Two new species of *Pleurogenoides* (Luhe, 1901) Travassos, 1921 (Digenea: Pleurogenidae) infecting two species of frogs of the Western Ghats, India

K. Shinad¹ · P. K. Prasad¹

Received: 1 November 2017 / Accepted: 9 January 2018 / Published online: 23 January 2018
© Indian Society for Parasitology 2018

Abstract Two new species of digenetic trematodes of the genus *Pleurogenoides* infecting the freshwater frogs, *Euphlyctis cyanophlyctis* and *Hoplobatrachus tigerinus* inhabiting the Western Ghats, India are described and illustrated. *Pleurogenoides euphlycti* n. sp. and *P. wayanadensis* n. sp. are separated from their congeners on the basis of differences in the morphology and morphometry. The prevalence of infection of *P. euphlycti* n. sp. in *E. cyanophlyctis* is 8.99% and in *H. tigerinus* 12.5% and the intensity is 6.5 and 7.0 respectively. Prevalence of *P. wayanadensis* n. sp. in *E. cyanophlyctis* is 4.49% and in *H. tigerinus* 6.25% and the intensity is 11.25 and 6 respectively. Since trematode parasites have complex life cycles, with multiple hosts that follow trophic level transfer, the parasite larvae may serve as reliable indicators of species diversity in an ecosystem.

Keywords *Pleurogenoides euphlycti* n. sp. · *Pleurogenoides wayanadensis* n. sp. · Digenea · Frog · *Euphlyctis cyanophlyctis* · *Hoplobatrachus tigerinus*

Introduction

The water skipper, *Euphlyctis cyanophlyctis* (Schneider, 1799) inhabits pools/standing waters in the plains and submountainous areas of the Western Ghats. It is often seen at the edge of water bodies with their eyes above water and is

widely distributed throughout South Asia (Frost 2014). *Hoplobatrachus tigerinus* (Daudin, 1803) is semi-aquatic, living in the fringes of rivers, reservoirs, ponds, paddy fields and marshes with floating vegetation. While studying the digenetic trematodes infecting frogs of the Wayanad region of the Western Ghats, two undescribed species of the genus *Pleurogenoides* (Luhe, 1901) Travassos 1921 infecting *E. cyanophlyctis* and *H. tigerinus* were encountered.

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, 1928, *P. medians* var. *equalis* Mehra and Negi, 1928, *P. prayagi* Mehra and Negi, 1928, *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. ovatus* (Rao, 1977), *P. amritsarensis* Gupta and Chopra, 1984, *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) have been recorded from India. Among these, life cycles of *P. ovatus* and *P. malampuzhaensis* have been elucidated (Janardanan and Prasad, 1991; Brinsh and Janardanan, 2014). The present paper describes two new species of *Pleurogenoides*, *P. euphlycti* n. sp. and *P. wayanadensis* n. sp. infecting two species of frogs, *E. cyanophlyctis* and *H. tigerinus* from the Wayanad region of the Western Ghats with their prevalence and intensity of infection.

Materials and methods

Study area

The study was carried out in the Wayanad region of the Western Ghats, which stands second only to the Eastern

✉ P. K. Prasad
pkprasad@gmail.com

¹ Department of Zoology, Ecological Parasitology and Tropical Biodiversity Laboratory, Kannur University, Mananthavady Campus, Wayanad, Kerala 670645, India

Himalayas as a treasure trove of biological diversity in India (Fig. 1). Western Ghats along with its geographical extension in the wet zone of Sri Lanka is considered one of the “hottest hotspots” of biodiversity. The map of the study area (Fig. 1) was prepared using QGIS 2.16.1 software.

Eighty nine *E. cyanophlyctis* and 16 *H. tigerinus* were collected during January 2016 to July 2017 from the water bodies using sweep hand net. Live specimens were brought to the laboratory, maintained in clean glass jars/aquariums and fed occasionally with insects. The frogs were narcotized

with chloroform, dissected and examined under a Labomed Luxeo4Z stereo zoom microscope for digenetic trematodes. The skin was removed and muscle tissues were macerated to detect the presence of metacercariae, if any. Internal organs like heart, liver, gallbladder, lungs, pancreas, intestine, kidney and urinary bladder were also dissected out from each frog, placed in separate Petri dishes containing 0.75% saline and examined under the dissecting microscope. Adult trematodes, when present, were carefully transferred to 0.75% saline in separate watch glasses and studied under

