Advances in life science research

Life-cycle stages of *Pleurogenoides cyanophlycti* Shinad and Prasadan, 2017 (Digenea: Pleurogenidae) from the Western Ghats, India

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ABSTRACT

During our studies on the digenean parasites of the Western Ghats, Wayanad region, to establish the trophic level transfer, the life-cycle stages, metacercaria and adult of *Pleurogenoides cyanophlycti* Shinad and Prasadan, 2017 were recovered and studied. The specimens were collected from different paddy fields and freshwater bodies of the Western Ghats, Wayanad region, Kerala. Two hundred and twenty six individuals of *E. cyanophlyctis* (Schneider, 1799), 50 of *I. aurantiaca* (Boulenger, 1904), 113 of *M. keralensis* (Dubois, 1980) and 28 of *Copera sp.* collected from tbe paddy fields and the nearby streams of the Western Ghats, Wayanad region from January 2016 to November 2020 were brought to the Laboratory. The frogs and damsel fly naiads were dissected and examined for trematodes in detail. The recovered parasites were studied using Nikon ECLIPSE Ni-U phase contrast research microscope. Trematodes were fixed and stained following the standard procedure. Photographs were taken with a Nikon Y-TV55 camera. The prevalence, the mean intensity of infection and the mean abundance were measured. The metacercariae were recovered from the damselfly naiads, *Copera sp.* Two individuals of *Copera sp.* were positive to this meta- cercaria. Prevalence of infection was 7.14% (two out of 28 Copera sp. were infected), mean intensity was 33 (66 *P. cyanophlycti* were recovered from two infected Copera sp.) and the mean abundance of infection was 2.35 (66 *P cyanophlycti* recovered from 28 *Copera sp.).* The adults were recovered from the frogs, *Euphlyctis cyanophlyctis, lndosylvirana aurantiaca and Minervarya keralensis.* Seventeen *E. cyanophlyctis,* one *I. aurantiaca* and two *M. keralensis* were positive to adult infection. The prevalence of natural infections were 7.52% (17 of 226 *E. cyanophlyctis* were infected) in *E. cyanophlyctis;* 2 % (One of 50 *I. aurantiaca* was infected) in *I aurantiaca* and l. 76 % (two of 113 *M.keralensis* were infected) in *M. keralensis.* The mean intensity of infection were 5.35 (91 *P cyanophlycti* recovered from 17 infected *E. cyanophlyctis)* in *E. cyanophlyctis;* three (three *P. cyanophlycti* from one infected *I. aurantiaca)* in *I. aurantiaca* and one(two *P. cyanophlycti* from two infected *M. keralensis)* in *M. keralensis.* The mean abundance of infections were 0.40 (91 *P. cyanophlycti* recovered from 226 *E. cyanophlyctis)* in *E. cyanophlyctis;* 0.06 (three *P. cyanophlycti* from 50 *I. aurantiaca)* in *I. aurantiaca* and 0.01(two *P.cyanophlycti* from 113 *M. keralensis)* in *M. keralensis.* Information on the life-cycle studies of trematode parasites is scanty. In Pleurogenoide, majority of species chooses odonate naiads and frogs as their hosts. Both the odonate naiads and frogs exist in the same ecological niche. Through trophic level transfer, the metacercaria reaches the definitive host frog when the frog feeds on the infected odonate naiad and develops into adult. In-depth ecological Parasitology studies are needed to elucidate the complete life cycle and to establi.sh the same in the laboratory.

KEYWORDS
Pleurogenoides cyanophlycti, Euphlyctis cyanophlyctis, Indosylvirana aurantiaca, Minervarya keralensis, Copera sp., life-cycle stages

lndroduction

To understand the ecology and evolution of parasites, the full characterization of their developing stages is essential. Biodiversity of parasites is being discovered at a high rate but very little efforts are being taken by researchers to elucidate their life-cycle stages. Here the morphology of metacercaria and adult of *P. cyanophlycti* obtained from their natural hosts. 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).

