Digenean parasites of the water skipper Euphlyctiscyanophlyctis(Schneider, 1799)Dicroglossidae) of the South Western Ghats, India

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Abstract. Five species of digenean parasites, *Diplodiscus mehrai*, *Tremiorchis ranarum*, *Halipegus mehransis*, *Ganeo glottoides* and *Ganeo tigrinum*, of the frog *Euphlyctis cyanophlyctis* (Schneider, 1799) (Anura: Dicroglossidae) from the Wayanad Region of the South Western Ghats are reported in this paper. Multiple infections were also recorded during the study. Prevalence of infection of *D. mehrai*, *T. ranarum*, *H. mehransis*, *G. glottoides* and *G. tigrinum* were 12%, 8%, 2%, 31% and 8%, respectively, and the intensity of infection were 1.7, 34.4, 2, 4.5 and 2.9, respectively.

Keywords: Digenean parasites; Frog; *Euphlyctis cyanophlyctis*; Western Ghats; Prevalence.

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Introduction

Amphibians, particularly anurans, harbour a variety of adult and larval trematodes involving freshwater gastropods as first intermediate hosts in their life-cycles (Duda and Verma, 1996). The trematode fauna of amphibians of India has been the subject of numerous investigations, including those by Mehra and Negi (1926a, b, 1928), Bhalerao (1926, 1936a, b, c, 1937), Srivastava (1933a, b, 1934a, b), Pande (1937), Chauhan (1954), Gupta and Agrawal (1966, 1967), Pandey (1968, 1969a, b, 1981), Gupta (1970, 1977), Mukherjee and Ghosh (1970, 1972), Singh (1977) Janardanan et al. (1987), Janardanan and

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Prasadan (1991), Brinesh and Janardanan (2014) and Shinad and Prasadan (2017a, b, 2018a, b). Singh (1977) made the first contribution to the trematode fauna of amphibians in Kerala. Later Janardanan et al. (1987) recorded Pleurogenoides ovatus Rao, 1977 from Rana tigrina (Hoplobatracus tigerinus) and later Janardanan and Prasadan (1991) elucidated its life cycle. The life cvcle of new species Р. а Malampuzhaensis was established by Brinesh and Janardanan (2014). Recently four new species of digenetic trematodes from amphibian hosts were reported from Western Ghats by Shinad and Prasadan (2017a, b, 2018a, b).

The water skipper *Euphlyctis* cyanophlyctis (Schneider, 1799) (Anura: Dicroglossidae) inhabits the pools/standing waters in the plains and sub-mountainous areas of the Western Ghats. It is widely distributed throughout South Asia (Frost, 2014). Despite its common occurrence in different types of freshwater bodies, the parasite fauna had not been a subject of any in depth study. During our studies on the digenean parasites of frogs of the Western Ghats, Wayanad Region, an analysis was made on the species composition of digenean parasites of *E. cyanophlyctis*. This paper deals with the digenean parasites found in E. cyanophlyctis of the Western Ghats, Wayanad Region 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 (latitude 11° 27' and 15° 58' North and 75° 47' and 70° 27' East longitude). Western Ghats is a treasure trove of biological diversity in India and is considered one of the "hottest hotspots" of biodiversity because of its very rich fauna and flora and the highest level of endemism.

Collection localities Birakuppa, Chandhanathodu, Chennalode, Chooralmala, Chundel, Ammana, Karappuzha Dam, Kavummandham, Makkimala, Ondayangadi, Pakkam, Panamaram, Payyampally, Peechangode, Pulpally, Thalappuzha and Wayanad District.

Period of collection: January 2016 to October 2017.

Prevalence and intensity of infection

Prevalence and intensity of infection were measured following Bush et al. (1997).

One hundred specimens of *E*. *cyanophlyctis* collected during the period from January 2016 to October 2017 from various water bodies using sweep hand net were brought to the Laboratory, maintained in cement cisterns and fed them occasionally with insects. The specimens were narcotized with chloroform, dissected, their skins were removed, and the muscle tissues were macerated to detect the presence of metacercariae. Internal organs were also dissected out from each frog, placed in separate Petri dishes containing 0.75% saline, macerated and examined under the stereozoom microscope. Adults, when present, were carefully removed, transferred to 0.75% saline in separate watch glasses and studied under Nikon ECLIPSE Ni-U phase contrast research microscope without supravital staining or after staining with neutral red.

Permanent slides of adult parasites were prepared after fixing them in 5% formalin under slight cover glass pressure and staining with acetocarmine, following the procedure outlined by Cantwell (1981).

Specimens were measured using the Nikon NIS Elements Imaging software. All measurements are in micrometers (μ m), as range followed by mean in parentheses. 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. Photographs were taken with a Nikon Y-TV55 camera.

