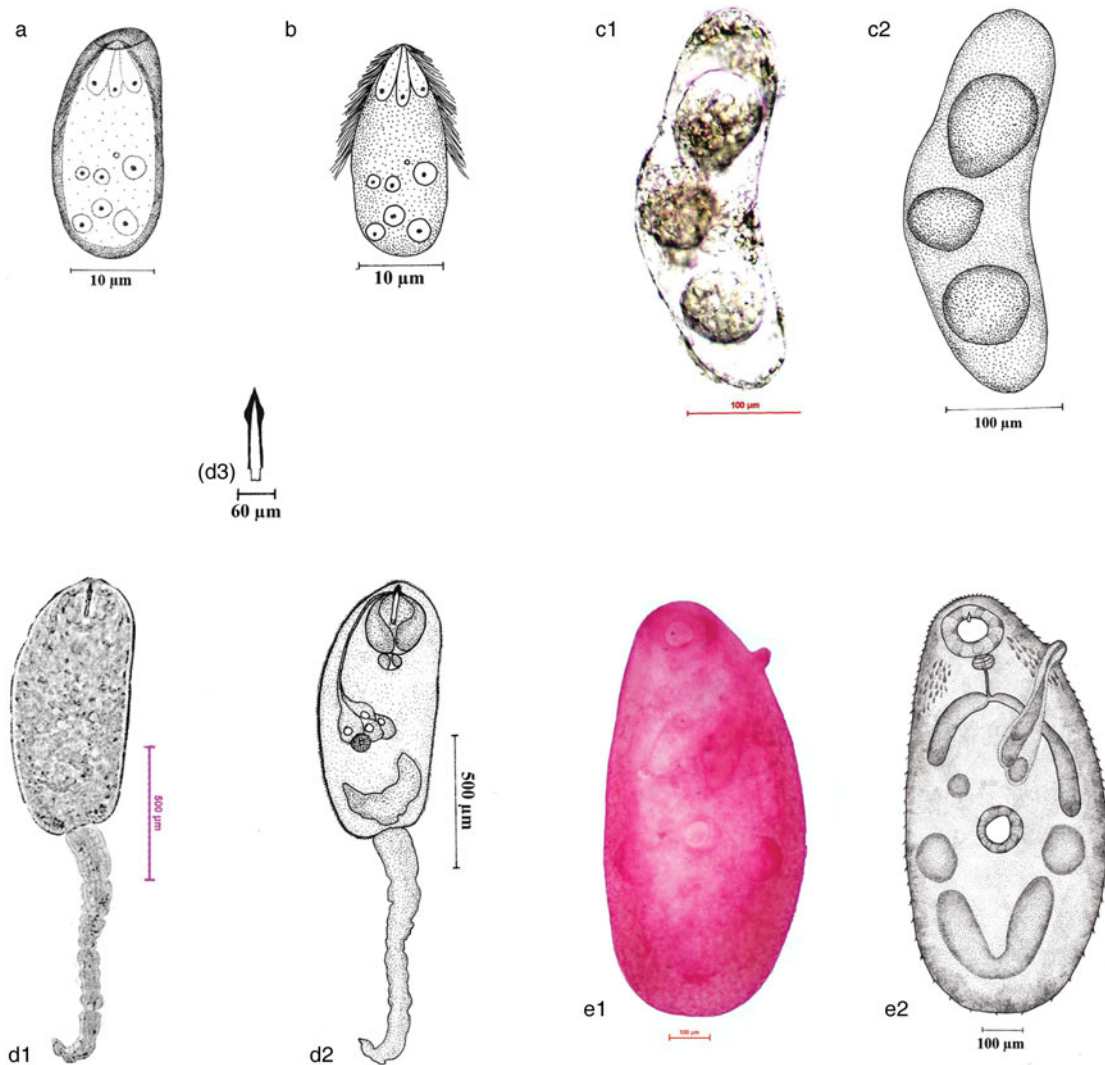


Fig. 1. Egg (a), miracidium (b), sporocyst (c1, c2), cercaria (d1, d2), stylet (d3) and 21-day-old excysted metacercariae (e1, e2) of *P. wayanadensis*.

Observations

Egg

Eggs (fig. 1a) ovoid (27.0×13.0), light yellow, operculate, numerous in numbers, contained fully developed miracidia. Eggs were directly collected from the adult. Attempts made to release miracidia were successful.

Miracidium

Miracidia (fig. 1b) are small, oval, with a pointed anterior portion. The body surface is covered with backwardly directed, moderately long cilia that reaches from the anterior portion to about one third of the body length. Penetration glands elliptical, uninucleated and placed one on either side of the median apical gland. Six moderate, uninucleated germinal cells occur in the posterior half of body. Miracidia measured 24.8×11.1 .

Sporocyst

First intermediate host. *Bithynia (Digoniostoma) pulchella*.

Site of infection. Hepatopancreas.

Accession number. Z-S/DP-42.

