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Studies on the distribution and diversity of helminth infection in *Xenentodon cancila* (Hamilton, 1822) in Mizoram, Northeast India

C. Malsawmtluangi and Lalramliana*

Department of Zoology, Pachhunga University College, Aizawl-796 001, India

***Corresponding Author Email :** lrl_zoo@yahoo.co.in

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Abstract

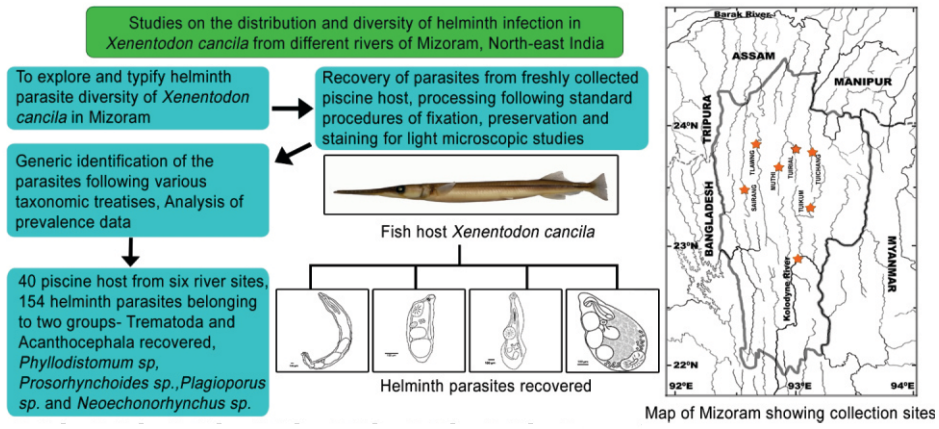
Aim: The aim of the present study was to document the diversity of endoparasitic helminth in fresh water garfish, *Xenentodon cancila* and to assess, their zoonotic potential if any.

Methodology: The collections of fishes were conducted in rivers of Mizoram and parasites were retrieved. Morphological identification was done by following various taxonomic keys.

Results : A total of 40 specimens of garfish were collected from six different locations. Only two taxonomic groups of helminth parasites were recorded; Trematoda (Platyhelminthes)-including three species- one each representing the genera *Prosorhynchoides*, *Phyllodistomum* and *Plagioporus*; and Acanthocephala- represented by *Neoechinorhynchus*. No cestode or nematodes were recorded from this piscine host.

Interpretation: The results revealed that prevalence, mean intensity and abundance were found to be highest in fishes collected from river Tuikum followed by Tuirial. No helminth infection was found in the fishes collected from Chhimtuipui and Tlawng Rivers. Further, no potential zoonotic helminths were recorded in the present studies. All the parasites reported in the present study constitute the first report from the state of Mizoram.

Keywords: Acanthocephala, Helminth parasites, Trematoda, *Xenentodon cancila*



Map of Mizoram showing collection sites

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Introduction

Fishes constitute an important part of the local delicacies and have been extensively used as a source of protein, especially for the rural area, in Mizoram, Northeast India. However, fishes serve as hosts to number of helminth parasites, some of which can even turn out to be potential zoonotic parasites. Helminths cause both infection and diseases in freshwater and marine fishes, their importance being related directly to the fish that may affect the general public health (Hoffman, 1967). Certain helminth parasites are a matter of major public health concern (Yooyen *et al*., 2006). In addition, they may also cause economic loss to farmers, as parasites also compete for food with the fish host, thus, depriving fish of essential nutrients and inhibiting growth leading to morbidity and mortality (Khalil and Polling, 1997).