The genus Pleurogenoides of the family Pleurogenidae Looss, 1899 was established by Travassos (1921) to accommodate the type-species *P. tener* described by Looss, 1898 as *Prosotocus tener*. The parasites usually infect the intestine of amphibians and reptiles and

occasionally of fishes.

The genus comprises species described from frogs worldwide. 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. Ou the basis of the length of intestinal caeca and position of genital pore, Mehra and Negi (1928) dropped the genus Pleurogenoides into nvo sub genera, *P (pleurogenes)* and *P. (telogonella).* Srivastava (1934) also dropped the genus Pleurogenoides and transferred the species under it to Pleurogenes. Macy (1936) retained the genus Pleurogenoides and the same was accepted by Kaw (1943) and Mukherjee and Ghosh (1970). Of the 26 species of Pleurogenoides recorded worldwide from amphibians (Brinesh & Janardanan, 2014), so far, nine species were reported from India, *P gastroporus* (Lube, 190l) Travassos, 1921; *P. sphaericus* (Klein, 1905) Travassos, 1921; *P. sitapurii* Srivastava, 1934; *P. orientalis* Srivastava, 1934; *P bufonis* Kaw, 1943; *P ovatus* Rao, 1977; *P.jamshedpurensis* Hasnain and Sahay, 1987; *P. ranchiensis* Dan and Hasnain, 1991 and *P. malampuzhensis* Brinesh and Janardanan, 2014.

Recently three new species of Pleurogenoides, *P cyanophlycti, P. euphlycti* and *P. wayanadensis* were described by Shinad and Prasadan (2017, 2018a) from *E. cyanophlyctis* and *H. tigerinus* of the Wayanad region of the Western Ghats, Kerala, India. Shinad and Prasadan (201Sb) recorded the prevalence, the intensity of infection and the mean abundance of *P. cyanophlycti, P. euphlycti, P. wayanadensis* and *P orientalis* from *E. cyanophlyctis.* Shinad and Prasadan (2019a) reported the prevalence, the intensity of infection and the mean abundance of *P euphlycti* and *P wayanadensis* from *H. tigerinus. Also* documented that of *P. cyanophlycti* from *Minervarya sp. (Fejervarya sp.)* (2019b). The phylogenetic position of *P cyanophlycti* and *P euphlycti* from the frog, *E. cyanophlycti* was described by Chaudhary et al. (2020) through molecular studies.

Madhavi et al. (1987) studied the life history of *P orientalis* (Srivastava, 1934) and considered *P. sawanensis* and *P. gastroporus* as synonyms of *P. orientalis.* Life-cycles of seven species of Pleurogenoides have been studied and established from different parts of the world: *P medians* (Mathias, 1924; Neuhaus, 1940; Buttner, 1951), *P. japonicus* (Okabe & Shibue, 1951; Shibue, 1953), *P. tener* (Macy, 1964), *P. orientalis* (Madhavi et al., 1987), *P ovatus* (Janardanan & Prasadan, 1991), *P malampuzhensis* (Brinesh & Janardanan, 2014) and *P. wayanadensis* (Prasadan et al., 2020b). Life cycles of all the seven species followed three-host pattern, differ *only* on the invertebrate hosts and the morphology/morphometry of developing stages (Prasadan et al., 2020b). Madhavi and Ratnakumari (1989) studied the growth and development of *P. orientalis* in the intermediate and definitive hosts. Reports are available on metacercariae from crabs, imago and naiads of dragonflies & 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). Of the life-cycle studies, that of *P ovatus, P malampuzhensis* and *P wayanadensis* were elucidated from Kerala (Janardanan & Prasadan, 1991; Brinesh & Janardanan, 2014; Prasadan et al., 2020b). The present paper describes the morphology of the metacercaria and adult of *P. cyanophlycti* obtained from natural infection from the definitive and intermediate hosts.