Body of sporocysts (fig. 1c1 and c2) white, ovoid, containing two to three germ balls; measured 357.5×116.8 . After eight to nine days of exposure to snails, miracidia developed into sporocysts. Sporocysts were recovered from the hepatopancreas of the five *B. (D.) pulchella* examined.

Cercaria

Natural infection with cercariae was observed in 22 of 348 *B. (D.) pulchella* (6.3%). Other species of snails – 246 *Indoplanorbis exustus* and 186 *Lymnaea luteola* – collected from the same geographic locations were refractory to infection with this cercaria. Under experimental conditions, cercariae emerged within 11–13 days exposure of eggs and/or miracidia to the snail. All the exposed snails were found infected. Cercariae were positively phototactic. They emerged throughout the daytime and the change of water acted as a cue for their emergence.

Description

Virgulate xiphidiocercariae (fig. 1d1 and d2); spinose, oval, body measured 941.9×453.5 . Ventrally attached aspinose tail has non-

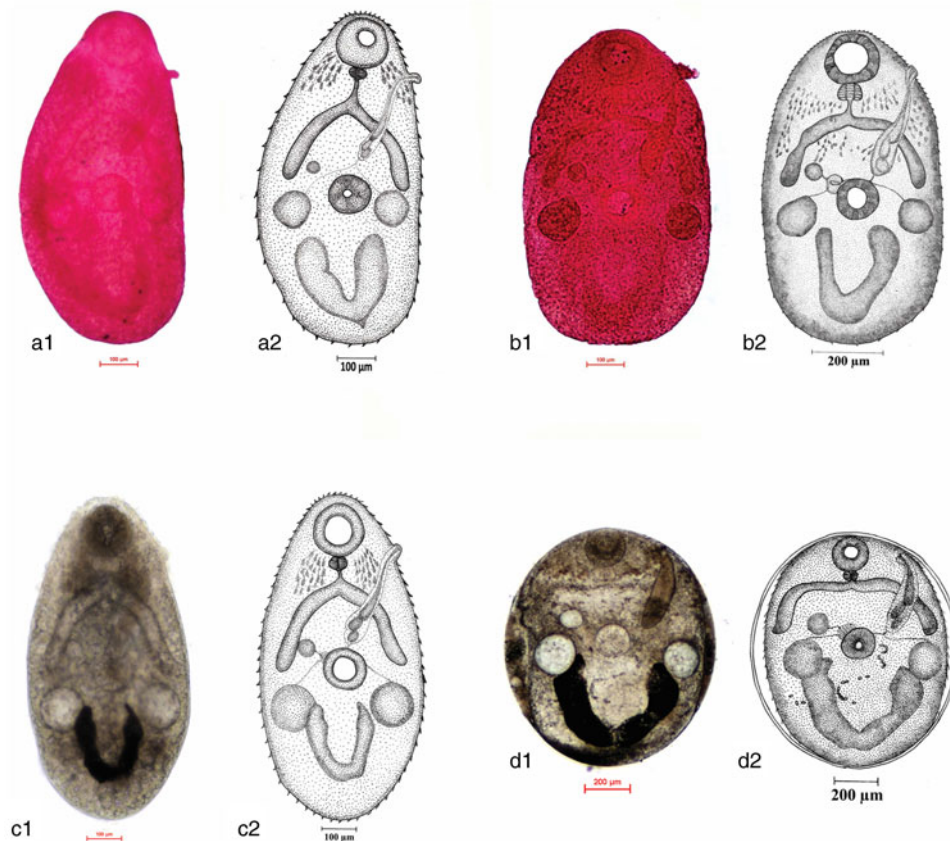


Fig. 2. Twenty-four-day-old excysted metacercaria (a1, a2), 27-day-old excysted metacercaria (b1, b2), 30-day-old excysted metacercaria (c1, c2) and 33-day-old encysted progenetic metacercaria (d1, d2) of *P. wayanadensis*.

uniform plications. Tail 976.8 in length and 139.5 in width. Oral sucker large, sub-terminal, 232.6×220.9 . Ventral sucker small, round, post-equatorial and 69.8×69.8 in diameter. Sharply pointed stylet (fig. 1d3) inserted into the dorsal wall of the oral sucker. Stylet, with thickening at the shoulders, measured 147.0×30.0 . Large, bi-lobed, inverted-comma-shaped virgula organ (290.7×93.0) occupied major portion of oral sucker.

Mouth sub-terminal. Pre-pharynx short followed by muscular pharynx, 58.1×93.0 . Four pairs of penetration glands located antero-lateral to ventral sucker, their ducts open at the base of stylet. Large, thin-walled, V-shaped excretory bladder at the posterior end measured 279.0×290.7 .

Metacercaria

Second intermediate host. Dragonfly naiads (*Orthetrum* sp.) and damselfly naiads (*Ischnura* sp. and *Copera* sp.).

Site of infection. Thorax and abdomen (*Orthetrum* sp.) and eyes (*Ischnura* sp. and *Copera* sp.).

Accession number. Z-N/O-12.

