Digenean Trematode parasites in two fresh water fishes *Ophiocephalus punctatus* and *Xenentodon cancila* from North west Himalayas

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**Abstract**

The study was conducted during September 2018-August 2019 to study the digenetic trematode infection in fresh water fishes of some of the water bodies viz. Gho-manhasan, Chakrali and Chadwal of Jammu region of J&K union territory. A total of 220 fishes comprising *Ophiocephalus punctatus* and *Xenentodon cancila* belonging to families Channidae and Belonidae respectively were examined. A total of 4 digenetic trematode parasites belonging to 4 different families i.e., *Euclinostomum heterostomum* (Clinostomidae Luhe, 1901); *Phyllodistomum tripathi* (Gorgoderidae Looss, 1901); *Genarchopsis piscicola* (Hemiuridae, Luhe, 1901), and *Bucephalopsis karvei* (Bucephalidae Poche, 1907) were detected. The overall prevalence of digenetic trematode infection was 65.90% and the mean intensity was 3.58. Among these *Genarchopsis piscicola* showed the highest prevalence (40.38%) with mean intensity 2.95 in the host fish *Xenentodon cancila*, while in other species the prevalence ranged between 26.23 and 34.62%. Present study authenticates the presence of several species of digenetic trematode parasites in the fish inhabiting freshwaters of J&K union territory.

**Keywords** Trematode · Metacercariae · Jammu · Digenetic · *Ophiocephalus punctatus* · *Xenentodon cancila*

**Introduction**

Fish constitutes an important source of food, nutrition, income and livelihood for millions of people around the world. Moreover, fish continues to be one of the most traded food commodities worldwide with more than half of fish exports by value originating in developing countries. Fishes provide a potential habitat to metacercariae of digenetic trematodes. Digenetic trematodes are a diverse group of parasites that use more than one host to complete their life cycle. Avian digenetic trematodes use fishes as secondary intermediate hosts to complete their life-cycles (Bullard and Overstreet 2008). Trematodes are the most diverse group of fish parasite and are present in larval or adult forms in majority of fish species and most of them exist as endoparasites. Digenetic trematode infections cause low weight gain, high mortality and immarketability of the infected fish. Currently, fish-borne zoonotic trematodes are the cause of health problems and also affect the public health in many Asian countries (Murrell et al. 2007; Tran et al. 2008; Phan et al. 2010). The present study was therefore undertaken to ascertain the spectrum of digenetic trematodes from fresh water fishes *Ophiocephalus punctatus* and *Xenentodon cancila* in Jammu region.

**Materials and methods**

A total of 220 fish samples were collected from different freshwater bodies of Jammu region; Gho-manhasan stream (32.69°N latitude, 74.68°E longitude), Chakrali stream (32.70°N latitude, 74.65°E longitude) and Chadwal stream (32.36°N, 75.35°E longitude) (Fig. 1). Collected fish samples were brought alive to laboratory. Fishes were anesthetized and thoroughly examined for presences of parasites by carefully dissecting under Stereoscope. The gastrointestinal tract, muscle tissue and other internal organs of the host fish, thus obtained were teased in 0.8% saline and examined for the recovery of the metacercarial or adult stage of trematode parasites. Trematode parasites were flattened between two slides or under slight pressure of slide and coverslip, post-fixed in AFA (Alcohol, Formalin and Acetic acid in 85:10:5) and stained with Borax carmine. Afterwards, specimens
were dehydrated in ascending series of ethanol, cleared in xylene and finally mounted on glass slides using DPX. Photomicrographs of stained specimens were studied and photographed using microscope Leica M205 C and identified by following the standard literature (Yamaguti 1971; Bray et al. 2008; Pandey and Agrawal 2013). The prevalence of infection and mean intensity (Table 1) were calculated following Bush et al. (1997).

\[
\text{Prevalence} = \frac{\text{No. of hosts infected} \times 100}{\text{Total no. of hosts examined}}
\]

\[
\text{Mean Intensity} = \frac{\text{No. of parasites recovered}}{\text{Total no. of infected hosts}}
\]