Consumption of raw or under cooked or processed fish can be the main source of infection for humans, with number of cases being reported from different geographical regions (Park *et al*., 2009). The worldwide number of people infected with small liver flukes only exceeds 45 million and many more are at risk (Keiser and Utzinger, 2009). More than 30,000 helminth parasites have been reported to infect fishes and many of them are harmful to their hosts. Dependency of the parasite on its host and exploitation of the fishes by the helminth parasites provide a useful research model in the field of ecology and evolutionary biology. Studies on the helminth parasitic spectrum in freshwater fishes have been carried out in different parts of the world. In India, many researchers have worked on the helminth parasites of both freshwater and marine fishes, describing new species and have made further advancements in this field using various molecular biological tools,(Dayal, 1949; Srivastava, 1982; Dhole *et al*., 2010). However,in the context of North-east India, reports on this field are meager, and very limited reports are available. Most of the reports are from Manipur, Meghalaya and Assam, where, Shomorendra *et al.* (2005) reported five helminth parasites of fishes from Loktak lake Manipur; Tandon *et al*. (2005),Thapa *et al*. (2008) and Jyrwa *et al*. (2014) reported and made a checklist on the helminth parasite of fishes from Meghalaya; Binky *et al*. (2011) reported three species of Nematode in *Anabas testudineus* in Assam; Singha *et al*. (2015) reported Nematode, Cestode, Trematode and Acanthocephala from three different species of fish from Dolu lake, Silchar, Assam and Koiri and Roy (2016) reported nine different species of helminth parasites in *Clarias batrachus* from Tripura.

In Mizoram, no work has been done to explore the diversity of helminths infecting freshwater fishes. Among many freshwater fishes, predatory fish species harbor a greater diversity and abundance of helminths, compared to herbivorous and planktivorous species (Luque and Poulin, 2004). The present study was undertaken to find out the helminth parasitic spectrum of the predatory fish, *Xenentodon cancila,* from Mizoram Northeast India.

Materials and Methods

Study area: The state of Mizoram covering an area of 21,081 km^2 with geographical isolates of 23.1645° N, 92.9376° E is situated in the southern part of North-eastern India, bordering Bangladesh in the south-west and Myanmar in the east. The major rivers of Mizoram are Tuirial, Tlawng, Tuirini, Tuivai, Mat, Kolodyne, Tuichawng, Karnafuli and Serlui, which can be categorized under three major drainage systems, Barak-Meghna drainage system, Indo-Bangladesh; Kolodyne (Kaladan) drainage system, Indo-Myanmar and Tuichawng-Karnafuli drainage system, Indo-Bangladesh.

Sample collection and morphological analysis: Fishes collected from different rivers were brought to the laboratory. Their external body surface was examined for the presence of ecto-parasites. Fishes were dissected and their internal organs such as heart, lungs, liver, spleen, stomach, intestine, swim bladder, gonads and mesenteries etc., were examined thoroughly for the presence of helminth parasites under dissecting microscope. The helminth parasites recovered were counted, stretched and they were gently flattened between a glass slide and a cover slip, tied with thread and fixed overnight in 70% ethyl alcohol inside a coplin jar. Whole mount preparations were made by staining with Borax carmine, and dehydrated through ascending grades of alcohol, cleared in methyl benzoate and finally mounted using Canada balsam. The permanent slides were observed and studied under stereo microscope. A high-resolution image of the parasites was obtained using stereo microscope in a series of adjacent focal planes. Measurements of the specimens were taken using stage and ocular micrometers. All measurements are expressed in mm unless otherwise stated.

For taxonomic identification of parasites, standard reference works of Yamaguti (1971,1963b, 1958). Keys to the Trematoda Vols. 1-3 (Gibson *et al*., 2002, Jones *et al*., 2005, Bray *et al*., 2008) were referred.

Analysis of prevalence data: Data were recorded on the prevalence and intensity of helminth infection in piscine hosts and the data were analyzed following Bush *et al*. (1997):

Prevalence (P) = the number of infected host with one or more individuals of a particular parasites species (or taxonomic group) divided by the number of hosts examined.

Mean intensity (MI) = the average intensity, i.e., the total number of parasites of a particular species found in a sample divided by the number of hosts infected.

Abundance (A) = the total number of individuals of a particular parasite species in a sample of a particular host species divided by the total number of hosts (including both infected and uninfected) of that species examined.

Statistical Analysis: One-way analysis of variance (ANOVA) was performed using SPSS 20.0 to ascertained and analyse the significant of variation (at p≤0.05) at different sites.

