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

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

Correspondence
PK Prasadan
Ecological Parasitology and Tropical Biodiversity Laboratory, Department of Zoology, Kannur University, Mananthavady Campus, Wayanad, Kerala, India

Description of *Diplodiscus cyanophlycti* n.sp. (Digenea: Paramphistomidae) and prevalence and intensity of two other digeneans infecting the water skipper, *Euphlyctis cyanophlyctis* from the Western Ghats, India

K Shinad and PK Prasadan

Abstract
Western Ghats, one of the hottest biodiversity hotspots in the world, has very rich amphibian diversity. In an attempt to map the digenetic trematode fauna of the frogs of this region, as a part of a major research project, we came across three species of trematode parasites infecting the water skipper, *Euphlyctis cyanophlyctis* (Anura, Dicroglossidae) collected during January 2016 to April 2017. Detailed study revealed that one species is new to science and is named *Diplodiscus cyanophlycti* n.sp. (Digenea, Paramphistomidae) after the name of its host. Other two trematodes were *Ganeo tigrinus*, and *Halipegus mehransis.* All the three species of parasites were recovered from the duodenum of *E. cyanophlyctis*. *D. cyanophlycti* n.sp. is new to the genus and is separated from its congeners on the basis of differences in the morphology and morphometry. Of the 68 *E. cyanophlyctis* studied, six were infected with *D. cyanophlycti* n.sp, 12 were infected with *G. tigrinus* and two with *H. Mehransis.* Prevalence of *D. cyanophlycti* n.sp. Infection is 8.82%, that of *G. tigrinus* is 17.64% and of *G. tigrinus* is 2.94%. Intensity of infection is higher (3.8) in *G. tigrinus* followed by *H. mehransis D. cyanophlycti* n.sp. Among the 17 *E. cyanophlyctis* infected with the three parasites, three exhibited mixed infections in different combinations. Thus the incidence of mixed infection is 17.65%.

Keywords: *Diplodiscus cyanophlycti* n.sp., digenean, frog, prevalence, *E. cyanophlyctis*

1. Introduction
The water skipper, *E. cyanophlyctis* [1] 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 [2]. A study on the digenetic trematodes of *E. cyanophlyctis* collected from diverse water bodies in the Wayanad region of the Western Ghats, India revealed infections with an undescribed species of *Diplodiscus* and two described species, *Ganeo tigrinus* and *Halipegus mehransis*. Of the 17 species of *Diplodiscus* recorded worldwide from amphibians, five species, viz- *Diplodiscus amphichrus* Tubangui, 1933, *D. amphichrus magnus* Srivastava, 1934, *D. mehrai* Pande, 1937, *D. lali* Pandey and Chakrabarty, 1968, and *D. chauhani* Pandey, 1969 are known from Indian amphibian hosts [3].
Trematode parasites have complex life cycles, requiring multiple hosts. If these parasites are present in an ecosystem, then one can infer that their respective hosts must also be present. Thus, these parasites may serve as reliable indicators of species diversity in an ecosystem. After mapping the trematode fauna of frogs of the Wayanad region of Western Ghats and elucidating their life cycles, in our ongoing project, emphasis will be given to study the services of larval trematodes as potential indicators of frog diversity. The paper describes one new species of the genus *Diplodiscus* from India and provides data on the prevalence and intensity of infection of *D. cyanophlycti* n.sp., *Ganeo tigrinus* and *Halipegus mehransis* infecting the same host.

2. Materials and Methods
2.1 Study Area: The study was carried out in the Western Ghats region of Wayanad (Figure 1) during the period from January 2016 to April 2017. The Western Ghats is second only to the Eastern Himalaya as a treasure trove of biological diversity in India.

The Western Ghats along with its geographical extension in the wet zone of Sri Lanka is now considered one of the "hottest hotspots" of biodiversity. The map of the study area (Figure 1) was prepared by using QGIS 2.16.1 software.