Fig. 1 Study area—Western Ghats—Wayanad region

Fig. 2 *Pleurogenoides euphlycti* n. sp. adult

Table 1 Taxonomic summary of *P. euphlycti* n. sp

Holotype	<i>Pleurogenoides euphlycti</i> n. sp. Accession No. Z-P/H-F 109. Deposited in the Helminth parasite collections, Ecological Parasitology and Tropical Biodiversity Laboratory, Department of Zoology, Kannur University, Mananthavady Campus, Wayanad-670645, Kerala, India	
Hosts	<i>E. cyanophlyctis</i>	<i>H. tigerinus</i>
Accession Nos.	Accession No. Z-F/E-15. (Deposited in the Herpetology collections, Department of Zoology, Kannur University)	Accession No. Z-F/H-16. (Deposited in the Herpetology collections, Department of Zoology, Kannur University)
Type localities	Birakuppa, Kavummandam, Chooralmala and Panamaram in Wayanad District	Panamaram in Wayanad District
Site of infection	Duodenum	Duodenum
Period of collection	January 2016 to July 2017	January 2016 to July 2017
Prevalence	Eight of 89 frogs (8.99%) screened were infected	Two of 16 frogs (12.5%) screened were infected
Intensity	Fifty two <i>P. euphlycti</i> recovered from eight infected frogs (6.5)	Fourteen <i>P. euphlycti</i> recovered from two infected frogs (7)

Nikon ECLIPSE Ni-U phase contrast research microscope with or without supravital staining using neutral red. Permanent whole mounts of adult trematodes were prepared after fixing them in 5% formalin under slight cover glass pressure and staining with acetocarmine, following the procedure outlined by Cantwell (1981). Measurements were taken with a calibrated ocular micrometer and all measurements are in micrometers (μm), as range followed by mean in parentheses. Figures were drawn with the aid of a prism type camera-lucida and details were added free hand from observations made on live specimens. Photographs were

taken with a Nikon Y-TV55 camera and Nikon NIS Elements Imaging software.

Results

Pleurogenoides euphlycti n. sp. (Fig 2; Table 1)

Description is based on the holotype and 65 paratypes. Body slightly ovoid, 368.2–490.9 (420.3) long, and 230.1–322.1 (254.6) wide, with a slightly ovoid anterior and broadly round posterior ends; body covered with

Table 2 Prevalence and intensity of infection of *P. euphlycti* n. sp. and *P. wayanadensis* n. sp

Host	Trematoda (Digenea)	Number (N)	Prevalence (%)	Intensity	Total mixed infections (incidence)
<i>E. cyanophlyctis</i>	<i>P. euphlycti</i> n. sp.	52	8/89 (8.99)	6.5	4 (50%)
	<i>P. wayanadensis</i> n. sp.	45	4/89 (4.49)	11.25	
<i>H. tigerinus</i>	<i>P. euphlycti</i> n. sp.	14	2/16 (12.5)	7	1 (50%)
	<i>P. wayanadensis</i> n. sp.	6	1/16 (6.25)	6	

Fig. 3 *Pleurogenoides wayanadensis* n. sp. adult

minute, backwardly directed spines which are closely set at the anterior and posterior regions and sparsely distributed in the central region. Oral sucker subterminal, almost circular, $61.4\text{--}92.1 \times 61.1\text{--}76.7$ (67.5×66.0) in size, almost equal to or slightly larger than ventral sucker. Ventral sucker circular, post-equatorial, $61.4\text{--}92.1 \times 61.4\text{--}76.7$ (69.0×66.0) in diameter. Mouth subterminal; pharynx muscular, almost spherical, 26.6×34.7 . Oesophagus short or absent, bifurcates into blunt, laterally inclined, baseball racket shaped intestinal caeca having equal arms $107.4\text{--}153.4 \times 30.7\text{--}61.4$ (139.6×52.2) in size, ending at the level of ovary and left testis.

Testes two, equally sized, $46.0\text{--}76.7 \times 46.0\text{--}61.4$ (53.7×49.1), spherical, equatorial or post-equatorial, symmetrical, one on either side of the ventral sucker in the lateral body margins. Cirrus sac Claviform, $153.4\text{--}230.1 \times 46.0\text{--}61.4$ (202.5×52.2), extends from the

equator 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-spinose cirrus. Ovary small, spherical or ovoid, $46.0\text{--}61.4 \times 30.7\text{--}46.0$ (49.1×38.4) in size, lies close to the level of right caecum, above the level of ventral sucker and right testis. Uterus post-acetabular and winding, mostly in the hind body. Eggs ovoid, operculate and measured 30.7×15.3 . Vitellaria well developed, present only at the posterior region of pharynx.

