

**OCCURRENCE OF PROTOZOAN PARASITES OF
CHANNA PUNCTATUS IN BANGLADESH**

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Abstract

Three species of myxozoa (*Henneguya chaudhuryi*, *Henneguya bengalensis* and *Myxobolus* sp.) and 3 species of ciliophora (*Trichodina pediculus*, *Epistylis lwoffii* and *Apisoma piscicolum*) and two actinosporean stages of myxozoa were identified. Some 51.72% of total host fishes which were found infected with at least one of the above mentioned parasites with average load of 95.93 ± 41.53 per infected host. High percentage (98.05) of *C. punctatus* possessed myxozoan infection and 1.95 had ciliophoran infection. The highest prevalence of parasitic infection was observed in host sample collected from Faridpur district and lowest (33.33%) in fish sample collected from Mymensingh. The association of parasitic infection of *H. bengalensis* and *Myxobolus* sp. with study areas was found statistically significant ($p=0.024$ and 0.049 , respectively). Protozoan parasites were most abundant in gills of hosts. Shannon Diversity Index indicated that host fishes were not infested by more parasites and the parasite community was poorly diverged in all study sites. However, Simpson's Diversity showed that, parasites community was moderately diverged in host fishes collected from Mymensingh district and in rest of the areas parasite community was poorly diverged.

Key words: Protozoa parasites, Prevalence, Diversity, *Channa punctatus*

Introduction

Parasitic infections are the major issues causing low productivity in fish farming as well as in open water bodies (Dogiel 1961, Kayis *et al.* 2009). Because of its inherent difficulty in perusal compared to other larger parasitic fauna, protozoan parasite exploration has been neglected for a long time. Both ecto- and endoparasitic protozoa occupy a very important role in fish growth retardation and nutrition that most often results in multiple fish pathogenesis as being one of the hazardous threats to fish health (Enayat 2011). Most common pathogenesis can be detected as: (i) massive destruction of skin and gill epithelium of fish with internal damage and (ii) loss of appetite and ability to swim properly; sometimes it can be fatal even in the case of mild infection (Enayat 2011). Parasites generally increase in abundance and diversity in more polluted waters

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which indicates the quality of the water (Poulin 1992). Apart from mortality and treatment expenses, growth retardation during disease outbreak can cause economic loss that influences against expansion of aquaculture (Omeji *et al.* 2011).

A considerable number of studies have been conducted on the protozoan parasites of *C. punctatus* in Bangladesh (Sanaullah 1996, Arthur and Ahmed 2002, Miah *et al.* 2013, Deb *et al.* 2015, Akther *et al.* 2018). From these studies a limited knowledge about the species identification, taxonomy, distribution and prevalence of protozoan infection was found in the host. However, precise information regarding prevalence and intensity of the protozoan parasites of this host was not mentioned. Therefore, it seems to be essential to know the current status of protozoan infestation in the wild fish of Bangladesh. The present study was an attempt to assemble a base line data of protozoan parasites of one of the important fish species, namely of *Channa punctatus* in Bangladesh.

Materials and Methods

Collection of host sample: The host species *Channa punctatus* was sampled from the freshwater bodies of Kishoreganj (Kuliar char- 24°10'40" N, 90°50'57" E), Mymensingh (Ishawrganj-24°41'16" N, 90°35'58" E), Faridpur (Dumain union 23°32'50" N, 89°31'22" E) and Jashore (Purondorpur, Jhikorgacha Upazila- 23°5'51" N, 89°5'53" E). A total of 29 fish specimens were collected alive with the help of fishermen from mid-April, 2018 to end of the March, 2019. Sample sizes collected from each area were not equal. However, area wise sample size was: Kishoreganj-5, Mymensingh-9, Faridpur-5 and Jashore-10 fishes.

Sample preparation and examination: Immediately after collection, the external surfaces of the fish were observed carefully using a magnifying glass. Specimens were kept moist during examination by spraying them with a fine mist of water. The collected host fishes were examined as soon as possible after capture. Samples were collected from the body slime, gill slime and blood of the host fishes which are generally the best site to colonize for protozoan parasites. Smears of body slime, gill slime and blood were placed on glass slides on the spot of collection of fishes and fixed them in ethanol solution for the observation in the Parasitology laboratory, Department of Zoology, University of Dhaka.

Klein's dry silver impregnation method: It was applied to detect the presence of ciliates in body slime and gill slime. Mucus was scraped gently off gills and skin with a scalped, spread thinly on a grease-free slide, and dry rapidly. The slide was covered with a 2% aqueous solution of silver nitrate (AgNO₃) for 8 min. After that they were rinsed

thoroughly with distilled water and were placed facing up in a dish of distilled water and expose to bright sunlight for 1 - 2 hours. Finally, they were allowed to dry and mount with a neutral medium, Canada balsam.