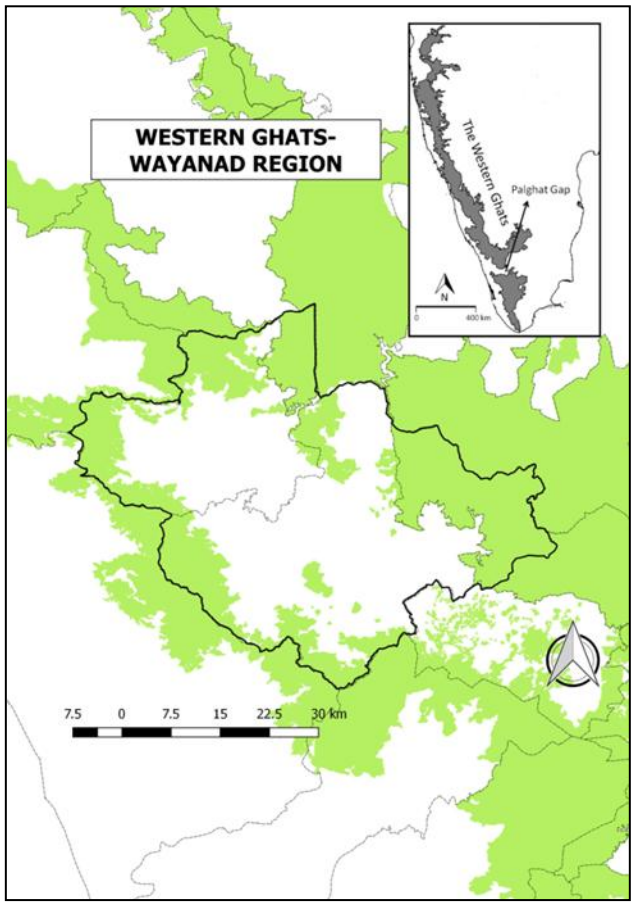

Fig 1: Study area – Western Ghats – Wayanad region

E. cyanophlyctis were collected 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 digeneans. The skin was removed, and muscle tissues were macerated to detect 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 with neutral red or methylene blue. 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 [4]. 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.

3. Results

3.1 *Diplodiscus cyanophlycti* n.sp. (Figure 2)

Description is based on the holotype and nine paratypes. Body stout, 813.0 – 3022.0 in length, conical with a blunt anterior and round posterior end; surface smooth and maximum width (383.5 – 1518.7) near mid-body. Mouth sub-terminal, followed by fairly well developed, spherical or ovoid pharynx with 46.0 – 184.1 x 30.7 – 122.7 in size. Oral sucker ovoid, subterminal, with 138.1 – 582.9 in length and 138.1 – 552.2 in width. Ventral sucker large, conspicuous, cup shaped, with a size of 276.1 – 797.7 x 398.8 – 966.4, located at posterior extremity, provided with an additional sucker with depression in centre. Intestinal caeca 490.9 – 1488.0 long, and 61.4 – 214.8 wide, extends along the lateral margin of body up to the anterior border of the posterior sucker (left caeca: 490.9 – 1488.0 x 61.4 - 214.8; right caeca: 490.9 – 1488 x 61.4 – 214.8). Testis single, round or ovoid, 122.7 – 214.8 x 92.0 – 214.8 in size, at the posterior third of body. Cirrus sac small; genital pore median, immediately behind the bifurcation. Vitelline follicle large, lateral, extends from the level of intestinal bifurcation to posterior sucker and meet in the median line anteriorly and posteriorly. Eggs 110.0 – 130.0 in number, large, oval, operculate, 76.7 – 122.7 x 46.0 – 92.0.

3.2 Taxonomic Summary

Type specimen: Holotype (No. Z-P/H-F 102) deposited in the Helminth parasites collection, 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-12) Deposited in the Herpetology collections, Department of Zoology, Kannur University, Mananthavady Campus, Wayanad-670645, Kerala, India

Type localities: Chundel, Panamaram, and Pulpally, Wayanad District, Kerala, India.

Site of infection: Intestine.

Period of collection: January 2016 to April 2017.

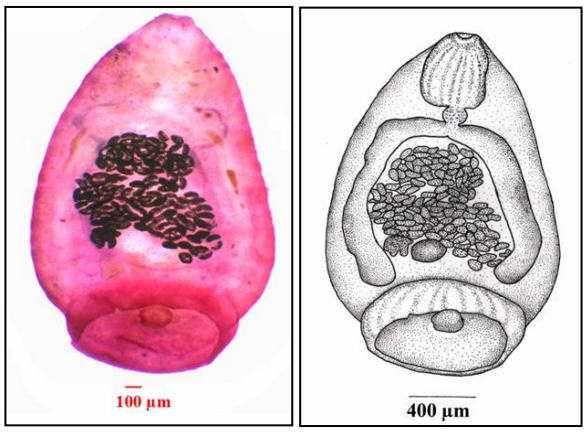

Fig 2: *Diplodiscus cyanophlycti* n. sp. adult (photograph and line drawing)

Table 1: Comparison of morphologic and morphometric (in µm) characters of *D. amphichrus*, *D. chauhani* and *D. cyanophlycti* n.sp.