Four of the 89 *E. cyanophlyctis* and one of the 16 *H. tigerinus* studied showed mixed infection with *P. euphlycti* n. sp. and *P. wayanadensis* n. sp. A total of 117 individual trematodes (Table 2) were documented during the study, of which 66 were *P. euphlycti* n. sp. and 51 were *P. wayanadensis* n. sp.

Table 3 Taxonomic summary of *P. wayanadensis* n. sp

Holotype	<i>Pleurogenoides wayanadensis</i> n. sp. Accession No. Z-P/H-F 111. Deposited in the Helminth parasite collections, Ecological Parasitology and Tropical Biodiversity Laboratory, Department of Zoology, Kannur University, Mananthavady Campus, Wayanad-670645, Kerala, India	
Hosts	<i>E. cyanophlyctis</i>	<i>H. tigrinus</i>
Accession Nos.	Accession No. Z-F/E-17. (Deposited in the Herpetology collections, Department of Zoology, Kannur University)	Accession No. Z-F/H-18. (Deposited in the Herpetology collections, Department of Zoology, Kannur University)
Type localities	Kavummandam and Panamaram in Wayanad District	Panamaram in Wayanad District
Site of infection	Duodenum	Duodenum
Period of collection	January 2016 to July 2017	January 2016 to July 2017
Prevalence	Four of 89 frogs (4.49%) screened were infected	One of 16 frogs (6.25%) screened were infected
Intensity	Forty five <i>P. wayanadensis</i> recovered from four infected frogs (11.25)	Six <i>P. wayanadensis</i> recovered from one infected frog (6)

***Pleurogenoides wayanadensis* n. sp. (Fig. 3; Table 3)**

Description is based on the holotype and 50 paratypes. Body ovoid 920.4–1288.6 × 444.9–647.3 (1127.5 × 527.7), with a slightly ovoid anterior and broadly round posterior ends; body covered with minute, backwardly directed spines that are closely set throughout the body. Oral sucker subterminal, circular, 184.1–214.8 (193.3) in diameter, larger than ventral sucker. Ventral sucker circular, equatorial and 138.1–153.4 (145.7) in diameter. Mouth subterminal; pharynx muscular, ovoid, wider than long, 46.0–76.7 × 92.0–138.1 (56.8 × 115.1). Oesophagus short or absent. Caeca equal, 337.5–613.6 × 92.0–122.7 (463.3 × 105.8) in size, reach up to the middle of testes, below the ventral sucker. Testes two, spherical, equatorial or post-equatorial, symmetrical, one on either side near the posterior margin of ventral sucker in the lateral body margins, 46.0–76.7 (64.4) in diameter. Cirrus sac Claviform, 398.8–512.9 × 76.7–107.4 (440.3 × 85.9), extends from the posterior margin of ventral sucker to the genital pore, at the left body margin near the mid level of oral sucker; it encloses bipartite seminal vesicle, well developed pars-prostatica, long and narrow ejaculatory duct and an un-spinose cirrus. Ovary spherical, zone of ventral sucker, between ventral sucker and right caecum and measured 46.0–76.7 (56.8) in diameter. Uterus post-acetabular and winding, mostly in the hind body. Eggs ovoid, operculate and measured 15.3 × 11.5. Vitellaria absent.

Discussion***Pleurogenoides euphycti* n. sp.**

The described species has similarity to *P. ovatus* in their body shape (Table 4). Detailed study revealed that all the species

reported, except *P. ovatus* and *P. malampuzhensis* are distinctly different in their characters. The species is different from *P. ovatus* on many morphological features and morphometry like body shape, size, distribution of spines, size of oral and ventral suckers, size of pharynx, length, shape and nature of caeca, shape and size of testes and ovary, nature and distribution of vitellaria, length and position of cirrus sac, and size of eggs.

The present species deserves comparison (Table 4) with *P. malampuzhensis* also. In spite of the similarities, it differs from *P. malampuzhensis* in many morphological features and in morphometry such as ratio of body length to width, size of oral and ventral suckers, shape and size of pharynx, caecum, testes, size and structure of cirrus sac, nature and distribution of vitellaria and size of eggs.

The remaining species of *Pleurogenoides* are distinctly different in their characters. The evident differences in the morphology and morphometry clearly indicate that the parasite is a different one and we are strongly convinced that it is a new species. Therefore, the present species is reported here as *Pleurogenoides euphycti* n. sp. after the genus name of the frog host.

***Pleurogenoides wayanadensis* n. sp.**

The described species bears similarity to *P. malampuzhensis* (Table 4). Detailed study revealed that the species is different from *P. malampuzhensis* on many morphological features and morphometry like body shape, length to width ratio, spine arrangements, size of oral and ventral suckers, size and shape of pharynx, caeca, testes and ovary, absence of vitellaria, size and position of cirrus sac, and the size and number of eggs.