In natural infections, metacercariae were found in the eyes of the damselfly naiads of the *Ischnura* sp. and *Copera* sp. collected from the paddy fields from where the infected *B. (D.) pulchella* were collected. In natural conditions, the prevalence of infection was 56.2% (nine out of 16 were infected) in *Ischnura* sp. and 100% (three specimens collected were infected) in *Copera* sp. The metacercariae were also found in the thorax of the dragonfly naiads *Orthetrum* sp. collected from the same paddy field. In

natural conditions, the prevalence of infection was 33.3% (four out of 12 were infected) in *Orthetrum* sp. Experimental infection has been successfully established in 15 naiads of *Orthetrum* sp. exposed to cercariae. The encysted metacercariae developed in the thorax and abdomen. Nine cysts were obtained from a single naiad exposed to cercariae. On the 14th day post infection, encysted metacercariae were obtained from the thorax.

On the 21st day post infection, encysted metacercariae were recovered from the thoracic region of dragonfly naiads. Cysts round; developing cysts wall bi-layered and thin. Excysted metacercariae measured 1023.5×499.3 . Tegument thin and spinose. Backwardly directed spines visible more clearly on live specimens. Stylet could still be seen in the oral sucker. The cephalic glands with ducts as a bunch were found on either sides of oral sucker. Oral sucker sub-terminal measured 139.1×163.0 and ventral sucker 110.5×114.7 . Mouth sub-terminal followed by developing pharynx (32.6×38.5), oesophagus (86.3×11.6) and intestinal caeca (355.5×51.0). Testes primordia visible as two round bodies just below the ventral sucker; right primordial testis measured 117.8×123.5 and left 136.5×125.2 in size. Ovarian primordium (74.2×63.1 in size) located in between the right intestinal caecum and ventral sucker. A developing cirrus pouch visible, measured 375.2×94.2 . The V-shaped excretory vesicle filled with excretory concretions present at the posterior part of body (fig. 1e1 and e2).

On the 24th day post infection, excysted metacercaria measured 889.5×440.3 . The stylet disappeared, cephalic glands present. Cuticular spines more visible, prominent in fore body. Oral sucker 143.0×152.0 and ventral sucker 105.4×106.3 in size. Pharynx

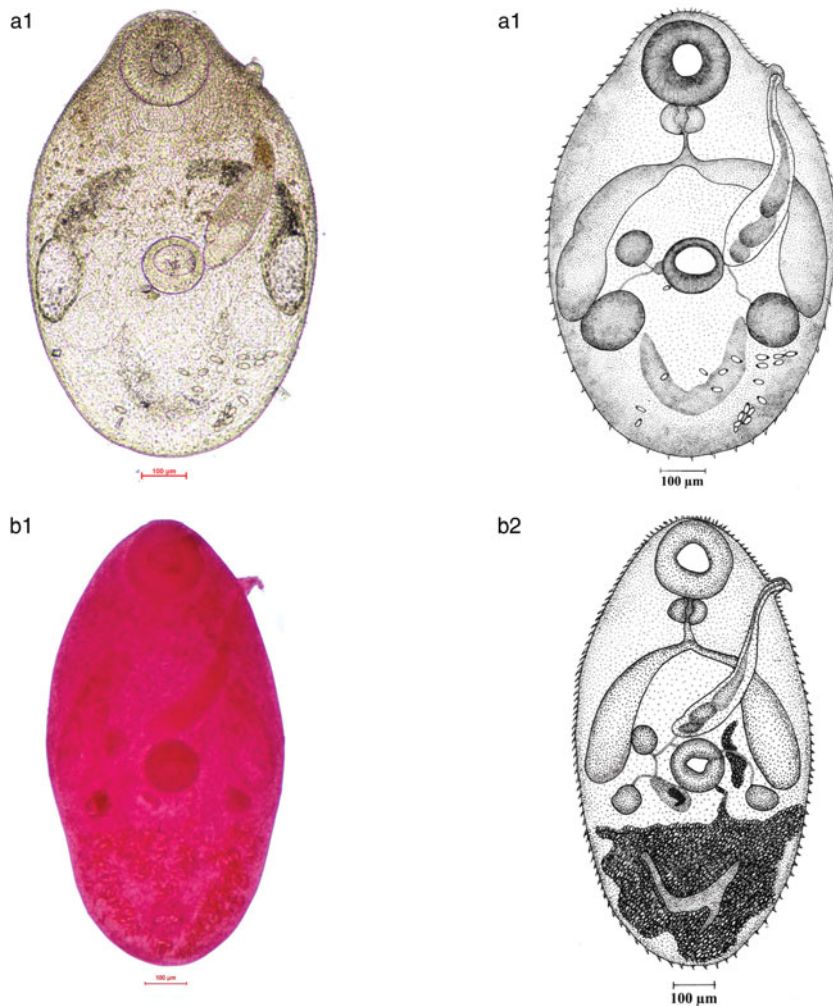


Fig. 3. Three-day-old adult (a1, a2) and 17-day-old adult (b1, b2) of *P. wayanadensis*.

measured 39.7×70.7 and oesophagus 42.3×29.5 in size. Intestinal caeca extend laterally a short distance anterior to developing testes (325.4×58.2). Size of the genital primordium reduced. Right testis measured 109.6×94.8 , left testis 107.6×103.6 and ovary 58.9×36.4 . Cirrus sac 261.3×66.1 in size (fig. 2a1 and a2).