**Results and discussion**

Based on morphological and morphometric criteria the recovered trematode parasites were identified as belonging to genera *Euclinostomum* (Rudolphi, 1809) Travassos, 1928 (Family Clinostomiodae Luhe, 1901); *Phyllodistomum* (Braun, 1899 (Family Gorgoderidae Looss, 1901); *Genarchoopsis* Ozaki, 1925 (Family Hemiuridae (Looss, 1899, Luhe, 1901) and *Bucephalopsis* Diesing, 1855 (Family: Bucephalidae Poche, 1907) all of which are redescribed.

1. *Euclinostomum heterostomum* (Rudolphi, 1806) Travassos, 1928 (Fig. 2A1 & A2).
   - **Host:** *Ophiocephalus punctatus* Bloch
   - **Site of infection:** Liver, Coelomic cavity, muscles.
   - **Locality:** Gho-manhasan, Chakrali.
   - **Prevalence:** 33.62% (39 infected out of 116 hosts).
   - **Mean intensity:** 4.68 (182 parasites were recovered from 39 infected host).

**Description**

Cysts of *E. heterostomum* were yellowish and spherical, excretory vesicle, intestinal caeca brownish and are observed on the external surface; excysted metacercariae body elongated, linguiform after fixing measures 4.36–6.54 mm × 2.18–2.49 mm; anterior end of the body truncated and posterior end is rounded; oral sucker sub-terminal, small surrounded by collar-like fold 0.19–0.38 mm × 0.16–0.27 mm; ventral sucker lager well...
Fig. 2 Euclinostomum heterostomum (Rudolphi, 1806) Travassos, 1928 (A1 & A2); Phyllodistomum tripathi Motwani & Srivastava, 1961 (B1 & B2); Genarchopsis piscicola Srivastava, 1933 (C1 & C2); Bucephaloopsis karvei Bhalerao, 1937 (D1 & D2). OS- Oral sucker; VS- Ventral sucker; O-Ovary; T-Testis; UT-Uterus; CD-Caecal diverticula; AO-Anal opening; OES-Oesophagus; GP-Genital pore; SV-Seminal vesicle; E-Eggs; VG-Vitelline gland; EV-Excretory vesicle; P-Pharynx; RH-Rhynchus; VF-Vitelline follicle; CS-Cirrus sac; GL-Genital lobe; GA-Genital atrium
developed, almost round measuring 0.712–0.872 mm × 0.625–0.721 mm; Pharynx rudimentary; oesophagus short bifurcates in front of acetabulum; intestinal caeca long with 7–12 lateral diverticulate branching; anterior testis U-shaped 0.115–0.159 × 0.36–0.462 mm; posterior testis Y-shaped 0.218–0.320 × 0.253–0.374 mm; cirrus sac oval, intertesticular; ovary small, round to oval, intertesticular 0.121–0.146 × 0.140–0.28 mm; uterine sac elongated and club shaped; excretory pore subterminal.

Remarks

The genus Euclinostomum was established by Travassos, 1928 which have well developed diverticulated caeca. Moreover, Euclinostomum is the type genus of subfamily Euclinostominae, found in the buccal cavity and in the oesophagus of piscivorous birds, primarily herons and egrets. Many reports of metacercarial and adult forms of E.heterostomum from freshwater fishes have been studied by several workers across the world (Jhansilakshmibai & Madhavi 1997: Britz et al. 1984: Taher 2009: Purivirojkul and Sumontha 2012; Senapin et al. 2014; Abro et al. 2016; Mansour 2019) from different host fishes. Present fluke exhibits similarities with E.heterostomum Rudolphi 1809; E.bhagavantami Jaiswal, 1957; E.minutus Bhutta and Khan 1975 and E.reticulatum Sudan; 1979 (Table 2). In the present fluke oral sucker is subterminal closely resembles to E.reticulatum but smaller than E.bhagavantami and E.minutus. The number of intestinal diverticulae differs in E.bhagavantami (9–12), in E.minutus (12–15) whereas resembles E.reticulatum with 7–12 diverticulae. The size of ovary, however show resemblance to E.minutus which is quite smaller than that of E.bhagavantami and E.reticulatum.