Materials and Methods

Study area

The study was carried out and the specimens were collected from different paddy fields and freshwater bodiesof the Wayanad region of the Western Ghats, Kerala. The Western Ghats is considered one of the "hottest hotspots of biodiversity in the world due to its very rich biodiversity and high endemism".

Collection and examination of frogs and damselfly naiads

Two hundred and twenty six individuals of *E. cyanophlyctis* (Schneider, 1799), 50 of *I. aurantiaca* (Boulenger, 1904), 113 of *M. keralensis* (Dubois, 1980) and 28 of *Copera sp.* collected from the paddy fields and the nearby streams of the Western Ghats, Wayanad region from January 2016 to November 2020, were brought to the laboratory and examined for digenetic trematodes. After narcotisation with chloroform, the frogs were dissected and the intestines were transferred to physiological saline, for the detection of trematode parasites, under a stereo zoom microscope (binocular LABOMED Luxeo 4Z). The collected damselfly naiads of the genus Copera 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.

Studies on developing stages of the trematode

The recovered *P. cyanophlycti* (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 cyanophlycti* were examined under the Nikon phase contrast research microscope (ECLIPSE Ni-U) 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 acetocarrnine, following the procedure outlined by Cantwell (1981). The prevalence, the mean intensity of infection and the mean abundance were measured following Bush et al. (1997).

Measurements, sketches and photographs

Nikon Y-TV55 camera attached to the Nikon ECLIPSE Ni-U phase contrast research microscope was used to take photographs. The trematodes were measured using the Nikon NIS Elements

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 10 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.

Results and Discussions

Metacercaria

Second intermediate host: Damselfly naiad (Copera sp.)

Site of infection: Thorax and abdomen

In natural infections the metacercariae were found in the thorax and abdomen of the damselfly naiads of the species *Copera sp.* collected from the paddy fields of Moolithode on September 2018 and September 2019. Two individuals of *Copera sp.* were positive for this metacercaria. ln natural conditions the prevalence of infection was 7.14% (two out of 28 *Copera sp.* were infected), the mean intensity of infection was 33 {66 *P cyanophlycti* were recovered from two infected *Copera sp.)* and the mean abundance of infection was 2.35 (66 P. cyanopblycti recovered from 28 *Copera sp.)* (Table 2).

Description (Figure l; Table 1)

Bi-layered cyst. Transparent and thin outer layer. Inner layer thick and opaque. Metacercarial body oval and spinose, 433.5 - 561.0 (507.1) long and 222.1 - 335.2 (281.6) wide. Oral sucker sub-terminal, 81.3 - 104.4 (94.5) x 97.7 - 123.7 (109.9) in size and larger than ventral sucker. Ventral sucker circular 54.1- 72.0 (62. 4) x 55.9 - 76.2 (66.0) and equatorially placed. Pharynx oval, 18.8 - 27.2 (23.7) x 38.7 - 54.1 (44.2) in size. Short oesophagus. Broad intestinal caeca. Right caecum measures 155.0 - 254.6 (210.6) x 29.3-58.37 (40.4) and left caecum 160.5-245.4 (211.7) x 27.3-60.6 {41.0), extend laterally a short distance posterior to developing testes. Cirrus sac claviform, 120.6 - 154.3 (139.0) x 18.9 - 34.9 (24.2). Ovary slightly oval, 23.8 - 29.3 (26.5) x 22.6 - 34.9 (28.8), rudimentary, pre-equatorial and placed near the right testis. Developing testes are slightly ovoid, at the right and left sides of ventral sucker. Right testis 35.l - 59.2 (47.2) x 32.0- 50.7 (41.4) and left testis 39.0- 57.3 (48.1) x 30.7 -38.61 (34.7). Excretory bladder, 113.4-201.4 (164.5) x 166.7 -231.3 (195.3) in size, with a sub-terminal excretory pore filled with excretory concretions occupied at the posterior part of body.