On the 27th day post infection, bi-layered cyst walls fully developed; outer layer transparent and thick; the inner layer thick and opaque. The excysted metacercaria measured 851.3×507.3 . Oral sucker 139.7×156.9 and ventral sucker 115.3×121.6 in size. Pharynx globular, muscular, 39.4×62.6 and oesophagus 42.8×26.5 in size. Intestinal caeca measured 343.6×56.2 . Right testis 101.7×99.5 and left testis 116.7×118.5 and ovary measured 56.3×52.6 . Vasa efferentia, vas deferens, seminal vesicle and oviduct discernible. Uterine coils empty. Cirrus sac 326.6×68.7 in size (fig. 2b1 and b2).

On the 30th day post infection, metacercaria measured 934.6×469.5 . The cyst wall well developed. Cuticular spines more visible; prominent in fore body. Oral sucker 156.5×147.8 and ventral sucker 121.7×126.1 . Pharynx 39.1×52.2 . Oesophagus 21.7×8.7 followed by intestinal caeca 313.0×47.8 . Right testis measured 126.1×121.7 , left testis 134.8×130.4 and ovary 52.2×47.8 . Cirrus sac 326.0×65.2 in size (fig. 2c1 and c2).

On the 33rd day post infection, progenetic metacercaria recovered from the abdomen of the libellulid naiad. Cyst measured 1052.0×936.8 and metacercaria measured 1031.0×810.5 . Oral

sucker 157.9×168.4 and ventral sucker 136.8×157.9 in size. Pharynx 42.1×63.2 and intestinal caeca measured 463.1×73.7 . Reproductive structures well developed. Right testis 168.4×152.6 , left testis 178.9×176.8 and ovary measured 94.7×94.7 . Cirrus sac 384.2×93.7 in size. Eggs present in initial coils of uterus, lying posterior to ovary (fig. 2d1 and d2).

Adult

Definitive host. *Euphlyctis cyanophlyctis*.

Site of infection. Duodenum.

Accession number. Z-F/E-21.

In natural infection, adults of *P. wayanadensis* were found in the duodenum of the common water skittering frog, *E. cyanophlyctis*. The prevalence of natural infection was 11.1% (two out of 18 were infected) in *E. cyanophlyctis*. Experimental infection has been successfully established in the frogs (ten in number) exposed to encysted metacercariae. The adult trematodes were developed in the duodenum of all exposed frogs.

On the third day post infection, immature adults were obtained from the duodenum of the frog. Body measured 999.8×647.7 . Cuticular spines more prominent. Oral sucker 191.3×202.3 and ventral sucker 126.1×139.1 . Pharynx 65.2×99.9 and oesophagus 56.5×17.4 . Intestinal caeca measured 526.0×108.7 . Right testis 121.7×121.7 , left testis 136.9×147.8