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.

Results

Five species of digenetic trematodes, *D. mehrai, T. ranarum, H. mehransis, G. glottoides* and *Ganeo tigrinum* were found infecting the duodenum of the frog *E. cyanophlyctis*.

Table 1. Digenean parasites with their prevalence and intensity of infection.

Name of the parasite	Prevalence of infection	Intensity of infection
Diplodiscus mehrai	12 out of 100 frogs (12%)	20 from 12 infected frogs (1.7)
Tremiorchis ranarum	8 out of 100 frogs (8%)	275 from 8 infected frogs (34.4)
Halipegus mehransis	2 out of 100 frogs (2%)	4 from 2 infected frogs (2)
Ganeo glottoides	31 out of 100 frogs (31%	139 from 31 infected frogs (4.5)
Ganeo tigrinum	8 out of 100 frogs (8%)	23 from 8 infected frogs (2.9)

Family Paramphistomidae Fischoeder, 1901

Genus: *Diplodiscus* Diesing, 1836

1. *Diplodiscus mehrai* Pande, 1937 (Figure 1)

The genus Diplodiscus of the family Diplodiscidae Cohn, 1904 was erected by Diesing (1836) and ascribed D. Subclavatus (Pallas, 1760) as its type species. So far 17 species have been added to this genus from amphibians. Of these six valid species have been reported from Indian amphibians by Srivastava (1934b) Pande (1937). Bhalerao (1937), Kaw (1950), Pandey and Chakrabarti (1968), Pandey (1969a), Mukherjee and Ghosh (1972), Dwivedi (1977), Singh (1977) and Shinad and Prasadan (2017a). They are *Diplodiscus* amphichrus Tubangui, 1933. D. amphichrus magnus Srivastava, 1934, D. mehrai Pande, 1937, D. lali Pandey and Chakrabarty, 1968, D. chauhani Pandey, 1969 and *D. cyanophlycti* Shinad and Prasadan, 2017.

Tubangui (1933) described *D. amphichrus* from *Rana* sp. in the

Philippines. Srivastava (1934a) recorded a new variety, D. amphichrus var. magnus from *E. cyanophlyctis* from UP and Pande (1937) added another species, *D. mehrai*, from the same host in Kumaon Hills. Singh (1954) regarded D. amphichrus var. *magnus* and *D. mehrai* as synonyms of *D. amphichrus*. Mukherjee (1966) further synonymised *D. japonicus* (Yamaguti, 1936) with D. amphichrus. Fischthal and Thomas (1968) raised the variety *magnus* of Srivastava to species rank and considered that D. amphichrus of Agarwal and Singh, (1979) as synonym of magnus and so were D. amphichrus D. japonicus and D. mehrai. Pandey and Chakrabarty, (1968) and Pandey (1969a) described two new species, *D. lalli* and *D*. chauhani from *H. tigrina* and *E*. cyanophlyctis, respectively and Pandey and Jain (1974) upheld the validity of D. *mehrai*. Nama and Khichi (1973) described a new sub species, D. amphichrus brevis from E. cynophlyctis and disagreed to the synonymy of D. *mehrai* to *D. amphichrus.* Srivastava (1982) suggested that *D. lali* and *D.* *chauhani* are synonyms of *D. amphichrus*. Srivastava (1982) also considered that the genus is represented by two distinct species *D.amphichrus* and *D.mehrai* in India. Recently a new species, *D. cyanophlycti* was added by Shinad and Prasadan (2017a).

Remarks. The species is characterized by conical, aspinose body with a blunt anterior end and broadly round posterior end. It has a sub terminal and slightly oval oral sucker without oral diverticula and a large posterior sucker at the posterior end of body; it has a small central additional sucker. Its mouth is sub terminal and intestinal caeca is wide, extend along the lateral margin of body up to the level of posterior sucker. It has a single testis at the middle of body and a small cirrus sac behind the intestinal bifurcation. Its eggs are large, oval and operculate.

Twelve out of 100 *E. cyanophlictis* were infected with *D. mehrai* and the prevalence of infection is 12%. Twenty *D. mehrai* were recovered from 12 frogs and therefore, the intensity of infection is 1.7 (Table 1).

Collection localities: Pakkam, Payyambally, Chundel, Birakuppa and Panamaram, Wayanad District.



Figure 1. Diplodiscus mehrai (A1 and A2).