Thus the present fluke differs from E.bhagavantami, E.minutus and E.reticulatum in body size, size of ovary and testes. The present form closely resembles Euclinostomum heterostomum in size and shape of body and various organs which is reported from host fish Ophiocephalus punctatus of Jammu region.

2. Phyllodistomum tripathi Motwani & Srivastava, 1961 (Fig. 2B1 & B2).

Host: Xenentodon cancila Hamilton
Site of infection: Intestine
Locality: Chadwal
Prevalence: 34.62% (36 infected out of 104 hosts).
Mean intensity: 2.83 (102 parasites were recovered from 36 infected host).

Description

Body dorsoventrally flattened, spatulate 1.58–3.72 mm long, 0.72–2.04 mm maximum width at level of middle region of hindbody; fore-body narrow, elongated 0.89–0.94 mm × 0.43–0.75 mm; hind-body expanded nearly with feebly developed semicircular puckering present on the lateral sides of hind body, 0.95–0.98 mm × 0.78–0.80 mm in size; oral sucker terminal, oval 0.19–0.26 mm × 0.20–0.28 mm; ventral sucker rounded, pre-equatorial slightly larger and wider than oral sucker 0.22–0.28 mm × 0.24–0.33 mm; pharynx and pharynx absent; oesophagus straight, narrow 0.12–0.17 mm long bifurcates into well developed two intestinal caeca which terminate close to hind body end; testes deeply lobed, intercaecal, post-ovarian; right testis post-equatorial 0.16–0.20 mm × 0.14–0.17 mm; left testis equatorial 0.22–0.24 mm × 0.18–0.20 mm; genital pore subterminal.
pre-acetabular; ovary submedian, lobed, pretesticular, 0.07–0.12 mm × 0.06–0.08 mm; two compact vitelline glands, right vitelline gland 0.06–0.07 mm × 0.03–0.04 mm and left vitelline gland 0.07–0.08 mm × 0.04–0.05 mm; eggs oval, non-operculate 0.02–0.03 × 0.01–0.02 mm.

Remarks

The genus *Phyllodistomum* was erected by Braun (1899) for *Distomum folium* Olfers, 1816 as its type species. Based on the narrow and tubular anterior region and spatulated hind body our specimen belonged to the genus *Phyllodistomum*. Different species of the genus *Phyllodistomum* have been reported by several workers (Cribb, 1987; Helt, 2003; Mendoza-Garfias et al. 2005; Ho et al. 2014) from fishes all over the world including that from India (*Ploossi* Kaw, 1950; *P.indianum* Jaiswal, 1957; *P.intestinalis* Motwani and Srivastava, 1961; *P.srivastavi* Rai, 1964; *P.cameroni* Aragwal, 1966; *P.megacotyle* Fotedar, 1969; *P.mansari* Sudan, 1979; *P.triangulata* Sarwat, 2011; *P.betwaensis* Sen, 2014; *P.punctatai* Jithila and Prasad, 2018). The present species resembles *P.loossi* in the relative size of suckers, shape and position of vitellaria, uterus and genital pore. It however differs in the absence of any papillae in suckers and the size of eggs which are smaller and more slender in present form. The present species shows similarities with *P.betwaensis* but different from *P.betwaensis* in size of body, size of oral and ventral sucker and position of Vitelline glands with respect to ovary (Table 3). The present species differ further from *P.punctatai*, a species reported from urinary bladder of *Channa punctata* in ratio of body length to width, size of oral and ventral sucker and various morphological and morphometry features. Present species resembles *P.tripathi* in having the lateral margins of hindbody provided with indentations, however differs in some morphological features and morphometry like length to width ratio of body, size and shape of ventral sucker and size of testes and ovary. Since, most of the characteristics of the present species bear a close resemblance with *P.tripathi* hence the identified as *P.tripathi* reported from freshwater fish *Xenentodon cancila* of Jammu.