Giemsa's stain after acid hydrolysis- To detect the parasites in blood sample, the slides were stained using Giemsa stain and cover slipped by DPX mountant. During this process smears were fixed in Schaudinn's fluid and rinsed well in distilled water. After that they were hydrolysed for 8 min in 1N HCl at >60°C. Again, they were rinsed for several times in distilled water and stained with stocked Giemsa's stain (diluted 1:20 with water at pH 7.0 - 7.2) for about 20 min and rinsed with tap water. Then they were allowed to dry directly and finally mounted with a neutral medium, Canada balsam.

The slides were carefully examined under microscope (40x and 100x) to note the presence or absence of protozoan parasites. Counts of parasites found in selected organ were recorded. The numbers of examined parasites were counted for statistical analysis and microscopic photographs were made for identification of species with the help of 10-megapixel digital camera.

Protozoans were identified according to the description of Lom and Dyková (1992), Eiras (2002), Kalavati and Nandi (2007) and Bashě and Abdullah (2010). Some parasites could not be identified up to species level (*Myxobolus* sp.) because these were not seemed to be matched with any of the available published description. Moreover, it appeared reasonable to make detailed observation to come to a conclusion.

Calculation: Prevalence, mean intensity and abundance of infection were calculated according to Margoles *et al.* (1982). Simpson's Diversity index (Simpson 1949) was used to evaluate for both richness and abundance of parasites within the samples, which was counted by the formula: $D = 1/C$ where, $C = \sum P_i^2$ ($P_i^2 = (N_i/N_T)^2$); here, P_i is the proportional abundance of the i^{th} species. Shannon's Diversity index (Shannon and Weaver 1949), which measures the "information content" of a sample unit, was used to measure the diversity and it was calculated by the formula: $H = -\sum_{i=1}^s P_i \ln P_i$ where, P_i is the proportion of individuals found in the i^{th} species and \ln is the natural logarithm. A greater number of species and a more even distribution both increased diversity as measured by H.

The most commonly used index of evenness is that based on the Shannon - Wiener index (Pielou 1977) which was calculated by the formula: $E = \frac{H}{\ln s}$.

Margalef Index of species richness (Margalef 1958) was used to evaluate the richness of parasites species within the samples, was calculated by the formula: $R = (S - 1)/\ln(n)$.

Data analysis: Statistical analyses were carried out using Microsoft Excel 2010 and IBM SPSS version 20. Fisher's Exact test (as the sample size was small fisher exact test was done instead of Chi square test) was performed and level of significance was set at $p \leq 0.05$.

Results and Discussion

During the investigation, the protozoan parasites were collected from body slime and gills but no parasites were found in blood samples of host fishes from the four study sites. A total of 1437 protozoan individuals were collected from different body parts of 15 infected *C. punctatus* (out of 29 fish examined). Among them 1409 (98.05%) were Myxozoan and 28 (1.95%) were Ciliophoran parasite. Six genera/species and two Actinosporean stages were encountered from *C. punctatus* during this study (Fig. 1). Of them there were 3 Myxozoan (*Henneguya chaudhuryi*, *Henneguya bengalensis* and *Myxobolus* sp.) and 3 Ciliophoran (*Trichodina pediculus*, *Epistylis lwoffii* and *Apisoma piscicolum*) and two Actinosporean stages of Myxozoan parasite were found (Table 1).

Among the parasites *Trichodina pediculus* was previously recorded in *C. punctatus* in Bangladesh (Deb *et al.* 2015, Akther *et al.* 2018). *Henneguya chaudhuryi* (Bajpai and Halder 1982, Chaudhary *et al.* 2017) and *Henneguya bengalensis* (Raychaudhuri and Chakravarty 1970) collected from *C. punctatus* were previously recorded in India but recorded as a new locality in the present study for the first time in Bangladesh (Table 2). The rest two parasite species, namely *Epistylis lwoffii* and *Apisoma piscicolum* were recorded first in the host fish in Bangladesh (Table 2). Additionally, two Actinosporean stages of Myxozoa were found in the present study. *Trichodina cyprinocola*, *T. pediculus*, *Trichodina* sp., *Chilodonella* sp., *Ichthyobodo* sp., *Actinophrys* sp., *Ichthyophthirius* sp. and *Myxobolus* sp. were recorded from *C. punctatus* in Bangladesh in various other studies (Deb *et al.* 2015, Miah *et al.* 2013, Akther *et al.* 2018) and *Trichodina cobitis* were found from *Channa striatus* in Bangladesh (Asmat *et al.* 2017).