Family: Brachycoelidae Johnston, 1912 Genus *Tremiorchis* Mehra and Negi, 1926

2. *Tremiorchis ranarum* Mehra and Negi, 1926 (Figure 2)

Mehra and Negi (1926a)proposed the genus Tremiorchis of the family Brachycoeliidae Looss, 1899 with *T. ranarum* from *H. tigerinus* as the type species. Ten species of this genus have been reported so far. They are T. ranarum Mehra and Negi, 1926, T. mehrai Rai, 1962, T. vitelloconfluentum (Sic) Rai, 1962, T. attenuates Karyakarte, 1973, T. tigrinanum Simha et al., 1974, T. varanum Verma, 1930, T. mathuraensis Swarup and Jain, 1977, T. spinophlyctis Karyokarte and Palladwar, 1977, T. jamshedpurensis Hussain, 1989, and T. jaini Srivastava and Saxena, 1983. Pandey and Agarwal (1981), with due juistification, considered T. mehrai, T. vitelloconfluentum, T. tigrinarum, Τ. *mathurensis* and *T. spiniphlyctis* as synonyms of T. ranarum. Rajendran and Janardanan (1993) elucidated the life cycle of *T. ranarum*.

Remarks. The species is characterized by elongate - oval body with spines on the surface, except at the posterior third. It has a sub terminal and roughly spherical oral sucker and a ventral sucker at about one - third distance from the anterior end of body. Mouth is sub terminal, pharynx is small and thick walled, oesophagus is long narrow and intestinal caeca terminates just behind the anterior margin of anterior testes. It has a round or transversely ovoid testes and a round ovary. Numerous small eggs are present.

Eight out of 100 *E. cyanophlictis* were infected with *T. ranarum* so the prevalence of infection was 8%. Two hundred and seventy five *T. ranarum* were recovered from eight frogs and the intensity of infection was 34.4 (Table 1).

Few frogs infected with *T. ranarum* exhibited multiple infections with adult trematodes.

Collection localities: Chundel, Panamaram and Pulpally, Wayanad District.



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Figure 2. *Tremiorchis ranarum* (B1 and B2).

Family: Hemiuridae Luhe, 1901 Genus *Halipegus* Looss, 1899 3. *Halipegus mehransis* Srivastava, 1933 (Figure 3)

The genus Halipegus was established by Looss. 1899 with H. ovocaudatus Vulpian, 1859. Altogether 22 species of this genus have been reported from different parts of the world. The nine species reported from India are *H. ovocaudatus* (Vulpian, 1859) Looss, 1899, H. longispina Klein, 1905, H. mehransis Srivastava, 1933, H. mehransis mehransis Srivastava, 1933, H. mehransis minutes Srivastava, 1933, H. spindalis Sruivastava, 1933, H. udaipurensis Gupta and 1967, H. occidualis Stafford, 1905, and H. ambalensis Gupta and Chopra, 1936. Rankin (1944), while reviewing the genus, synonymised H. longispina and H. spindalis with H. ovocaudatus and H. mehransis, respectively. Srivastava (1933a) recorded a new variety of H. Mehransis, H. mehransis var. Minutum. Bhalerao (1936c) abolished the variety and considered it as a synonym of H. mehransis.

Some more species were described under the genus *Halipegus* from India. Klein (1905) described *H. longispina* from Indian frog. Rankin (1944) treated *H. longispina* as synonym of *H. ovocaudatus*, the type species of the genus. Later Rankin (1944) synonymised both species as that of *H. Mehransis*. Gupta and Agrawal (1967) described *H. udaipurensis* from E. *cyanophlyctis* and Gupta and Chopra (1986), *H. ambalensis* from the same host. Pandey (1975) synonymised *H. udaipurensis* and *H. ambalensis* with *H. mehransis*.

Remarks. The species is characterized by smooth, spindle shaped body with slightly tapering anterior end and a blunt posterior end. Sub terminal, circular oral sucker and large, prominent, muscular ventral sucker. Its mouth is sub terminal and intestinal caeca extend laterally up to hind end of body. Its testes are oval, post-acetabular and obliquely side by side and cirrus sac is poorly developed. Genital pore is median, ventral to pharynx and ovary is post testicular and intercaecal. Uterus with large number of closely packed transverse coils occupy most of the intercaecal field in the forebody.

Two out of 100 *E. cyanophlictis* were infected with *H. mehransis* and the prevalence of infection is 2%. Four *H. mehransis* were recovered from two frogs and the intensity of infection is 2 (Table 1).

Multiple infection was observed in one *E. cyanophlictis* with *H. Mehransis*, *G. glottoides* and *D. mehrai*.

Collection locality: Pakkam, Wayanad District.



Figure 3. Halipegus mehransis (C1 and C2).