The present species deserves comparison (Table 3) with *P. ovatus* also. In spite of the similarities, it differs from *P. ovatus* in many morphological features and in

Table 4 Comparison of morphological and morphometric characters (in μm) of *P. ovatus*, *P. malampuzhensis*, *P. euphlycti* n. sp. and *P. wayanadensis* n. sp

Characters	<i>P. ovatus</i> Rao, (1977)	<i>P. malampuzhensis</i> Brinesh and Janardanan, (2014)	<i>P. euphlycti</i> n. sp.	<i>P. wayanadensis</i> n. sp.
Body L $\times$ B	Elliptical, discoid, spinose, 1342.0–2293.0 $\times$ 905.0–1310.0 (1702.0–1065.0)	Ovoid, spinose, 780.0–1404.0 $\times$ 603.0–820.0 (1071.0 $\times$ 734.0)	Ovoid, spinose, 368.2–490.9 $\times$ 230.1–322.1 (420.3 $\times$ 254.6).	Ovoid, spinose, 920.4–1288.6 $\times$ 444.9–647.3 0(1127.5 $\times$ 527.7)
Oral sucker L $\times$ B	Spherical, 140.0–250.0 $\times$ 156.0–250.0 (181.0 $\times$ 203.0)	Spherical, 132.0–234.0 (215.0)	Sub terminal, almost circular, 61.4–92.0 $\times$ 61.4–76.7 (67.5 $\times$ 66.0).	Sub terminal, circular, 184.1–214.8 (193.3)
Ventral sucker L $\times$ B	Circular, 172.0–265.0 $\times$ 187.0–250.0 (214.0 $\times$ 212.0)	Spherical, 100.0–172.0 (135.0)	Circular, equatorial, 61.4–92.0 $\times$ 61.4–76.7 (69.0 $\times$ 66.0)	Circular, equatorial, 138.1 - 153.4 (145.7).
Pharynx L $\times$ B	Short, globose, muscular, 47.0–94.0 $\times$ 62.0–125.0 (66.0 $\times$ 86.0)	Globular, muscular, 49.0–78.0 (60.0)	Almost spherical, muscular, 26.6 $\times$ 34.7	Ovoid, muscular, wider than long; 46.0–76.7 $\times$ 92.0–138.1 (56.8 $\times$ 115.1)
Oesophagus L $\times$ B	Short, narrow, 47.0–125.0 $\times$ 16.0–31.0 (75.0 $\times$ 27.0)	Conspicuous, 58.0–78.0 (66.0)	Short or absent.	Short or absent.
Caeca L $\times$ B	Short, blunt, ending at the level of testes, 312.0–546.0 $\times$ 62.0–109.0 (443.0 $\times$ 76.0)	Extend a short distance beyond posterior margin of testes and curved inwardly, 454.0–625.0 $\times$ 45.0–75.0 (525.0 $\times$ 68.0)	Equal, ends at the level of ovary and left testis, laterally inclined, baseball racket-shaped, 107.4–153.4 $\times$ 30.7–61.4 (139.6 $\times$ 52.2)	Equal, ends at the middle of testes, below the ventral sucker, blunt, laterally inclined, 337.5–613.6 $\times$ 92.0–122.7 (463.3 $\times$ 105.8)
Testes L $\times$ B	Two, ovoid, symmetrical, at the lateral margins, immediately behind caecal ends; right testis 187.0–515.0 $\times$ 140.0–374.0 (317.0 $\times$ 243.0); left testis 234.0–484.0 $\times$ 187.0–390.0 (331.0 $\times$ 248.0)	Two, ovoid, symmetrical, covered with inwardly curved intestinal caeca; right testis 125.0–281.0 $\times$ 109.0–218.0 (212.0 $\times$ 170.0); left testis 109.0–265.0 $\times$ 110.0–265.0 (204.0 $\times$ 178.0)	Two, spherical, equally sized, equatorial or post-equatorial, symmetrical, at the lateral body margins, one on either side of the ventral sucker; 46.0–76.7 $\times$ 46.0–61.4 (53.7 $\times$ 49.1)	Two, spherical, equatorial or post-equatorial, symmetrical, at the lateral body margins, one on either side near the posterior margin of ventral sucker. 46.0–76.7 (64.4)
Cirrus sac L $\times$ B	Retort-shaped, lies ventral to the left intestinal caecum and extends obliquely from the anterior side of ventral sucker to the genital pore at the left margin near the level of oral sucker, 655.0–1201.0 $\times$ 157.0–296.0 (810.0 $\times$ 214.0); it encloses S-shaped tubular seminal vesicle, pars prostatica, ejaculatory duct and an un-spined cirrus	Claviform, 412.0–624.0 $\times$ 109.0–187.0 (518.0 $\times$ 160.0), extends from anterior region of ventral sucker, to left body margin and opens through genital pore at the level of pharynx; it encloses bipartite seminal vesicle, well developed pars-prostatica, long and narrow ejaculatory duct and un-spined cirrus	Claviform, 153.4–230.1 $\times$ 46.0–61.4 (202.5 $\times$ 52.2), extends from the equator to the genital pore, at the left body margin near the level of oral sucker; encloses bipartite seminal vesicle, well developed pars-prostatica, long and narrow ejaculatory duct and un-spined cirrus	Claviform, 398.8–512.9 $\times$ 76.7–107.4 (440.3 $\times$ 85.9), extends from the posterior margin of ventral sucker to the genital pore, at the left body margin near the mid level of oral sucker; encloses bipartite seminal vesicle, well developed pars-prostatica, long and narrow ejaculatory duct and un-spined cirrus
Ovary L $\times$ B	Spherical or ovoid, 172.0–374.0 $\times$ 125.0–312.0 (243.0 $\times$ 212.0)	Ovoid, 94.0–123.0 $\times$ 72.0–156.0 (114.0 $\times$ 103.0)	Spherical or ovoid, 46.0–61.4 $\times$ 30.7–46.0 (49.1 $\times$ 38.4)	Spherical, 46.0–76.7 (56.8), intercaecal in the ventral sucker zone, between ventral sucker and right caecum.
Vitellaria	Two sets of grape like follicles, extending from the level of oesophagus to ovary	Extend from mid-level of oral sucker to mid-level of ventral sucker, confluent in forebody	Well developed, only at the posterior region of pharynx	Diffused
Eggs L $\times$ B	Ovoid, operculate, 31.0–33.0 $\times$ 14.0 (32.0 $\times$ 14.0)	Ovoid, operculate, 40.0–56.0 $\times$ 15.0–26.0 (50.0 $\times$ 23.0)	Ovoid, operculate, length is twice the width; 30.7 $\times$ 15.3	Ovoid, operculate, 15.3 $\times$ 11.5.