Adult

Definitive hosts: *E. cyanophlyctis,I. aurantiaca* and *M. kera/ensis*

Site of infection: Duodenum

Natural infections with the adults of the present fluke were found in the duodenum of three species of frogs; *E. cyanophlyctis* collected from the Chundel, Karakkuni, Makkitnala, Panamaram, Payyampally (Koodalkadavu), Peechangode, Pulpally and Valliyoorkavu (Kammana) during January 2016 to September 2018; I. aurantiaca collected from Makkimala on January 2016 and 2018; I. aurantiaca collected from Makkimala on January 2016 and *M. keralensis* collected from Peechangode on February 2016. Seventeen *E. cyanophlyctis,* one *I. aurantiaca* and two *M. keralensis* were positive to this adult trematode.

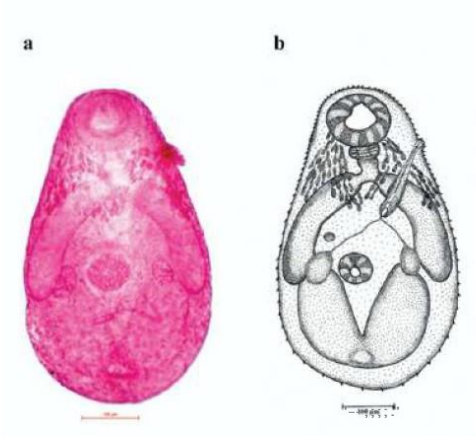

Figure 1 *Pleurogenoides cyanoplilycti* Shinad and Prasadan, 2017 Metacercaria (a and b)

Description (Figure 2; Table 1)

Description is based on the holotype and 95 paratypes. Body oval, 797.7 - 1410.4 (I 040.1) long, and 368.2- 767.0 (587.5) wide, with blunt anterior and broadly round posterior ends; body covered with minute, backwardly directed spines which are closely set at the anterior region but widely spaced at the posterior. Oral sucker sub terminal, almost round, 153.4- 230.1 (185.6) x 153.4-214.8 (187. 1) in diameter, larger than ventral sucker. Ventral sucker spherical, equatorial and 92.0 - 168.7 (133.5) x 92.0 - 168.7 (135.0) in diameter. Mouth sub terminal; pharynx muscular, ovoid (46.0- 65. 4 (53.7) x 61.4- 138.1 (96.6) in size). Oesophagus short, bifurcates anterior to ventral sucker into short, blunt, laterally inclined, unequal intestinal caeca; right caecum longer, 230.l - 836.2 (602. 2) in length and 30.7 - 138.1 (61.4) in width; left caecum 230.1 - 690.2 (460.2) x 46.0 - 88.7 (76.6) ending slightly behind testes.

Testes two, spherical or oval, equatorial or post-equatorial, symmetrical, one on either side of the ventral sucker in the lateral body margin; right testis 92.0 - 224.8 (130.4) x 61.4 - 168.7 (107.4) and left testis 76.7 - 214.8 (125.8) x 61.4 - 122.7 (96.6). Cirrus sac claviform (260.8 - 521.6 (406.5) x 61.4 - 184.1 (121.2) in size), extends from the anterior margin of ventral sucker to the 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 spinosed cirrus. Ovary spherical, intercaecal, located anterior to right testis and measured 61.4 - 276.1 (I 16. 6)x61.4-276.l (105.8) in diameter. Uterus post-acetabular and winding mostly in the hind body. Eggs ovoid, operculate and measured 15.3 - 30.7 (26.1) x 7.7 - 15.3 (11.5). Vitellaria 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 the pharynx, but not meeting together.