Family: Lecithodendriidae (Luhe, 1901) Odhner, 1901 Genus *Ganeo* Klein, 1905

4. *Ganeo glottoides* Klein, 1905 (Figure 4)

The genus *Ganeo* of the family Lecithodendriidae (Luhe, 1901) Odhner, 1901 was proposed by Klein (1905) with G. glottoides as its type from E. hexadactylus from Tamil Nadu. Since then 17 species have been added to the genus. They are *G. glottoides* Klein, 1905, G. glottoides glottoides Klein, 1905, G. attenuates Srivastava, 1933, G. bufonis Fotedar, 1959, G. gastricus Srivastava, 1933, G. glottoidea madrasensis Mehra and Negi, 1928, G. korkei Bhalerao, 1936. G. kumaonensis Pande. 1937. G punjabensis Gupta, 1954, G. srinagarensis Kaw, 1950, G. tigrinum Mehra and Negi, 1928, G. gazipurensis Pandey and Chakrabortv. 1968. G. ankholensis Hafeezullah and Dutta. 1985. G. vitellosinistrum Dwivedi and Chauhan, 1971, G. kawi Dwivedi and Chauhan, 1971, G. bahurai Ghosh and Chauhan, 1982, G. lucknowensis Gupta and Jahan, 1976. Fotedar (1959) while reviewing the genus synonymised G. kumaonensis with G. tigrinum. Mukherjee and Ghosh (1970) synonymised G. attenuates with G. tigrinum. G. kawi and G. gazipurensis were synonymised with G. bufonis by Rao (1974) and Gupta and Jahan (1976) respectively. Later Gupta (1977)considered G. bufonis as a synonym of G. tigrinum. Rao and Kameswari (1976) synonymised G. korkei and G. punjabensis with *G. tigrinum*.

Remarks. The species has an elongate oval body with bluntly pointed anterior end, and broadly round posterior end. Body is covered with small spines which are closely set at the preacetabular zone and gradually decrease in number behind ventral sucker. It has a small, circular, sub terminal oral sucker and a slightly larger equatorial ventral sucker. Its mouth is sub terminal and pharynx is thick walled and globular. It oesophagus and has long narrow intestinal caeca. Its testes are obliquely placed one behind the other in front of ventral sucker and round ovary is just below the posterior testes. Uterus is

coiled, filled with oval operculate eggs. Urinary bladder is V-shaped and vitellaria on the lateral margin up to the posterior one – fourth. Cirrus sac is above ventral sucker. Its gonopore opens on the lateral margin just above the position of caecal bifurcation.

Thirty one out of 100 *E. cyanophlictis* were infected with *G. glottoides* and the prevalence of infection is 31%. One hundred and thirty nine *G. glottoides* were recovered from 31 frogs and the intensity of infection is 4.5 (Table 1).

Multiple infection was observed in few frogs with *G. glottoides G. tigrinum*, *D. mehrai*, *H. mehransis and T. Ranarum*.

Collection localities: Makkimala, Peechangode, Kammana, Pakam, Chundel, Panamaram, Chooralmala, Bairakuppa, Kavummandam and Pulpally, Wayanad District.



Figure 4. Ganeo glottoides (D1 and D2).

5. *Ganeo tigrinum* Mehra and Negi, 1928 (Figure 5)

G. tigrinum was described from *H. tigerinus* by Mehra and Negi (1928) and from *Rana* and *Bufo* species by other workers (Mukherjee and Ghosh 1970, 1972; Agarwal and Singh, 1977). It was also reported from two more hosts, *H. crassus* and *Fejervarya* sp. by Diengdoh (1989) and Tandon *et al.* (2001), respectively. Brinesh and Janardanan (2012) described the life cycle of *G. tigrinum.*

Remarks. The species is characterised by elongate-oval body with bluntly pointed anterior end and broadly round posterior end. Body is covered with small spines which are closely set at the pre-acetabular zone and gradually decreased in number behind ventral sucker. Its oral sucker is small. circular. sub terminal, smaller than ventral sucker. Mouth is sub terminal, pharynx is thick walled, globular, and oesophagus is long, narrow and intestinal caeca reaches up to the upper margin of posterior onefifth. Its obliquely placed Testes are larger than ovary, slightly tandem and located above the ventral sucker. Anterior testis is smaller than posterior testis. Vitellaria are present on the lateral margin up to the posterior one-fourth. Cirrus sac is above ventral sucker and gonopore opens on the lateral margin just above the position of caecal bifurcation.

Eight out of 100 *E. cyanophlictis* were infected with *G. tigrinum* and the prevalence of infection is 8%. Twenty three *G. tigrinum* were recovered from eight frogs and the intensity of infection is 2.9 (Table 1).

Multiple infection was observed in a few frogs with *G. tigrinum, G. glottoides* and *D. mehrai.*

Collection localities: Kammana, Pakam, Payyambally, Chundel and Panamaram, Wayanad District.



Figure 5. Ganeo tigrinum (E1 and E2).

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Conflict of interest

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

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