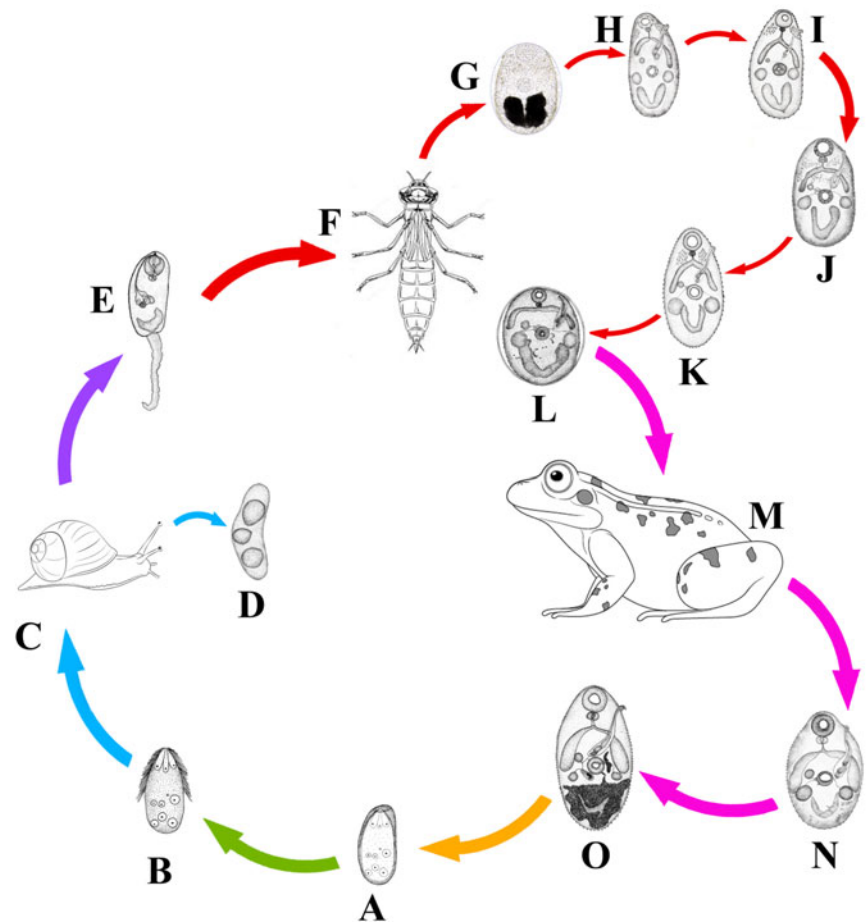


Fig. 4. Life cycle of *P. wayanadensis*. (A) egg; (B) miracidia; (C) snail host; (D) sporocyst; (E) cercaria; (F) naiad host; (G) 14-day-old encysted metacercaria; (H) 21-day-old excysted metacercaria; (I) 24-day-old excysted metacercaria; (J) 27-day-old excysted metacercaria; (K) 30-day-old excysted metacercaria; (L) 33-day-old encysted progenetic metacercaria; (M) frog host; (N) three-day-old adult; (O) 17-day-old adult.

and ovary measured 82.6×84.8 . Cirrus sac 465.1×99.9 in size. Eggs measured 30.4×10.7 (fig. 3a1 and a2).

On the 17th day post infection, mature adults of *P. wayanadensis* were recovered (fig. 3b1 and b2). Duodenum of *E. cyanophlyctis*, force-fed with 33-day-old metacercariae contained mature *P. wayanadensis*. The prepatent period was 14–19 days. Seventeen-day-old flukes measured 1071.4×571.4 . Mature flukes were identical to those recovered from field-collected *E. cyanophlyctis*.

Discussion

Experimental infection studies can provide crucial information when certain life cycle stages cannot be found in nature and different life cycle stages suspected of belonging to the same species are found in the same habitat (Blasco-Costa & Poulin, 2017). These studies can provide more proof, along with morphological matching of life cycle stages recovered from the naturally infected hosts. Thus, the experimental infection study is an important tool for life cycle resolution. Information on the life cycle studies of trematode parasites is scanty. This paper describes the life cycle of *P. wayanadensis* (fig. 4) from egg to egg-producing adult as elucidated during the course of the present study. *Pleurogenoides wayanadensis* has a three-host life cycle. The adult is found in the duodenum of *E. cyanophlyctis*. Eggs from the adult trematode released miracidia, which entered the snail *B. (D.) pulchella*, developed into sporocysts and then into cercariae. Sporocysts were found in the hepatopancreas

of the snail. The emerged cercariae were then encysted to form metacercariae in the eyes, thorax and abdomen of the naiads. When the definitive host, frog, was fed on the encysted metacercariae, the metacercariae developed into adult parasites. The life cycle is completed in about 58–65 days: the molluscan phase in 11–13 days, 33 days in the naiad host, where the metacercariae become infective, and a 14–19-days prepatent period in the definitive host (fig. 5).

The life cycle of *P. wayanadensis* is similar to that of *Pleurogenoides medians* (Mathias, 1924; Neuhaus, 1940; Buttner, 1951), *Pleurogenoides japonicus* (Okabe & Shibue, 1951; Shibue, 1953), *P. tener* (Macy, 1964), *Pleurogenoides orientalis* (Madhavi et al., 1987), *Pleurogenoides ovatus* (Janardanan & Prasad, 1991) and *Pleurogenoides malampuzhensis* (Brinsh & Janardanan, 2014); all the seven species have three host life cycles, and differ only with regard to the morphology/morphometry of larvae (table 1) and the invertebrate hosts (table 2).

The virgulate xiphidiocercaria of *P. wayanadensis* is comparable with the cercariae of *P. medians* (Mathias, 1924; Neuhaus, 1940; Buttner, 1951), *P. tener* (Macy, 1964), *P. orientalis* (Madhavi et al., 1987), *P. japonicus* (Okabe & Shibue, 1951; Shibue, 1953), *P. ovatus* (Janardanan & Prasad, 1991) and *P. malampuzhensis* (Brinsh & Janardanan, 2014). The present cercaria is distinguishable from other cercariae by the shape and structure of stylet, virgula organ and excretory bladder, and size of ventral sucker, number of penetration glands and morphometry. The cercariae of *P. wayanadensis* were positively