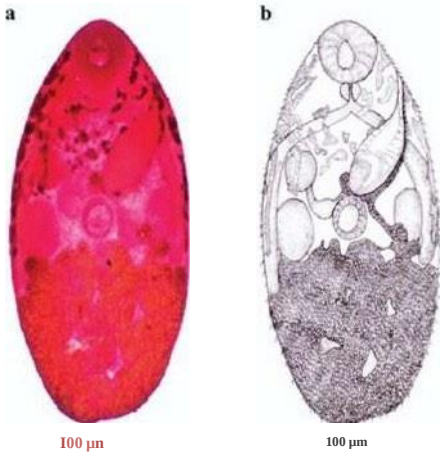

Figure 2 ***Pleurogenoides cyanophlycti*** **Sbinad and Prasadan, 2017 Mature adult {a and b)**

Mature adult (a and b) Imaging softw2018; *l. aurantiaca* collected from Makkimala on January 2016 and *M. keralensis* collected from Peechangode on February 2016. Seventeen *E. cyanophlyctis,*one *I. aurantiaca* and two *M. keralensis* were positive to this adult trematode.

Table I Measurements of metacercaria and adultof ***P. cyanophlycti***

Characters	Life-cycle stages	
	Metacercaria	Adult
Body LxW	433.5-561.0 x222.I-335.2 (507.1X 281.6)	7'.17.7 - 14I0.4 X 368.2 - 767.0 (1040.1 *X* 587-1)
OralSucker LxV	81.3- 104.4 X 97.7-123.7 (94.5, 109.9)	153.4 -230.1X 153.4-214.8 (185.6 X 187.1)
VentraJ Sucker LxW	54.1- 72.0 X)5.9-76.2 (62.4, 66 0)	92.0-16$.7 X 92,Q- 168.7 (133.5 X 135.0)
Pharynx LxW	I8.8- 27.2 x38.7- 54,I (23.7, 44.2)	46.0-6S4 x 61.4- 138.1 (53.7 X 96.6)
Anltrior Testis LxW	35.1-59.2 X 32.0-50.7 (47.2 *X* 41.4)	92.0-224.8x6l.4- 168.7(130.4x 107.4)
Posterior Testis LxW	39.0-57.J X 30.7-38.61 (48.1 X .l4.7).	76.7-214.8 X 6L4- 122.7 (125.8 X 96.6)
Ovary LxW	23.8- 29.3 X 22,6-34.9 (26.5 , 28.8)	61.4-276.1x 61.4-276.1 (II6.6 x 105.8)
Ci„·usSar LxW	120.6 - I54J X 18.9 - 34.9 [139.QX 24.2).	260.8-521.6x6L4-184.I (406.5 X 121.2)
Excretory Organ	113.4-201.4, 166.7-231.3 (164 5 X I95.3)	Nil
Egg	Nil	J53-307x7.7-15.3(26.l x 11.5)
Caeca LxW	160.5- 245.4 , 27.3- 6-06 (21Ux 41.0)	230.I -836.2x 30.7- 138.1(602.2 X 61.4)

Table 2 Prevalence of infection, mean intensity of infection and mean abundance of ***P. cyanophlycti***

Hosts	u,ubcr orhosu Examine-d	Infect('d	Thenu1t1bcrof *P.9·tn1ophb•cti* obtained	Ptevale1k':e (%)	Mean intensity	Menn Abundnnce
Copera sp.	28	2	66	7.10	33	2.35
E.cyanopMyclis	226	17	91	7.52	5.35	0.40
1.auram/oca	50	I	3	2	3	0.06
t.1. keralentis	113	2	2	1.76	I	0.01