Results and Discussion

The present study gives the first report on the presence of helminth parasites in the fish host, *Xenentodon cancila* from Mizoram, North-east India. During the study period, a total of 40 fishes were examined from 6 different locations, where 154 helminth parasites were recorded belonging to two different groups, Trematoda and Acanthocephala. Prevalence, Abundance and Mean Intensity was found to be highest in the fishes collected from Tuikum followed by Tuirial, Tuichang and Muthi Rivers, but no parasites were retrieved from the fishes collected from Chhimtuipui and Tlawng Rivers (Table 1). Statistical analysis confirmed significant variations on the prevalence of parasites within the six collection sites ($F = 8.160$, $df = 5, 34$; and $p < 0.05$). Furthermore, the occurrence of the four species of parasites in the host *X. cancila* showed significant variations ($F = 15.854$, $df = 3,156$; $p < 0.05$). The trematode parasites collected belonged to the genus *Prosorhynchoides* sp., *Plagioporus* sp., *Phyllodistomum* sp. while *Neoechinorhynchus* sp. belonged to Acanthocephala. The genus *Prosorhynchoides* sp. was found to be the most prevalent group of parasites (50%) followed by *Plagioporus* sp. (17.5%), *Phyllodistomum* sp. (7.5%) while *Neoechinorhynchus* sp. (5%) was the least prevalent among the helminth parasites collected. Mean intensity was also found to be highest in *Prosorhynchoides* sp. (6.3) followed by *Neoechinorhynchus* sp. (2.5), *Plagioporus* sp. (2.43) and *Phyllodistomum* sp. (2). The abundance of *Prosorhynchoides* sp. (3.15) was highest, followed by *Plagioporus* sp. (0.45), *Phyllodistomum* sp. (0.15) and *Neoechinorhynchus* sp. (0.12) (Table 2). The overall prevalence, mean intensity and abundance was highest in the fishes collected from the river Tuikum, located in Serchhip district of Mizoram followed by the fishes collected from river Tuirial. No cestode or nematode parasites were collected during the study period.

Parasitic spectrum: Trematoda

Family: Bucephalidae Poche, 1907; Genus: *Prosorhynchoides* Dollfus, 1929; Host: *Xenentodon cancila;* Locality: Tuikum; Site of infection: Intestine

Description: Body small, inversely pear-shaped, broader at the anterior region than posterior. Body measured 0.168-1.10 mm in length and maximum width was 0.441-0.576 mm at post oral region. Rhynchus (anterior sucker) was large. Digestive cecum globular sac like, usually lies at level of anterior testis. Ovary pretesticular, testes somewhat oval, cirrus sac cylindrical, extended from level of anterior or posterior testis to slightly posterior end of the body. Genital pore terminal at posterior extremity. Vitellaria compact, in 2 groups each with 14–15 rounded follicles arranged in lateral body spaces, anterior extent of vitellaria upto rhynchus.

Remarks: The genus *Prosorhynchoides* was established by Dollfus (1929), with the type species being *Prosorhynchoides ovatus* Linton 1900, having a simple sucker for a rhynchus. *Based*

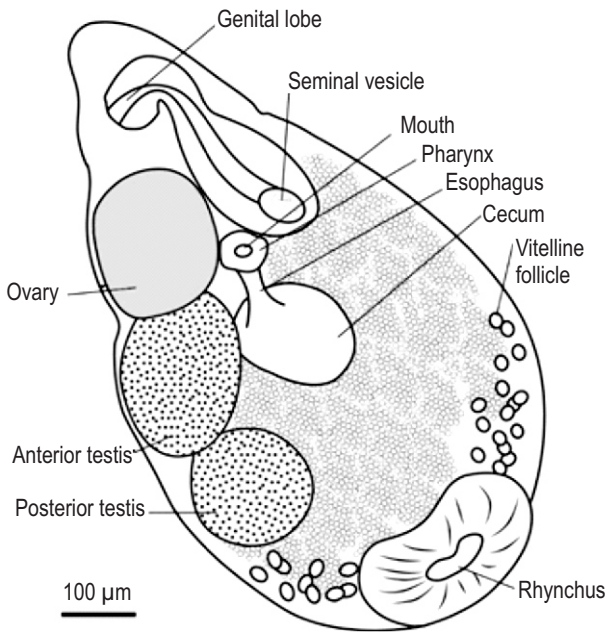

Fig. 1 : Ventral view of *Prosorhynchoides sp*.