Characters	*D. amphichrus* [5]	*D. chauhani* [6]	*D. cyanophlycti n.sp.*
Body LxB	Body conical with a blunt anterior and round posterior end; 1420.0 – 3042.0 x 718.0 – 1232.0 (2196.0 x 987.0); aspinose	Body conical with a blunt anterior end and broadly round posterior end; 2122.0 – 6396.0 x 764.0 – 2340.0 (3557.0 x 1332.0); aspinose	Body stout, conical with a blunt anterior and round posterior end; 813.0 – 3022.0 x 383.5 – 1518.7 (1815.2 x 806.2); aspinose
Oral sucker LxB	Sub terminal, oval; 140.0 – 312.0 x 234.0 – 421.0 (232.0 x 328.0); oral diverticula present	Sub terminal, slightly oval; 281.0 – 749.0 x 328.0 – 796.0 (480.0 x 509.0); oral diverticula absent	Ovoid, sub terminal; 138.1 – 582.9 x 138.1 – 552.2 (332.4 x 284.6)
Ventral sucker LxB	Large; at the posterior end of body; 499.0 – 796.0 x 686.0 – 1170.0 (668.0 x 891.0); a small central additional sucker	Large; at the posterior end of body; 671.0 – 1217.0 x 796.0 – 2044.0 (911.0 x 1260.0); a small central additional sucker	Large, conspicuous, cup shaped, located at posterior extremity, provided with an additional sucker with depression in centre; 276.1 – 797.7 x 398.8 – 966.4 (523.3 x 683.5)
Pharynx LxB	Absent	Absent	Spherical or ovoid; 46.0 – 184.1 x 30.7 – 122.7 (102.3 x 76.7)
Testes LxB	Single, round; 140.0 – 468.0 x 125.0 – 452.0 (240.0 x 237.0); at the middle of the body	Single; at the middle of the body or slightly posterior; 250.0 – 499.0 x 328.0 – 624.0 (390.0 x 443.0)	Single, round or ovoid; at the posterior third; 122.7 – 214.8 x 92.0 – 214.8 (156.8 x 148.3)
Eggs LxB	Few, oval, operculate	Oval, operculate	110 – 130 in number, large, oval, operculate; 76.7 – 122.7 x 46.0 – 92.0 (95.9 x 67.1)
Caeca LxB	Wide; extend along the lateral margin of body up to the anterior border of the posterior sucker; 749.0 – 1700.0 x 109.0 – 203.0 (1187.0 x 161.0)	Wide; extend along the lateral margin of body up to the level of posterior sucker; 1420.0 – 4212.0 x 125.0 – 421.0 (2287.0 x 218.0)	Extends along the lateral margin of body up to the anterior border of the posterior sucker; 490.9 – 1488.0 x 61.4 – 214.8 (915.3 x 131.3); both caeca (490.9 – 1488.0 x 61.4 - 214.8) equally long.

Table 2: Summary of digeneans from *E. cyanophlyctis* collected from the Western Ghats Wayanad region, India.

Trematode	Number (N)	Prevalence (%)	Intensity	Total mixed infections (Incidence)
D. cyanophlycti n.sp.	10	6/68 (8.82)	1.7	3 (17.65%)
G. tigrinus	45	12/68 (17.64)	3.8	
H. mehransis	4	2/68 (2.94)	2	

Among the 17 *E. cyanophlyctis* infected with the three parasites, three exhibited mixed infections in different combinations. Thus the incidence of mixed infection is 17.65%. A total of 59 individual trematodes belonging to three genera (Table 2) were documented during the study. Of that, 10 individuals were of an undescribed species of *Diplodiscus*, 45 of *G. tigrinus* and two of *H. mehransis.*