The Actinosporean stage of Myxozoa had not been reported as yet in *C. punctatus* host in this locality. Wolf and Markiw (1984) discovered that an Actinosporean (triacinomyxon) is a required alternate life cycle stage of *M. cerebralis*. Similar life cycles have now been described for around 25 species assigned to the genera *Myxobolus*, *Henneguya*, *Sphaerospora*, *Ceratomyxa*, *Myxidium*, *Zschokkella*, *Thelohanellus*, *Hoferellus*, and *Tetracapsula* (Kent *et al.* 2001).

Among the parasites found, *Trichodina pediculus* had the highest prevalence (24.14%) and *Apisoma piscicolum* showed the lowest prevalence (3.45%) (Table 3). Mean intensity of parasitic infection varied from 1.5 ± 0.41 to 240 ± 62.81 in *C. punctatus* (Table 3). Of

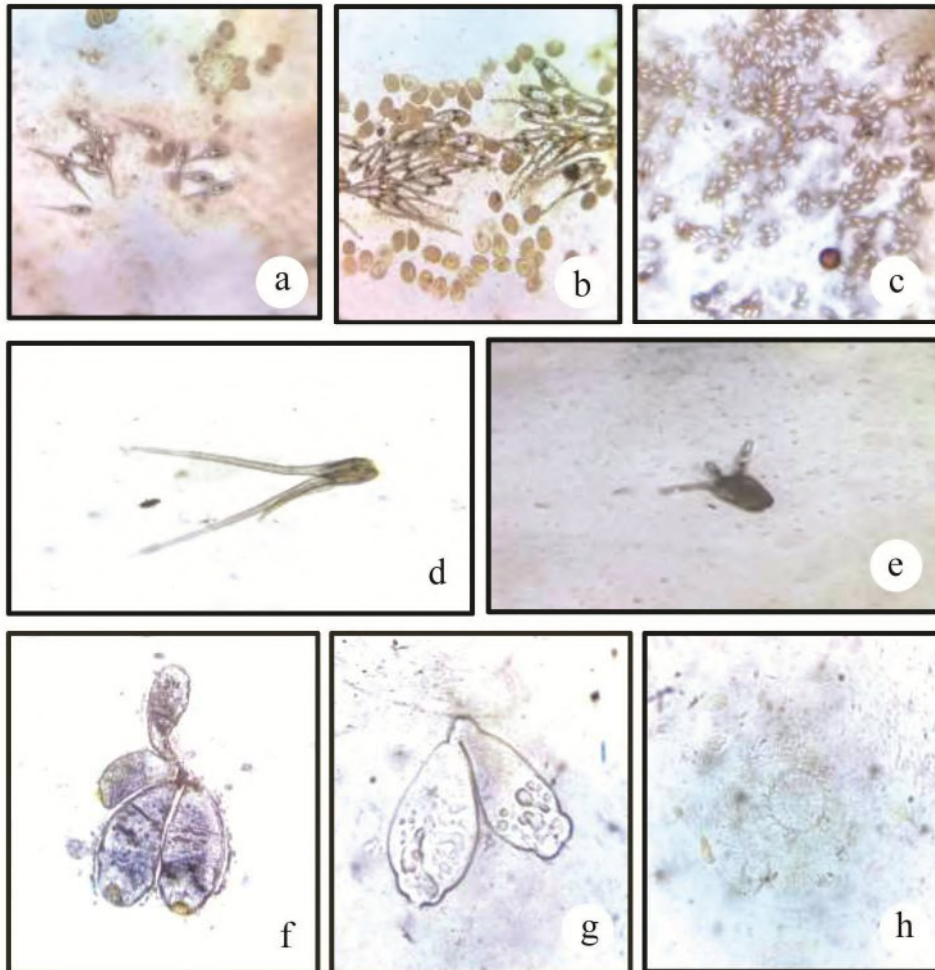


Fig. 1. Photomicrograph of different protozoan parasites. (a) *H. bengalensis* (100x), (b) *H. chaudhuryi* (100x), (c) *Myxobolus* sp. (100x), (d-e) Actinosporean stage of Myxozoa (observed in 100x), (f) *A. piscicolum* (100x), (g) *E. lwoffii* (40x), (h) *T. pediculus* (100x). Fig. a, b, c, f = Stained with silver nitrate impregnation.

the parasites found in the different body parts of *C. punctatus*, *Henneguya bengalensis* (55.05%) was the most abundant and Actinosporean stage showed the lowest abundance (0.21%) (Table 3).

