Description of *Pleurogenoides cyanophlycti* n. sp. (Digenea: Pleurogenidae) infecting *Euphlyctis cyanophlyctis* from the Western Ghats, India

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Abstract *Pleurogenoides cyanophlycti* n. sp. (Digenea, Pleurogenidae) from the intestine of the water skipper, *Euphlyctis cyanophlyctis* (Anura, Dicroglossidae), inhabiting the Western Ghats, India is described and illustrated. *Pleurogenoides cyanophlycti* n. sp. is new to the genus and is separated from its congeners on the basis of differences in morphology and morphometry. It is named after the host, *E. cyanophlyctis*.

Keywords *Pleurogenoides cyanophlycti* n.sp · Digenea · Frog · Prevalence · *E. cyanophlyctis*

Introduction

The water skipper, *E. cyanophlyctis* (Schneider, 1799) inhabits the pools/standing waters in the plains and sub-mountainous 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). 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) Janardanan et al., 1987, *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. Life cycle of two species of *Pleurogenoides*, *P. ovatus* and *P. malampuzhaensis* have been elucidated from Kerala (Janardanan and Prasad 1991; Brinsh and Janardanan 2014). The present paper describes a new species of *Pleurogenoides*, *P. cyanophlycti* n. sp. from *E. cyanophlyctis* from the Wayanad region of the Western Ghats.

Materials and methods

Study area

The study was carried out in the Wayanad region of the Western Ghats (Fig. 1). Western Ghats stands second only to the Eastern Himalaya as a treasure trove of biological diversity in India. 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 by using QGIS 2.16.1 software.

Sixty-eight specimens of *E. cyanophlyctis* were collected during the period from January 2016 to April 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 their body parts examined under a stereo zoom dissecting microscope for digenetic trematodes. The skin was removed, and the muscle tissues were macerated to detect

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Fig. 1 Study area—Western Ghats–Wayanad region

the presence of metacercariae. Internal organs like heart, liver, gall bladder, lungs, pancreas, intestine, kidney, and urinary bladder were also dissected out from each frog, placed in separate Petri dishes containing 0.75% saline, macerated and examined under the dissecting microscope. Adults, when present, were carefully removed and transferred to 0.75% saline in separate watch glasses. Adult trematodes were studied under a 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 then staining with acetocarmine, following the procedure outlined by Cantwell (1981). Measurements were taken with the aid of a calibrated ocular micrometer. 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 BG

330/120 camera attached to a Leica DM 500 research microscope with the support of Bio wizard software.

Results

Pleurogenoides cyanophlycti n. sp. (Fig. 2)

Description is based on the holotype and seventy-six paratypes. Body oval, 797.7–1410.4 long, and 368.2–767 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 subterminal, almost round, 153.4–230.1 $\times$ 153.4–214.8 in diameter, larger than ventral sucker. Ventral sucker spherical, equatorial and 92.0–168.7 $\times$ 92.0–168.7 in diameter. Mouth subterminal; pharynx muscular, ovoid (46.0–65.4 $\times$ 61.4–138.1 in size). Oesophagus short, bifurcates anterior to ventral sucker into short, blunt, laterally inclined, unequal intestinal caeca; right caecum longer, 230.1–836.2 in length and 30.7–138.1 in width; left caecum 230.1–690.2 $\times$ 46.0–88.7 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 $\times$ 61.4–168.7 and left testis 76.7–214.8 $\times$ 61.4–122.7. Cirrus sac claviform (260.8–521.6 $\times$ 61.4–184.1 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 $\times$ 61.4–276.1 in diameter. Uterus post-acetabular and winding mostly in the hind body. Eggs ovoid, operculate and measured 15.3–30.7 $\times$ 7.7–15.3. 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.

Taxonomic summary

Type specimen: Holotype (No. Z-P/H-F 101) deposited in the Helminth parasite collections, Ecological Parasitology and Tropical Biodiversity Laboratory, Department of Zoology, Kannur University, Mananthavady Campus, Wayanad-670645, Kerala, India.

Type host: Water skipper frog, *E. cyanophlyctis* (Z-F/E-11). Deposited in the Herpetology collections, Department of Zoology, Kannur University, Mananthavady Campus, Wayanad-670645, Kerala, India.

Type localities: Makkimala, Chundel, Peechangode, Valliyookavu, Payyampally,

Panamaram and Pulpally, Wayanad District, Kerala, India.

Site of infection: Duodenum.

Period of collection: January 2016 to April 2017.

Prevalence: Fifteen of 68 frogs (22.06%) screened were infected.

Intensity: Seventy seven *P. cyanophlycti* recovered from fifteen infected frogs (5.1).

Of the 68 *E. cyanophlyctis* examined, 15 were found infected with a total of 77 parasites. The prevalence of infection was 22.06%, while the intensity of infection stood at 5.1.