3. *Genarchopsis piscicola* Srivastava, 1933 (Fig. 2C1 & C2).

- **Host:** *Xenentodon cancila* Hamilton
- **Site of infection:** Stomach
- **Locality:** Chakrali, Chadwal
- **Prevalence:** 40.38% (42 infected out of 104 hosts).
- **Mean intensity:** 2.95 (124 parasites were recovered from 42 infected host).

### Description

**Body cylindrical, more or less fusiform,** 1.28–2.57 × 0.42–0.79 mm; oral sucker oval, sub-terminal 0.24–0.38 × 0.26–0.47 mm; ventral sucker equatorial, highly muscular and well developed 0.56–0.79 × 0.58–0.80 mm; prepharynx absent; pharynx 0.03–0.07 × 0.09–0.12 mm; oesophagus absent; testes two, roughly triangular or oval in outline, extracaeal; right testis 0.15–0.17 × 0.12–0.14 mm; left testis 0.15–0.17 × 0.13–0.14 mm; pars-prostatica tubular and well developed; metraterm absent; genital pore present below pharynx near intestinal bifurcation; ovary 0.13–0.18 × 0.09–0.15 mm post-testicular, oval, median or dextrally placed between testes and vitelline glands two, oval, compact 0.15–0.20 × 0.08–0.012 mm; eggs oval, operculated bear long filaments 0.032–0.035 × 0.009–0.016 mm.

### Table 2: Comparative measurements of *Euclinostomum heterostomum* with other species of *Euclinostomum* (in mm)

| Characters          | *E. bhagavantami* (Ukoli, 1966) | *E. minutus* (Bhutta and Khan, 1975) | *E. reticulatum* (Pandoh, 1992) | *E. heterostomum* (Purivirojkul and Sumontha 2013) | *E. heterostomum* Present species |
|---------------------|-------------------------------|------------------------------------|--------------------------------|--------------------------------------------------|----------------------------------|
| Mean body size      | 12.7 × 3.37                   | 4.484–6.66 × 1.66–2.424            | 6.40–10.08 × 1.76–3.26         | 5.78–7.77 × 2.25–2.73                            | 4.36–6.54 × 2.18–2.49            |
| Oral sucker         | 0.53 × 0.38                   | 0.235–0.302 × 0.245–0.392          | 0.19–0.32 × 0.24–0.48          | 0.199 × 0.264                                   | 0.19–0.38 × 0.16–0.27            |
| Ventral sucker      | –                             | 0.235–0.392 × 0.245–0.39           | 0.96–1.47 × 0.96–1.76          | 0.978 × 0.930                                   | 0.712–0.872 × 0.625–0.721        |
| Number of diverticula | 9–12                         | 12–15                              | 6–13                          | 10–12                                           | 7–12                             |
| Ovary               | 0.6 × 0.26                    | 0.245–0.333 × 0.235–0.393          | 0.09–0.20 × 0.08–0.24          | 0.179 × 0.149                                   | 0.121–0.146 × 0.140–1.28         |
| Anterior testes     | –                             | 0.48–1.20 × 0.08–0.24              | 0.13 × 0.179                   | 0.115–0.159 × 0.36–0.462                        | 0.218–0.320 × 0.253–0.374 mm     |
| Posterior testes    | –                             | 0.40–1.12 × 0.19–0.40              | 0.530 × 0.680                   |                                                  |                                  |
| Hosts               | –                             | *Channa punctatus*                 | *Trichoplos vittata, Betta splendens, Betta imbellis* | *Ophiocephalus punctatus*           |
| Locality            | –                             | Pakistan                          | Jammu (J&K) India             | Israel              | Jammu (J&K) India                             |
The genus *Genarchopsis* was erected by Ozaki (1925) with *G. goppo* as the type species from the intestine of *Mogurnda obscura*, Japan. Several species of *Genarchopsis* have been reported viz. *G. ovocaudata* Srivastava, 1933 from *Ophiocephalus punctatus*; *G. piscicola* Srivastava, 1933 from *Channa punctatus*; *G. anguillae* Yamaguti, 1938 from *Anguilla japonica* G. folliculate Bhaduria and Dan- dotia, 1954 from *Mastacembelus sp.* and *Channa sp.*; *G. thapari* Dwivedi, 1965 from *Bufo melanostictus*; *G. punctati* Agarwal, 1966 from *Ophiocephalus punctatus*; *G. cameroi* Kakaji, 1969 from *Mystus seenghala*; *G. cuchia* Kakaji, 1969 from *Amphipnous cuchia*; *G. avitellarium* Varma and Sahay, 1983 from *Ophiocephalus punctatus*; *G. kalriai* Bilqees and Khan, 1990 from *Channa marulius*; *G. fellicola* Shimazu, 1995 from *Rhinogobius brunneus; G. gibsoni* Shaikh et al., 2011 from *Ophiocephalus striatus*. The present parasite resembles closely with *G. piscicola*, *G. goppo* and *G. gibsoni* (Table 4). The present form differ markedly from Ozaki’s species *G. goppo* in larger body size, size and ratio of suckers, position of genital pore, absence of oesophagus pouch, location of gonads, position of vitellaria and position of ventral sucker. It differs from *G. gibsoni* in various morphological features and morphometry in body size, size of oral and ventral sucker, position and shape of testes and ovary. But the present form shows comes very close to *G. piscicola* in position of oral sucker, size and ratio of suckers, absence of oesophagus, shape and disposition of testes and position of gential pore. The present species is redescribed as *G. piscicola* and reported from different host *Xenentodon cancila* of Jammu region.