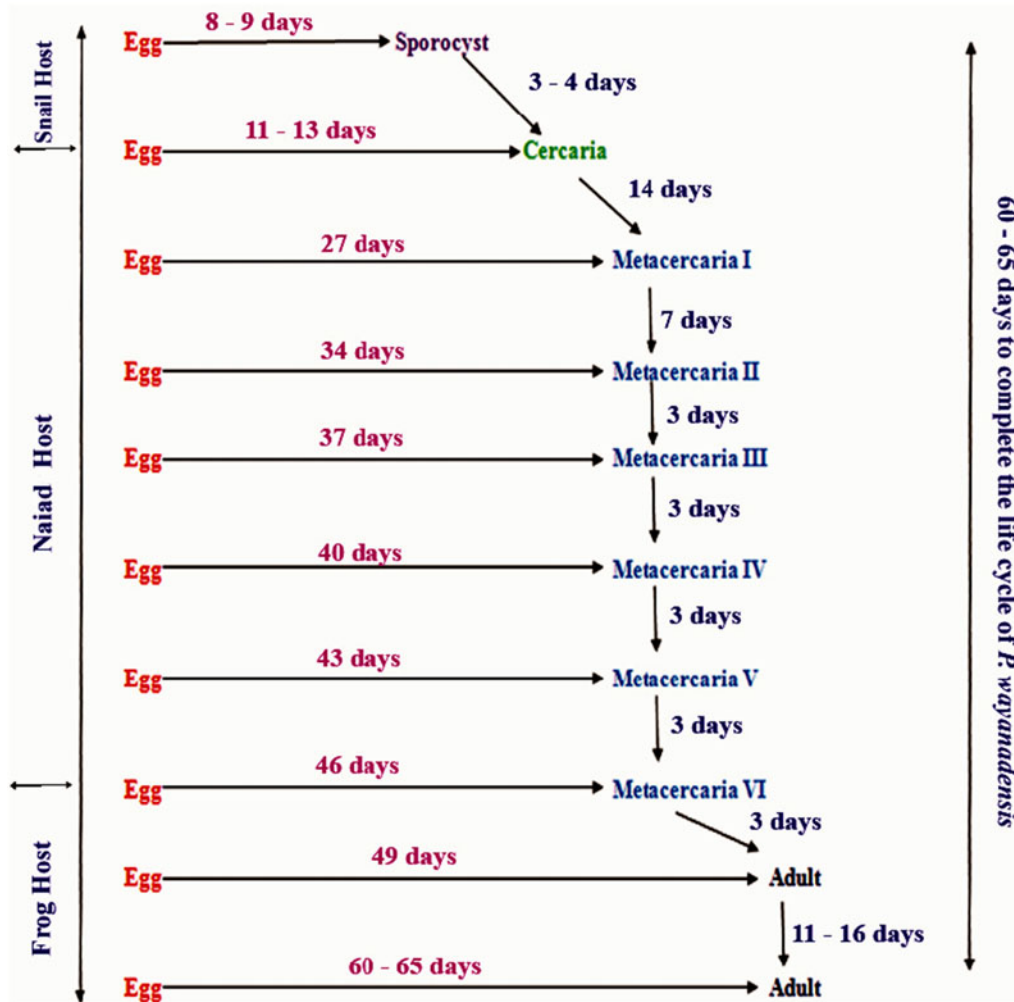


Fig. 5. Milestones in the life cycle of *P. wayanadensis*.

phototactic. They emerge throughout the day and the change of water acts as a cue for their emergence.

Habitat of the bithynid snail *B. (D.) pulchella* varies from permanent or temporary freshwater bodies, marshes, swamps and shallow ponds. They are mostly found in mud, on rocks or attached to grass or other aquatic vegetation. For the present study, the snails were collected from the paddy fields with loose soil and muddy substratum. The dragonfly naiads and the frogs were also seen on the same habitat where the snails existed. The cercaria larvae can easily get access to the dragonfly/damselfly naiads, the second intermediate host. To complete the life cycle successfully, nearly all digenetic trematodes choose a confined habitat/ecological niche where all the hosts are available.

The metacercaria of *P. wayanadensis* exhibited progenetic development. Progenesis is the phenomenon of sexual maturation in an organism still in a morphologically juvenile stage (Gould, 1977). Among parasites, precocious egg production has been known since the report by Von Siebold (1835). This phenomenon was reported in trematodes for the first time by Dollfus (1924) with his observations on egg production by the metacercariae of *P. medians* (Pleurogenidae). Facultative truncation of life cycle is only shown by some individuals within a species. *Pleurogenoides wayanadensis* exhibits facultative progenesis at

the metacercarial stage with immature eggs in their initial coils of uterus. Normal and progenetic metacercariae were observed together in the thorax and abdomen of the dragonfly naiads. Progenesis has survival value and is adaptive (Janardanan & Prasad, 1991). Facultative progenesis is an option for metacercariae in second intermediate hosts, and it will be selected in any unstable or temporary habitat in which intermediate hosts become stranded in marginal pools where no definitive hosts are available for the completion of the life cycle of those trematodes (Poulin & Cribb, 2002). Metacercariae of *P. medians*, *P. japonicus*, *Pleurogenoides sitapurii* and *P. ovatus* are the other species under the genus *Pleurogenoides* that exhibit progenesis and undergo considerable growth and development inside the second intermediate hosts. The progenetic development in the metacercariae of *P. medians* encysted in the dragonfly naiads was reported by Buttner (1951). The metacercaria of *P. japonicus* was reported from shrimps (Okabe & Shibue, 1951) and that of *P. sitapurii* in the crab, *Paratelphusa ceylonensis* (Dissanaike & Fernando, 1960). Metacercariae of *P. ovatus* were recovered from the connective tissues, hepatopancreas and musculature of the freshwater crab, *Paratelphusa hydrodromous* (Janardanan & Prasad, 1991).