4. Discussion

The genus *Diplodiscus* of the family Diplodiscidae Cohn, 1904 was erected by Diesing, 1836 and ascribed *D. Subclavatus* Pallas, 1760 as its type species. Since then 17 species have been added to this genus from amphibians. Of these, five valid species have been reported from Indian amphibians by Srivastava [7], Bhalerao [8], Pande [9], Kaw [10], Pandey and Chakrabarti [11], Pandey [6], Mukherjee and Ghosh [12], Dwivedi [13] and Singh [14]. The Indian species are *D. amphichrus* Tubangui, 1933, *D. amphichrus magnus* Srivastava, 1934, *D. mehrai* Pande, 1937, *D. lali* Pandey and Chakrabarty, 1968, and *D. chauhani* Pandey, 1969[3]. Srivastava [15] considered *D. mehrai* Pande, 1937, *D. lali* Pandey and Chakrabarti, 1968, *D. chauhani* Pandey, 1969 and *Diplodiscus sp.* Anjaneyulu, 1967 as synonyms of *D. amphicrus.* Tubangui [16] described *D. amphichrus* from *Rana* sp. in the Philippines. Srivastava [17] described a new variety, *D. amphichrus* var. *magnus* from *E. cyanophlyctis* from UP and Pande [9] added another species, *D. mehrai*, from the same host in Kumaon Hills, differentiating it from the earlier known species in the presence of pre-bifurcal genital pore. Singh [18], considering the position of genital and excretory pores only minor variations, regarded *D. amphichrus* var. *magnus* and *D. mehrai* as synonyms of *D. amphichrus.* Mukherjee[19] further synonymised *D. japonicus* Yamaguti, 1936 with *D. amphichrus*. Agrawal [20] re-described the latter and *D. Lali*, and supported the synonymy suggested earlier. Fischthal and Thomas [21] raised the variety *magnus* of Srivastava to species rank and considered that *D. amphichrus* of Agarwal as synonym of *magnus* and so were *D. amphichrus*, *D. japonicus* and *D. mehrai*. Pandey and Chakrabarty [11] and Pandey [6] described two new species, *D. lali* and *D. chauhani* from *H. tigrinus* and *E. cyanophlyctis*, respectively and Pandey and Jain [22] upheld the validity of *D. mehrai*. Nama and Khichi [23] described a new sub species, *D. amphichrus brevis* from *E. cyanophlyctis* and disagreed to the synonymy of *D. mehrai* to *D. amphichrus*. Srivastava [15], suggested that *D. lali* and *D. chauhani* are synonyms of *D. amphichrus* and stated that the genus is represented only by two distinct species *D. amphichrus* and *D. mehrai* in India. *D. amphichrus* appears to have a wide distribution in India, reported from many localities [20, 12, 23, 6] with frogs as their common hosts. From North-East India, *D. amphichrus* was reported by Diengdoh [24] and Tandon *et al*. [25], from two hosts, *Polypedates leucomystax* and *Rhacophorus maximus*.

The present species exihibits similarities with *D. amphichrus* (Table 1). It differs from *D. amphichrus* in various morphological features like absence of oral diverticula, length of caeca and size of testes. Apart from that measurements and proportions are different from that of *D. amphichrus* (Table 1). 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 *D. cyanophlycti* n.sp. after the name of the host species.

The morphological features and morphometry of the *Ganeo sp.* obtained from *E. cyanophlyctis* during the present study are almost similar to that of *G. tigrinus* and therefore, the present species is reported here as *G. tigrinus*. Twelve out of 68 *E. cyanophlyctis* studied were infected with *G. tigrinus*.

The prevalence is 17.64% (Table 2) and the intensity of infection is 3.8. In the life cycle studies of *G. tigrinus* carried out by Brinesh and Janardanan [26], 55 of 989 *Hoplobatrachus tigerinus* (prevalence is 5.56% and the intensity is between three to six) were found infected with *G. tigrinus*. Higher prevalence in *E. cyanophlyctis* may be due to its aquatic habit and non-selective mode of feeding.

Although the morphological features and the morphometry of the *Halipegus sp.* obtained from *E. cyanophlyctis* during the present study showed some differences, from *H. mehransis*, the present digenean is reported as *H. mehransis*. Two out of 68 *E. cyanophlyctis* were infected. The overall prevalence is 2.94% (Table 2) and the intensity of infection is two. Data on the prevalence and intensity of *H. mehransis* is available only in a study carried out by Muraledharan[27]. Prevalence was 3.92% in *E. hexadactylus* (two out of 51) and 0.81% in *H. tigrinus* (one out of 124). Prevalence of *H. mehransis* in the present study is almost similar to that documented in *H. tigrinus* by Muraleedharan [27].

5. Conclusion

Trematode parasites have complex life cycles, requiring multiple hosts. If these parasites are present in an ecosystem, then one can infer that their respective hosts must also be present. Thus, these parasites may serve as reliable indicators of species diversity in an ecosystem. Diverse assemblages of larval trematode parasites are easily sampled in intermediate host snails. Through their life cycles these parasites are functionally coupled with the surrounding free-living diversity of vertebrate and invertebrate animals. The present study is a part of an ongoing research project to prepare a database on trematode parasites infecting frogs of Western Ghats Wayanad region. After elucidating and establishing the life cycles of trematode parasites of frogs, the larval trematodes can be universally taken as indicators of frog diversity. The present report is the first step in the process.

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6.1 Author's contribution

Dr. P K Prasadan designed and guided the study. Mr Shinad carried out the survey, collected and studied the adult trematode in detail. The manuscript was written by both the authors.

6.2 Compliance with ethical standards

6.3 Conflict of interest

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

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