4. *Bucephalopsis karvei* Bhalerao, 1937 (Fig. 2D1 & D2)

*Host: Xenentodon cancila Hamilton*

Site of infection: Intestine

Locality: Chadwal

Prevalence: 26.93% (28 infected out of 104 hosts).

Mean intensity: 3.92 (110 parasites were recovered from 28 infected host).

### Description

**Body** small pear-shaped with broader anterior end and a pointed posterior end measuring 0.57–0.98 mm × 0.31–0.52 mm; tegument covered with spines; rhynchus (anterior sucker) large, spherical, terminal, 0.11–0.23 mm × 0.13–0.20 mm; pharynx muscular, oval 0.05–0.06 mm × 0.04–0.07 mm; esophagus tubular opens into digestive cecum; cecum globular sac-like, median 0.06–0.09 mm × 0.06–0.01 mm; testis oval, unequal, anterior testis 0.12–0.14 mm × 0.08–0.09 mm, posterior testis 0.11–0.13 mm × 0.07–0.09 mm; cirrus sac cylindrical, extent from level of testis to posterior of ovary.
body 0.24–0.28 mm × 0.05–0.07 mm; \textit{pars prostatica} opens into short ejaculatory duct; ovary oval, pre-testicular, 0.06–0.08 × 0.07–0.09 mm; egg numerous oval, 0.017–0.018 × 0.009–0.012 mm.