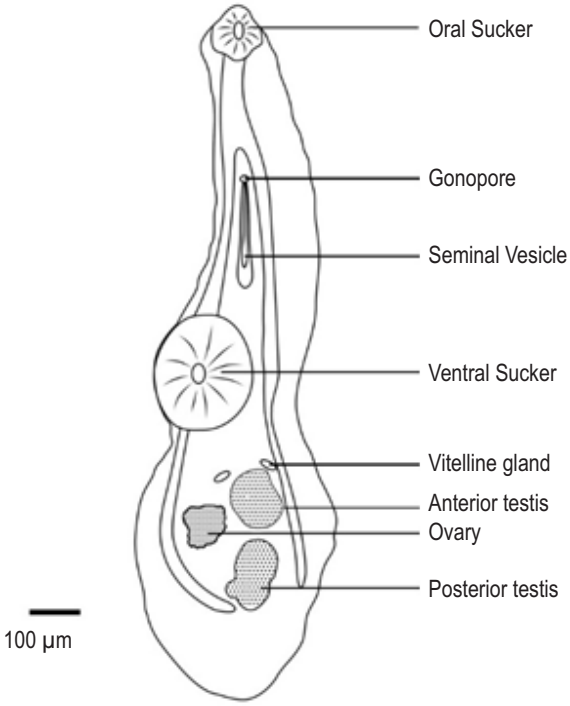

Fig. 2 : Ventral view of *Phyllodistomum* sp.

Table 1 : Diversity of helminth parasites in different collection sites

Locality (Rivers)	No of host examined	No of host infected	Total No of parasites	Prevalence (%)	Mean intensity	Abundance
Tuirial	15	11	58	73.34	5.28	3.86
Tuichang	9	6	17	66.67	2.84	1.89
Tuikum	5	4	73	80	18.25	14.6
Chhimtuipui	2	-	-	-	-	-
Tlawng	5	-	-	-	-	-
Muthi	4	2	6	50	3	1.5

Table 2 : Prevalence of helminth parasites in *Xenentodon cancila*

Helminth parasites	No of host examined	No of host infected	Total No of parasites	Prevalence (%)	Mean Intensity	Abundance
Phyllodistomum sp.	40	3	6	7.5	2	0.15
Prosorhynchoides sp.	40	20	126	50	6.3	3.15
Plagioporus sp.	40	7	17	17.5	2.43	0.42
Neoechinorhynchus sp.	40	2	5	5	2.5	0.12

on the presence of a simple sucker-like rhynchus, ovary anterior to the testes, and a ventrally located mouth. The present specimen belonged to the genus *Prosorhynchoides.*

Several species of the genus *Prosorhynchoides* have been described from the freshwater fishes in India including the checklist, redescription and reviewing of the genus (Chauhan, 1954; Srivastava and Chauhan, 1973; Pandey and Tiwari, 1984; Sen, 2014; Maurya *et al*., 2018). Our specimen was found close to *P. karvei* retrieved from *X. cancila* collected from Gomti river, Lucknow. Comparing their similarities both were more or less similar in size, having a small oval pear shaped body and ovary pre-testicular, the mouth opened at the mid level of the posterior testis and both had globular saclike cecum, and the uterus extended upto the rhynchus (filling most of the anterior body), and possessed ovary well above the level of pharynx, but differed in the extent of the cirrus sac as in our specimen the tubular cirrus sac extended up to the level of anterior testis and differed in the no of their vitelline follicles and by not having any spines on its teguments. This is the first report on the occurrence of the genus *Prosorhynchoides* from Mizoram.

Family: Gorgoderidae Looss, 1899; Genus: *Phyllodistomum Braun*, 1899; Host: *Xenentodon cancila;* Locality: Tuirial; Site of infection: Intestine

Description: Body dorsoventrally flattened, slightly spatulate, divided into a narrow tubular, curved fore body and posterior part of the body extended into bolbous shape. Measuring1.48mm in length and 0.45mm in width across the widest portion. Oral sucker, terminal, slightly oval measuring 0.18× 0.15mm, mouth opening ventrally, no noticeable papillae on oral sucker. Ventral sucker measuring 0.27×0.23mm was larger than oral sucker. Muscular pharynx present. Ovary oval parallel to anterior testis, post-equatorial, testes two in number located in the broadest part of hind body, post-equatorial, tandem, inter-caecal and lobed.