The relict hypothesis (Stunkard, 1959; Riggs & Ulmer, 1983) explains progenesis of the metacercariae as a reminiscence of an

Table 1. Measurements of cercaria, metacercaria and adult of *P. wayanadensis*.

Characters	Life cycle stages							
	Cercaria	21-day-old metacercaria	24-day-old metacercaria	27-day-old metacercaria	30-day-old metacercaria	33-day-old metacercaria	Three-day-old adult	17-day-old adult
Body LxW	941.9 × 453.5	1023.5 × 499.3	889.5 × 440.3	851.3 × 507.3	934.6 × 469.5	1031.0 × 810.5 (encysted 1052.0 × 936.8)	999.8 × 647.7	1071.4 × 571.4
Tail LxW	976.8 × 139.5	-	-	-	-	-	-	-
Body spines	Present	Present	Present	Present	Present	Present	Present	Present
Oral Sucker LxW	232.6 × 220.9	139.1 × 163.0	144.0 × 152.0	139.7 × 156.9	156.5 × 147.8	157.9 × 168.4	191.3 × 202.3	185.0 × 185.0
Ventral Sucker LxW	69.8 × 69.8	110.5 × 114.7	105.4 × 106.3	115.3 × 121.6	121.7 × 126.1	136.8 × 157.9	126.1 × 139.1	125.0 × 125.0
Stylet LxW	147.0 × 30.0	26.1 × 8.7	-	-	-	-	-	-
Virgula LxW	290.7 × 93.0	-	-	-	-	-	-	-
Pharynx LxW	58.1 × 93.0	32.6 × 38.5	39.7 × 70.7	39.4 × 62.6	39.1 × 52.2	42.1 × 63.2	65.2 × 99.9	60.0 × 115.1
Oesophagus LxW	-	86.3 × 11.6	42.3 × 29.5	42.8 × 26.5	21.7 × 8.7	-	56.5 × 17.4	45.0 × 22.5
Caeca LxW	-	355.5 × 51.0	325.4 × 58.2	343.6 × 56.2	313.0 × 47.8	463.1 × 73.7	526.0 × 108.7	463.3 × 105.8
Anterior testes LxW	-	117.8 × 123.5	109.6 × 94.8	101.7 × 99.5	126.1 × 121.7	168.4 × 152.6	121.7 × 121.7	65.0 × 77.5
Posterior testes LxW	-	136.5 × 125.2	107.6 × 103.6	116.7 × 118.5	134.8 × 130.4	178.9 × 176.8	136.9 × 147.8	70.0 × 72.5
Ovary LxW	-	74.2 × 63.1	58.9 × 36.4	56.3 × 52.6	52.2 × 47.8	94.7 × 94.7	82.6 × 84.8	70.0 × 60.0
Cirrus sac LxW	-	375.2 × 94.2	261.3 × 66.1	326.6 × 68.7	326.0 × 65.2	384.2 × 93.7	465.1 × 99.9	440.3 × 85.9
Excretory organ	V-shaped	V-shaped	V-shaped	V-shaped	V-shaped	V-shaped	V-shaped	V-shaped

Table 2. Summary of hosts in the life cycle of the seven species of *Pleurogenoides*.

	Snail hosts (first intermediate host)	Second intermediate hosts	Definitive hosts	References
<i>P. medians</i>	<i>Lymnaea limosa</i>	Dragonfly naiads (<i>Aeshna</i> and <i>Libellula</i>)	Frogs (<i>Rana nigromaculata</i>)	Mathias (1924), Buttner (1951)
<i>P. japonicus</i>	<i>Bulimus kiushuensis</i>	Shrimps (<i>Neocaridina denticulata</i>)	Frogs (<i>Rana nigromaculata</i>)	Okabe & Shibue (1951), Shibue (1953)
<i>P. tener</i>	<i>Bithynia subdiella</i>	Dragonfly naiads (<i>Trithemis annulata</i> , <i>Crocothemis erythraea</i> , <i>Anax imperator</i> and <i>A. Parthenope</i>)	Lizards (<i>Chalcides ocellatus</i>) Frogs (<i>Rana mascereniensis</i>)	Macy (1964)
<i>P. orientalis</i>	<i>Alocinma travancorica</i>	Dragonfly naiads (<i>Tholymis tillarga</i> and <i>Tremea limbata</i>)	Frogs (<i>E. cyanophlyctis</i>)	Madhavi et al. (1987)
<i>P. ovatus</i>	<i>B. (D.) pulchella</i>	Crabs (<i>Paratelphusa hydrodromous</i>)	Frogs (<i>H. tigerinus</i>)	Janardanan & Prasadani (1991)
<i>P. malampuzhensis</i>	<i>B. (D.) pulchella</i>	Dragonfly naiads (<i>Gomphidae</i> sp.)	Frogs (<i>H. tigerinus</i>)	Brinesh & Janardanan (2014)
<i>P. wayanadensis</i>	<i>B. (D.) pulchella</i>	Dragonfly naiads (<i>Orthetrum</i> sp.) and Damselfly naiads (<i>Ischnura</i> sp. and <i>Copera</i> sp.)	Frogs (<i>E. cyanophlyctis</i>)	Present paper

ancestral two-host cycle, whereas, according to the novelty hypothesis, progenetic species we observe today are secondarily derived from an ancestral three-host life cycle.