**Remarks**

Diesing (1855) erected \textit{Bucephalopsis} as subgenus of \textit{Bucephalus} Von Baer, 1827 for \textit{Bucephalus hairreanus} Lacaze-Duthiers, 1834 and was later raised to full generic status by Laebour (1908). Srivastava and Chauhan (1973) treated Bucephaloidea as a junior synonym of Prosorhynchoides and transferred \textit{Bucephaloidea sp.} to the Genus \textit{Prosorhynchoides} based on the presence of simple sucker (rhynchus) and a pretesticular ovary in it. Several species of Genus \textit{Bucehalopsis} (\textit{Prosorhynchoides}) have been reported from India; these include \textit{P.fusiformis} (Verma, 1936) Srivastava and Chauhan 1973; \textit{P.garuai} Verma, 1936; \textit{P.magnum} Verma, 1936; \textit{P.karvei} (Bhalerao, 1937) Srivastava and Chauhan 1973; \textit{P.thapari} (Dayal, 1948) Bott and Cribb, 2005. Maurya et al., (2018) synonymized \textit{P.lateroporus}, \textit{P.gaurii}, \textit{P.chauhani}, \textit{N. (P.) jhansiansis} and \textit{P.canciliansis} with \textit{P.karvei}. The present form belongs to the genus \textit{Bucephalopsis} Diesing, 1855. Bhalerao (1937) reported \textit{B. karvei} from the intestine of \textit{Xenentodon cancila}. The present adult worms were also recovered in large number from the intestine of \textit{Xenentodon cancila} and closely resemble \textit{B.karvei} in body shape, position of testes and ovary, but differ in some morphological and morphometric characters. The present adult worms differs from \textit{P.garuai}, \textit{P.fusiformis} and \textit{B. thapari} in various morphological and morphometric characters size and shape of body, size of rhynchus, position of ovary and testes and also size and disposition of cirus sac and uterus (Table 5). This bucephalid is new locality record observed for the first time in Jammu region, J&K.
Table 5 Comparative measurements of *Bucephalopsis* (*Prosorhynchoides*) karvei with other species of *Bucephalopsis* (*Prosorhynchoides*) (in mm)

| Characters         | *Prosorhynchoides garui* (Verma, 1936) | *P fusiformis* (Verma, 1936) | *P karvei* (Bhalerao, 1937) | *P karvei* Present species |
|--------------------|----------------------------------------|-----------------------------|-----------------------------|---------------------------|
| Body size          | 5.73–6.20×1.38–1.90                    | 1.24–2.52×0.39–0.84         | 0.5–0.96×0.27–0.57          | 0.57–0.98×0.31–0.52       |
| Rynchus (anterior sucker) | 0.56–0.69×0.58–0.69                  | 0.17–0.24×0.18–0.24         | 0.16–0.24×0.142–0.227       | 0.11–0.23×0.13–0.20       |
| Pharynx            | 0.25–0.29×0.25–0.33                    | 0.07–0.08×0.07–0.084        | 0.044–0.06×0.044–0.06       | 0.05–0.06×0.04–0.07       |
| Cecum              | 0.75–1.26×0.21–0.50                    | 0.21–0.46×0.18–0.23         | 0.68–0.09×0.68–0.09         | 0.06–0.09×0.06–0.01       |
| Anterior testis    | 0.71–0.86×0.38–0.66                    | 0.18–0.29×0.17–0.26         | 0.108–0.120×0.082–0.09      | 0.12–0.14×0.08–0.09       |
| Posterior testis   | 0.58–0.67×0.42–0.54                    | 0.16–0.25×0.15–0.21         | 0.108–0.120×0.082–0.09      | 0.11–0.13×0.07–0.09       |
| Cirrus sac         | 0.60–0.92×0.30–0.35                    | 0.46–0.71×0.10–0.14         | 0.23–0.29×0.056–0.063       | 0.24–0.28×0.05–0.07       |
| Ovary              | 0.46–0.67×0.29–0.42                    | 0.14–0.21×0.15–0.19         | 0.056–0.082×0.056–0.082     | 0.06–0.08×0.07–0.09       |
| Eggs               | 0.023–0.028×0.015–0.017                | 0.013–0.023×0.008–0.015      | 0.018–0.002×0.009–0.013     | 0.017–0.018×0.009–0.012   |
| Host               | Silonia silondia                       | Eutropiichthys vacha       | –                           | Xenentodon cancila        |
| Locality           | Prayagraj                              | Prayagraj                   | –                           | Jammu (J&K)               |

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Author’s contribution S.R and S.L designed the study S.R collected the data. Both the authors were involved in writing and reviewing of the manuscript.

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Declarations

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

Ethical approval No approval by an ethical committee was required to achieve the goals of the present study.

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