The prevalence of natural infection were 7.1% (two out of 28 *Copera sp* were infected) 7.52% (17 of 226 *E. cyanophlyctis* were infected) in *E. cyanophlyctis;* 2 % (One of 50 *I. aurantiaca* was infected) in *I. aurantiaca* and 1.76 % (two of 113 *M. keralensis* were infected) in *M. keralensis.* The mean intensity of infection were 33 (66 metacercariae from two infected *Copera sp.)* 5.35 (91 adults recovered from 17 infected *E. cyanophlyctis)* in *E. cyanophlyctis;* three (three adults from one infected *I. aurantiaca)* in *I. aurantiaca* and one(two adults from two infected *M. keralensis)* in *M. keralensis.* The mean abundance of infection were 2.35 (66 metacercariae from 28 Copera sp.) 0.40 (91 *P cyanophlycti* recovered from 226 E. cyanophlyctis) in E. cyanophlyctis; 0.06 (three *P. cyanophlycti* from 50 I. aurantiaca) in *I. aurantiaca* and 0.01 (two P. cyanophlycti from 113 M. keralensis) in *M. keralensis* (Table 2).

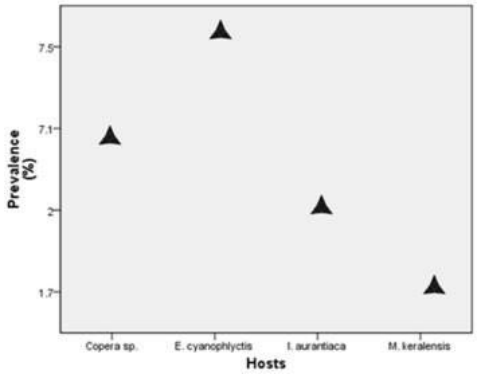

Figure 3 Prevalence of infection

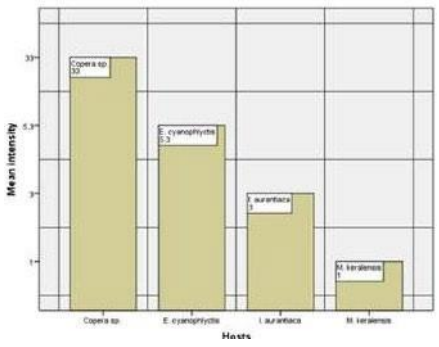

Figure 4 Mean intensity of infection

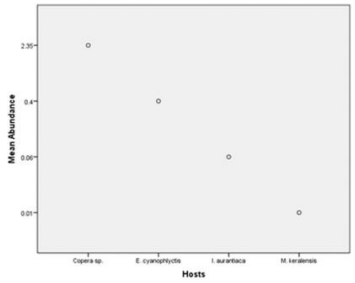

Figure 5 Mean abundance of infection

Information on the life-cycle studies of trematode parasites is scanty. This paper describes the morphology of metacercaria and adult of *P. cyanophlycti* obtained from natural infection from the definitive and intermediate hosts. The metacercariae were recovered from the thorax and abdomen of the damselfly naiads of the species *Copera sp.* Two individuals of *Copera sp.* were positive for this metacercaria. The adults were recovered from the duodenum of the frogs, *E. cyanophlyctis, I. aurantiaca* and *M. keralensis.* Seventeen individuals of *E. cyanophlyctis,* one individual of I. aurantiaca and two individuals of *M keralensis* were positive for this adult trematode. The present metacercaria and adult of *P. cyanophlycti* is distinct from the metacercariae and adults of other Pleurogenoides species in its morphological features and dimensions.