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*. *Phyllodistomum* Braun, 1899 (Trematoda: Gorgoderidae) comprising more than 110 species, and is one of the two largest genera of trematodes (Kudinova 1994; Cribb *et al.,* 2002). Different species of the genus *Phyllodistomum* have been described by several workers in India; these are *P. spatulaeforme* Odhner, 1902; *P. lewisi* Srivastava, 1938; *P. macronium (*Dayal, 1938) Yamaguti, 1958; *P. callichrius* Dayal, 1942; *P. vachius* Dayal, 1949; *P. loossi* Kaw,1950; *P. singhiai* Gupta, 1951; *P. vittatusi* Gupta, 1955; *P. parorchium* Jaiswal, 1957; *P. indianum* Jaiswal, 1957; *P. chauhani* Motwani and Srivastava, 1961; *P. tripathi* Motwani and Srivastava, 1961; *P. srivastavi* Rai, 1964; *P. cameroni* Agarwal, 1966. Recently *P. triangulata* Sarwat, 2011; *P. laximibai* Sen and Siddiqui,2013; *P. betwaensis* Sen, 2014;*P. batrachii* Vankara *et al*., 2016 and *P. punctati* Jithila and Prasadan, 2018). Our specimen differs in the shape and size of the oral sucker and the vitelline gland when compared with *P. laxmibai* described from the same fish host *X. cancila*. *P. batrachii* resemble our specimen with regards to the size and overall morphology, however, the size of the acetabulum and the anterior part is markedly different from our specimen. *P. punctati* from *C. punctata* in the Western Ghat, is much broader and bigger in size and the oral sucker is larger than the ventral sucker (vs. the ventral sucker is larger than oral sucker in our specimen). Nevertheless, the present study is the first report on the occurrence of the genus *Phyllodistomum* from the state of Mizoram.

Family: Opecoelidae Ozaki, 1925; Genus: *Plagioporus Stafford,1904;* Host: *Xenentodon cancila;* Locality: Tuikum; Site of infection: Intestine

Description: Body elongate measuring 0.8–0.97x0.24–0.25 mm, a spinose, forebody distinctly tapered, oral sucker terminal, oral sucker visibly smaller than acetabulum 0.15x0.14 mm which is located in anterior of the body, testes tandem in posterior third of the body anterior testis 0.11x0.13 mm, posterior testis 0.14x0.15 mm eggs large measuring 0.06x 0.02 mm and elliptical in shape, Ovary ovoid just anterior to anterior testis, pharynx located behind the anterior sucker, Vitellaria extending to the anterior region near the acetabulum.

Remarks: In India, the genus *Plagioporus* Stafford, 1904 is represented by only one species, *Plagioporus panchax* found in freshwater fishes. Our specimen was much smaller in size as compared to *P. panchax and P. (Caudotestis) mujibi* described by Bilquees (1972) from Pakistan. Mukesh and Gambhir (2016) have reported a new species *P. (Caudotesti)* minutus in loach, *Schistura manipurensis* from Manipur which was smaller in size as compared to our specimen. The present study gives the first report on the occurrence of *Plagioporus* sp. from the state of Mizoram.

Acanthocephala; Family: Neoechinorhynchidae Van Cleave, 1919; Genus : *Neoechinorhynchus* Hamann, 1892; Host: *Xenentodon cancila;* Locality: Tuichang; Site of infection: Intestine

Description: Acanthocephala was found in the fishes collected from Tuichang river only. Male body was 5.6mm long and the broadest region measured 0.5mm. Trunk aspinose, small, cylindrical bowed or straight, proboscis short somewhat globular, proboscis receptacle subcylindrical somewhat short, lemnisci digitiform to filiform, lemnisci twice as long as proboscis sheath, testis in posterior half of the trunk, testis oval, cement gland syncytial and cement reservoir rounded, everted bursa, proboscis receptacle short, simple, single-walled sac. Lemnisci slender, elongate, unequal.