In the earlier studies, phylogenetic reconstructions revealed that trematodes have a primitive cycle involving a mollusc as the first host and a predatory vertebrate as the definitive host. According to these studies, trematodes, after a very long time, had adjusted their developmental schedule so as to incorporate a trophic link, as a second intermediate host, to increase the chances of transmission toward the vertebrate host (Rohde, 1994; Ewald, 1995; Cribb et al., 2003).

Investigations following analysis of character convergence strongly support the idea that the two-host life cycles are derived from more ancient three-host life cycles (Carney & Brooks, 1991; Smythe & Font, 2001). Progenesis must, therefore, be considered as a novelty in the evolution of trematode life cycles. It can be interpreted that a simpler life cycle is easier to complete and the phenomenon of progenesis is to reduce the transmission events. Earlier studies (Buttner, 1951; Grabda-Kazubaska, 1976; Font, 1980; Poulin & Cribb, 2002) revealed that a large number of metacercariae perish with their intermediate hosts in normal three-host life cycles. Therefore, this may be a strategy where all metacercariae become potential breeders if they adopt progenetic development.

In the present study, the metacercariae were found in the eyes of the field-collected damselfly naiads *Ischnura* sp. and *Copera* sp., and in the thorax of the dragonfly naiads *Orthetrum* sp. In the experimental studies, dragonfly naiads of *Orthetrum* sp. were infected with the metacercariae of *P. wayanadensis*. The *P. medians* (Mathias, 1924; Neuhaus, 1940; Buttner, 1951), *P. tener* (Macy, 1964), *P. orientalis* (Madhavi et al., 1987) and *P. malampuzhensis* (Brinesh & Janardanan, 2014) are the other species under the genus *Pleurogenoides* that used odonate naiads as second intermediate hosts. The present metacercaria is distinct from the metacercariae of other *Pleurogenoides* species in its morphological features and dimensions.

Here, the eggs produced by the adult *P. wayanadensis* get deposited in the faecal matter of the definitive host frog, *E. cyanophlyctis*, and released miracidia larvae in the surrounding water. The miracidia enters the snail host, *B. (D.) pulchella*,

where it develops into sporocyst and cercaria. The cercaria larva becomes metacercaria in the damselfly naiads of the *Ischnura* sp. and *Copera* sp. and dragonfly naiads of the *Orthetrum* sp. that exist in the same ecological niche. Through trophic-level transfer, the metacercaria reaches the definitive host frog when the latter feeds on the infected damselfly and dragonfly naiad, develops into an adult parasite and completes the life cycle.

In this study, natural infection of cercariae was found only in *B. (D.) pulchella*, and all the other snail species of the area were negative to this cercarial infection. The metacercariae were found infected in both the dragonfly and damselfly naiads. Adult flukes were found in the duodenum of the frog species *E. cyanophlyctis* and *H. tigerinus*, and all other frog species of the area were negative to this infection.

Acknowledgements. The authors are grateful to the Kerala State Council for Science, Technology and Environment (KSCSTE), Government of Kerala, for providing financial assistance (Major Research Project, SRS/220/2015/KSCSTE-completed) to carry out this study. The author C.S. is grateful to the Department of Science and Technology (DST) INSPIRE program for providing the Inspire Fellowship. The permission accorded by the Department of Forest and Wildlife, Government of Kerala, order numbers WL10-63909/2016 and KFDHQ-6782/2019-CWW/WL10 for collecting frogs and naiads, respectively, from the Wayanad forest region is also gratefully acknowledged. The authors are indebted to Prof K.P. Janardanan (retired), renowned parasitologist, for critically reviewing the manuscript.

Financial support. This study was partially supported (major research project, number SRS/220/2015/KSCSTE) by the Kerala State Council for Science, Technology and Environment (KSCSTE), Government of Kerala (the project was completed in 2019).

Conflicts of interest. None.

Ethical standards. 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.

Author contributions. P.K.P. designed the experiments and guided the study. His research scholars, K.S. (working on the trematode parasites of frogs), C.S. (conducting research on the metacercaria larvae of the Western Ghats) and

K.A. (conducting research on the cercarial fauna of Wayanad Region of the Western Ghats) performed the experiments and studied the trematodes in detail. All authors contributed equally in the manuscript writing and analysis of the data.

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