Discussion

The present species exhibits similarities with *P. ovatus*, *P. orientalis* and *P. malampuzhensis* (Table 1). It differs from *P. ovatus* in various morphological features and morphometry like body shape, length to width ratio, size of oral and ventral suckers, size of oesophagus, length of caeca, nature and position of caecal tips, shape and size of testes and ovaries, nature and distribution of vitellaria, length of cirrus sac, shape of seminal vesicle and the size of eggs.

The present species deserves comparison (Table 1) with *P. malampuzhensis* also. In spite of the similarities, it differs from *P. malampuzhensis* in many morphological features and morphometry including the ratio of body length to width, size of oral and ventral suckers, shape and size of

Fig. 2 *Pleurogenoides cyanophlycti* n.sp., adult

Table 1 Comparison of morphometric characters (in μm) of *P. orientalis*, *P. ovatus*, *P. malampuchensis* and *P. cyanophlycti* n.sp

Characters	<i>P. orientalis</i> Srivasatava, 1934	<i>P. ovatus</i> Rao, 1977	<i>P. malampuchensis</i> Brinsh and Janardanan, 2014	<i>P. cyanophlycti</i> n. sp.
Body L $\times$ B	Ovoid, spinose, 1400.0–1960.0 $\times$ 764.0–1420.0 (1869.0 $\times$ 1103.0)	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, 797.7–1410.4 $\times$ 368.2–767.0 (1040.1 $\times$ 587.5)
Oral sucker L $\times$ B	Spherical, 203.0–343.0 $\times$ 203.0–359.0 (276.0 $\times$ 278.0)	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 spherical, 153.4–230.1 $\times$ 153.4–214.8 (185.6 $\times$ 187.1)
Ventral sucker L $\times$ B	Spherical, 156.0–281.0 $\times$ 172.0–281.0 (220.0 $\times$ 226.0)	Circular, 172.0–265.0 $\times$ 187.0–250.0 (214.0 $\times$ 212.0)	Spherical, 100.0–172.0 (135.0)	Spherical, equatorial, 92.0–168.7 $\times$ 92.0–168.7 (133.5 $\times$ 135.0)
Pharynx L $\times$ B	47.0–78.0 $\times$ 94.0–140.0 (64.0 $\times$ 120.0)	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)	Ovoid, 46.0–65.4 $\times$ 61.4–138.1 (53.7 $\times$ 96.6)
Caeca L $\times$ B	Extends to the level of anterior margin of testes, 608.0–1170.0 $\times$ 47.0–94.0 (973.0 $\times$ 70.0)	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)	Unequal in length; blunt, laterally disposed, ending slightly behind testes without curving inwardly, right caecum 230.1–836.2 $\times$ 30.7–138.1 (602.2 $\times$ 61.4); left caecum 230.1–690.2 $\times$ 46.0–88.7 (460.2 $\times$ 76.6)
Oesophagus L $\times$ B	Absent	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
Testes L $\times$ B	Two, spherical, symmetrical, immediately behind intestinal caeca; right testis 234.0–406.0 $\times$ 187.0–406.0 (329.0 $\times$ 287.0); left testis 203.0–406.0 $\times$ 172.0–406.0 (312.0 $\times$ 278.0)	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 or oval, equatorial or post-equatorial, symmetrical, at the lateral body margins, one on either side of the ventral sucker; Right testis slightly larger; right testis 92.0–224.8 $\times$ 61.4–168.7 (130.4 $\times$ 107.4); left testis 76.7–214.8 $\times$ 61.4–122.7 (125.8 $\times$ 96.6)
Cirrus L $\times$ B	437.0–874.0 $\times$ 109.0–250.0 (696.0 $\times$ 183.0)	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 unspined 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 unspined cirrus	Claviform, 260.8–521.6 $\times$ 61.4–184.1 (406.5 $\times$ 121.2), 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 unspined cirrus
Ovary L $\times$ B	Spherical, 140.0–296.0 (222.0)	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, 61.4–276.1 $\times$ 61.4–276.1 (116.6 $\times$ 105.8)
Vitellaria	Extend from mid-level of oral sucker to mid-level of ventral sucker, confluent in forebody	2 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, extending from the midlevel of oral sucker to the anterior margin of testes, on each side, converging medially on either side of the pharynx, but not meeting together
Eggs L $\times$ B	Ovoid, operculate, 24.0–26.0 $\times$ 14.0–16.0	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, 15.3–30.7 $\times$ 7.7–15.3 (26.1 $\times$ 11.5)

pharynx, length of caecum, nature and position of caecal tips, shape and size of testes, size and structure of cirrus sac, nature and distribution of vitellaria and size of eggs.

The present species shows some similarities with *P. orientalis* too. It differs from *P. orientalis* in many morphological features and morphometry like the body size, size of oral and ventral suckers, length and nature of caecum, size and position of testes, size of ovary, 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 cyanophlycti* n. sp. after the name of the host.

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Author's contribution Dr PKP designed and guided the study. Mr KS 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.

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