Remarks: Hamann (1892) described the genus *Neoechinorhynchus* with type species *N. ruttili*. *Neoechinorhynchus* are parasites of freshwater, brackish water and marine fishes, also of amphibians and chelonian. Even though in our specimen the proboscis was not everted but considering the body shape and size, arrangement and shape of the testis, unequal lemnisci, shape of cement gland and cement reservoir confirmed genus *Neoechinorhynchus.* Bhatacharya (1998) reported 140 species of Acanthocephala from fishes in India, indicating high degree of regional endemism. A total of 18 species of *Neoechinorhynchus* sp. were reported from fishes in India (Tripathi, 1959; Chandra *et al*., 1985). Since the proboscis is not fully everted in our specimen, the number of spines cannot be ascertained, and, the absence of female specimen makes it difficult to identify upto the species level. This is the first report on the occurrence of *Neoechinorhynchus* sp. from the state of Mizoram. The high prevalence, mean intensity and abundance of parasites of fishes collected from Tuikum and Tuirial Rivers may be attributed to the fact that both these rivers flow near human settlement, especially river Tuirial which is located near the city dumping ground. This is in agreement with the reports of Moller

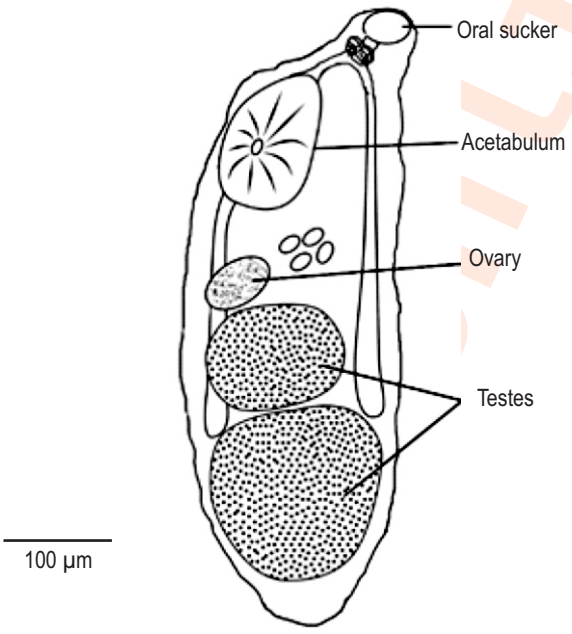

Fig. 3 : Ventral view of *Plagioporus* sp.

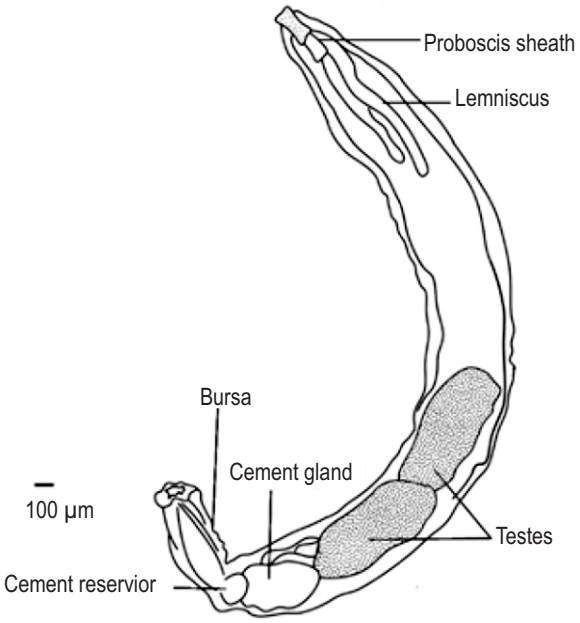

Fig. 4 : Ventral view of *Neoechinorhynchus* sp. male.

and Anders (1986) who concluded that fish from more polluted water tend to harbor more helminth parasites than those from less polluted waters. Subsequently, People living near fresh water bodies were found to have a 2.15-fold higher risk of parasitic infections than those living farther from the water (Keiser and Utzinger, 2005). However, no parasites were recovered from the fishes collected from Tlawng and Chhimtuipui Rivers which may be attributed to the availability of fewer fish host.