The life cycles of seven species of Pleurogenoides have been studied and established in the world, *P. medians* (Mathias, 1924; Neuhaus, 1940; Buttner, 1951), *P. japonicus* (Okabe & Shibue, 1951; Shibue, 1953), *P. tener* (Macy, 1964), *P. orientalis* (Madhavi et al., 1987), *P. ovatus* (Janardanan & Prasadan, 1991) *P. malampuzhensis* (Brinesh & Janardanan, 2014) and *P. waywzadensis* (Prasadan et al., 2020b). In the present study, the second intermediate host of *P. cyanoph/ycti* were similar to that of *P. medians, P. tener, P. orientalis, P. malampuzhensis*and *P. wayanadensis,* all the six species have odonate naiads as the second intermediate host. Among these five Pleurogeuoides species; *P. medians, P. tener, P orientalis, P. malampuzh.ensis* and *P. wayanadensis* uses dragonfly naiads as second intermediate hosts. In *P wayanadensis* including dragonfly naiads, damselfly naiads are also a second intermediate host. *Pleurogenoides cyanophlycti* uses damselfly naiads as second intermediate host. Shrimps and crabs are the second intermediate hosts of *P. japonicus* and *P. ovatus* respectively. All the eight Pleurogenoides including *P cyanophlycti* the final definitive host were frogs. But in *P. tener* including frogs, lizards also served as a final host (Table 3)

Table 3 Summary of hosts in the life-cycle stages of the eight species of Pleurogenoides

Pleurogenoides species	II[nd] Intermediate Hosts	Definitive Hosts	References
P. medians	Dragonfly naiads (*Aeshna* and *Libellula*)	Frogs (*Rana nigromaculata*)	Mathias, 1924; Neuhaus, 1940 and Buttner, 1951
P. japonicus	Shrimps (*Neocaridina denticulata*)	Frogs (*Rana nigromaculata*)	Okabe and Shibue, 1951 and Shibue, 1953
P. tener	Dragonfly naiads (*Trithemis annulata, Crocothemis erythraea, Anax imperator* and *A. Parthenope*)	Lizards (*Chalcides ocellatus*) Frogs (*Rana mascerenensis*)	Macy, 1964
P. orientalis	Dragonfly naiads (*Tholymis tillarga* and *Tramea limbata*)	Frogs (*Euphlyctis cyanophlyctis*)	Madhavi et al., 1987
P. ovatus	Crabs (*Parathelphusa hydrodromous*)	Frogs (*Hoplobatrachus tigerinus*)	Janardanan and Prasadan, 1991
P. malampuzhensis	Dragonfly naiads (*Gomphidae* sp.)	Frogs (*Hoplobatrachus tigerinus*)	Brinesh and Janardanan, 2014

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 them, 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).

Reports are available on metacercariae from crabs, imago and naiads of both dragonflies and damselflies and larvae of other insects (Dissanaike & Fernando, 1960; Muraleedharan & Pantle, 1967; K.rasnolobova, 1970; Prakash & Pantle, 1970; Vojtkova, 1970; Janardanan et al., 1987; Dhanumkumari, 2000, Brinesh & Janardanan, 2014; Bolek et al., 20I9). We conclude that in Pleurogcnoides, majority of species chooses odonate naiads and frogs as the second intermediate and final definitive hosts. Both odonate naiads and frogs exist in the same ecological niche. So that through trophic level transfer, the metacercaria reaches the definitive host frog when the frog feeds on the infected odonate naiad and develops into adult parasite. We conclude that

-depth ecological Parasitology studies are needed to elucidate the life cycle stages and to establish the same in the laboratory.

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. One of the authors (Ms Sherin) 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 No WLI0-63909/2016 and Order No KFDHQ-6782/2019-CWW/WLl0 for collecting frogs and naiads respectively from the Wayanad forest region is also gratefully acknowledged.

Authors' contribution

Dr Prasadan designed the experiments and guided the study. His research scholars, Mr Shinad K (working on the trematode parasites of frogs), Mrs Sherill Chacko (doing research on the metacercaria larvae of the Western Ghats) and Mrs Arusha K (doing research on the Cercaria fauna of Wayanad Region of the Western Ghats) studied the trematodes in detail All authors contributed equally in the manuscript writing and analyzing the data.

Funding information

This study was partially supported (Major Research project - SRS/220/2015/KSCSTE) by the Kerala State Council for Science, Technology and Environment (KSCSTE), Government of Kerala (the project is completed in 2019).

Compliance with ethical standards

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

The authors declare that there is no conflict of interest among the authors.

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