morphometry such as ratio of body length to width, size of oral and ventral suckers, size and shape of caecum, testes, ovary, size and structure of cirrus sac, absence of vitellaria and size and number of eggs.

The remaining species of *Pleurogenoides* are distinctly different in their characters. The evident differences in the morphology and morphometry clearly indicate that the parasite is a different one and we are strongly convinced

that it is a new species. Therefore, the present species is reported here as *Pleurogenoides wayanadensis* n. sp. after the name of the study area, Wayanad.

Acknowledgements The authors are grateful to the Kerala State Council for Science, Technology and Environment (KSCSTE), Government of Kerala for providing financial assistance in the form of a major research project (SRS/220/2015/KSCSTE) to carry out this study. The permission accorded by the Department of Forest and

Wildlife, Government of Kerala (Order No WL10-63909/2016) for collecting frogs from the Wayanad forest region is also gratefully acknowledged. The authors are indebted to Prof. K. P. Janardanan for critically going through the manuscript.

Author's contribution Dr P. K. Prasadán 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.

Compliance with ethical standards

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

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.

References

- Brinsh R, Janardanan KP (2014) The life history of *Pleurogenoides malampuzhensis* sp. nov. (Digenea: *Pleurogenidae*) from amphibious and aquatic hosts in Kerala, India. *J Helminthol* 88:230–236. <https://doi.org/10.1017/S0022149X13000084>
- Cantwell GE (1981) Methods for invertebrates. In: Clark G (ed) *Staining procedures*. Williams and Wilkins, Baltimore, pp 255–280
- Frost DR (2014) *Amphibian species of the world: an online reference*. Version 6.0. Electronic database accessible at <http://research.amnh.org/herpetology/amphibian/index.html>. *Amer Mus of Nat Hist*, New York
- Janardanan KP, Prasadán PK (1991) Studies on the life cycle of *Pleurogenoides ovatus* Rao 1977 (Trematoda: Pleurogenetinae). *J Helminthol* 65:43–50
- Rao R (1977) On a new species of *Pleurogenoides* Travassos, 1921 (Lcithodendriidae Odhner, 1910) and *Pleurogenoides sitapuri* (Srivstava, 1934) from frogs in Hyderabad. *Riv Di Parasitol* 38(1):23–29