Of all the specimen collected in this study, the genus *Prosorhynchoides* sp. was the most prevalent and abundant group whereas Acanthocephala showed minimum prevalence. Similar to our findings, Kundu *et al.* (2015) reported that among the helminth parasites collected from *C. punctata* in West Bengal, an Acanthocephalan group showed minimum prevalence. Contrastingly, Ningthoukhongjam *et al*. (2015), from Manipur, reported that the Acanthocephalan infection in *C. striata* was as high as 100%. Gautam *et al*. (2018) reported that an Acanthocephalan, *Pallisentis* sp., was the most prevalent and abundant group in *Channa* sp. (46.5% in *C. punctata* and 59.11 % in *C. striata*). The differences in prevalence, mean intensity and abundance of the parasites may be attributed to various environmental factors such as physico-chemical factors of the water, geographical location of the collection sites and fauna present in and around the habitat etc., which can affect the host-parasite relationship. Many studies were performed on the helminth parasitic fauna of freshwater fishes, its frequency and distribution in many parts of the world.

Fish borne helminth zoonoses is an important part of food borne helminth zoonoses (Eiras *et al*., 2018). At least 13 species of Diphyllobothrium have been reported from humans, with infections by *D. latum* and *D. dentriticum* being the most prevalent, (Adam *et al*., 1997). With approximately 20,000 reported worldwide Anisakidosis is the most representative fish-borne nematodiasis. Most cases of human infections are caused by *Anisakis simplex*, *A. physeteris* and *Pseudoterranova decipiens* (Hochberg *et al*., 2010). *Capillaria phillipinensis* which cause intestinal capillariasis had been considered to be one of the important zoonoses. It was first found in northern Luzon, Phillipines (*Cross et al.*, 2007) and further reported from North-east Thailand (Saichua *et al*., 2008). Another important fish borne parasite, *Clonorchis sinensis*, is prevalent in Asian countries including South Korea, China, northern Vietnam and far-eastern Russia (Rim, 2005; Qian *et al*., 2016). Currently, over 15 million people are infected with *C. sinensis* worldwide, and 1.5–2 million people show symptoms or complications (Hong and Fang, 2012). Most cases of clonorchiasis occur in low- or middle-income countries of Asia and almost 5591 people died from the infection each year (Furst *et al.*, 2012). Considering their zoonotic importance and their effect on humans it would be interesting to find out their occurrence in our study. However, we did not come across any of these important zoonotic helminths and no records were available within our study areas.

Helminth parasites are important as they not only cause diseases in fishes but also, form an essential component of global biodiversity (Poulin and Morand, 2004). In India, studies on the diversity of helminth parasites of different freshwater fishes has been carried out by several researchers, however, reports on the parasites of this particular fish *X. cancila* is meagre. Sharmin *et al*. (2003) had reported the endoparasitic helminth of *X. cancila* in Bangladesh, in which they showed the presence of trematode, Acanthocephala and a Nematode, but, all the species that they reported were different from this study. In Bangladesh, Hoque *et al*. (2006) reported two monogenean and one cestode from *Mystus aor* where they reported *Bucephalopsis karvei* (a synonym of *Prosorhynchoides karvei*) from *X. cancila*, similar to our present report. Beevi and Radhakrishnan (2012) reported the metazoan parasites of freshwater fishes from Kerala and found that the family Belonidae (to which *X. cancila* belong) showed the lowest parasitic fauna. The present studies contribute in enriching the data on helminth parasitic diversity of *X. cancila* from Mizoram, North-east India. Kennedy *et al*. (1986) demonstrated that the parasite fauna of freshwater birds and mammals are richer and more diverse as compared to fresh water fishes, and that, species richness and mean intensity of parasites of freshwater fishes is less than that of marine fishes. This conforms our findings in which only three Digenea and one Acanthocephala species have been reported and no cestode or nematode are retrieved.

In conclusion, the helminth communities of the freshwater fish, *X. cancila,* in Mizoram are species-poor, and that, considerable proportion of fish from the region is uninfected or lightly infected. A detailed account on the helminth parasitic infection of fishes in the region covering larger areas and wider species of fish hosts need to be investigated. Further work is required to supplement the morphological study with molecular data in order to accurately identify the helminth parasites.

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