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

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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. cyanophlycti 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 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 [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.

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

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<tr>
<td>Body LxB</td>
<td>Body conical with a blunt anterior and round posterior end; 1420.0 – 3042.1 x 718.8 – 1322.0 (2196.0 x 987.0); aspinose</td>
<td>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</td>
<td>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</td>
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<tr>
<td>Oral sucker LxB</td>
<td>Sub terminal, oval; 140.0 – 312.0 x 234.0 – 421.0 (232.2 x 328.0); oral diverticula present</td>
<td>Sub terminal, slightly oval; 281.0 – 749.0 x 328.0 – 796.0 (480.0 x 509.0); oral diverticula absent</td>
<td>Ovoid, sub terminal; 138.1 – 582.9 x 138.1 – 552.2 (332.4 x 284.6)</td>
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<td>Ventral sucker LxB</td>
<td>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</td>
<td>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</td>
<td>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)</td>
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<tr>
<td>Pharynx LxB</td>
<td>Absent</td>
<td>Absent</td>
<td>Spherical or ovoid; 46.0 – 184.1 x 30.7 – 122.7 (102.3 x 76.7)</td>
</tr>
<tr>
<td>Testes LxB</td>
<td>Single, round; 140.0 – 468.0 x 125.0 – 452.0 (240.0 x 237.0); at the middle of the body</td>
<td>Single; at the middle of the body or slightly posterior; 250.0 – 499.0 x 328.0 – 624.0 (390.0 x 443.0)</td>
<td>Single, round or ovoid; at the posterior third; 122.7 – 214.8 x 92.0 – 214.8 (156.8 x 148.3)</td>
</tr>
<tr>
<td>Eggs LxB</td>
<td>Few, oval, operculate</td>
<td>Oval, operculate</td>
<td>110 – 130 in number, large, oval, operculate; 76.7 – 122.7 x 46.0 – 92.0 (95.9 x 67.1)</td>
</tr>
<tr>
<td>Cae ca LxB</td>
<td>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)</td>
<td>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)</td>
<td>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 ceca (490.9 – 1488.0 x 61.4 – 214.8) equally long.</td>
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Among the 17 E. cyanophlycti 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. mehransi.

4. Discussion

The genus Diplodiscus of the family Diplodiscidae Cohn, 1904 was erected by Diesing, 1836 and ascribed D. Sabelavatus Pallas, 1760 as its type species. Since then 17 species have been added to this genus from amphibia. 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, 1969⁴. Srivastava [15] considered D. mehrai Pande, 1937, D. lali Pandey, 1968, D. chauhani Pandey, 1969 and Diplodiscus sp. Anjaneyulu, 1967 as synonyms of D. amphichrus. Tubangui [16] described D. amphichrus from Rana sp. in the Philippines, Srivastava [17] described a new variety, D. amphichrus var. magnus from E. cyanophlycti from UP and Pande [18] 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 [19], considering the position of genital and excretory pores only minor variations, regarded D. amphichrus var. magnus and D. mehrai as synonyms of D. amphichrus. Mukherjee⁹ 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. cyanophlycti, 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. cyanophlycti and disagreed for 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 exhibits 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 Gameo sp. obtained from E. cyanophlycti 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. cyanophlycti 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 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 Muraleedharan [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.

7. References
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