Chaudhary *et al.* (2017) reported higher prevalence (59.3%) of *C. punctatus* infected by *Henneguya chaudhuryi* in India which was relatively higher in comparison with that of the present study. Deshpande and Verma (2015) reported that approximately 28.1% *Channa striatus* were found to be infected with *Myxobolus* sp. in India which was recorded higher than in this study. Deb *et al.* (2015) reported that almost 3.33% *C. punctatus* were infected by *Trichodina pediculus* and around 33.33% of host fish infected

Table 1. List of protozoan parasites recorded from *Channa punctatus*.

Group of the parasites	Name of parasites	Sampling area	Site of infection
Myxozoa	<i>Henneguya chaudhuryi</i>	Jashore	Gill
	<i>Henneguya bengalensis</i>	Faridpur, Jashore	"
	<i>Myxobolus</i> sp.	Kishoreganj	"
	Actinosporean stage	Mymensingh	Body slime
	<i>Trichodina pediculus</i>	Mymensingh, Faridpur, Jashore	Body slime, Gill
Ciliophora	<i>Epistylis lwoffii</i>	Faridpur, Jashore	Body slime
	<i>Apiosoma piscicolum</i>	Jashore	"

Table 2. Updated list of protozoan parasites from *Channa punctatus* in this region (Bangladesh, India and Pakistan).

Parasites	References*
<i>Henneguya chaudhuryi</i> Δ	Bajpai and Halder 1982, Chaudhary <i>et al.</i> 2017, Present study
<i>Henneguya bengalensis</i> Δ	Raychaudhuri and Chakravarty 1970, Gupta and Khera 1987, Present study
<i>Myxobolus</i> sp.	*
Actinosporean stage Ω Δ	Present study
<i>Trichodina pediculus</i> §	Deb <i>et al.</i> 2015, Present study
<i>Epistylis lwoffii</i> Ω Δ	Present study
<i>Apiosoma piscicolum</i> Ω Δ	"

*References of parasites identified up to genus level have not been included in this chart. Ω New host record; Δ New locality record in Bangladesh; § Previously recorded in Bangladesh.

by *Trichodina cyprinocola* in their study. Approximately 32.50% of *C. punctatus* were found to be infected with *Trichodina* sp. (Miah *et al.* 2013) in Bangladesh and interestingly Trichodinids were neither host specific nor site specific (Thilakarathine *et al.* 2003). Prevalence of *Apiosoma* sp. was recorded 8.33% (Mofasshalin *et al.* 2012)

previously in Bangladesh from skin of *Cirrhinus reba* but no infection status was recorded in *C. punctatus* in Bangladesh. *Apisoma piscicolum* showed a quite wide range of host variability such as Europe, Asia and South Africa (Li *et al.* 2007). Mixed infections of *Epistylis lwoffii* and *Apisoma piscicola* were found in the fry of *Salvelinus fontinalis* in Canada (Cone and Odense 1987). Similar findings were also recorded in the present study.

Table 3. Overall prevalence (%), mean intensity and abundance (%) of different species of protozoan parasites in *C. punctatus*.

Name of parasites	No. of fish		Prevalence (%)	Parasites collected	Mean Intensity (\pm Sd)	Abundance (%)
	Examined	Infected				
<i>Henneguya chaudhuryi</i>	29	2	06.90	480	240.0 \pm 62.81	33.40
<i>Henneguya bengalensis</i>	29	4	13.79	791	197.8 \pm 85.33	55.05
<i>Myxobolus</i> sp.	29	2	06.90	135	67.5 \pm 22.36	9.39
Actinosporean stage	29	2	06.90	03	1.5 \pm 0.41	0.21
<i>Trichodina pediculus</i>	29	7	24.14	14	2.0 \pm 1.02	0.97
<i>Epistylis lwoffii</i>	29	3	10.34	10	3.3 \pm 1.11	0.70
<i>Apisoma piscicolum</i>	29	1	03.45	04	4.0 \pm 0.74	0.28

In *C. punctatus*, single species of parasitic infections was found to be higher than multiple species infection at a time. In fact, 48.28% host fish had no parasitic infection. None of the host harboured more than 3 parasitic species at a time. No previous record was available on multiple infections of protozoan parasites in the host fish. However, Kaur and Katoch (2016) reported 65.15% mixed infection of Myxozoan species at a time in native carp fish and that result was slightly similar to this study.

The occurrence of protozoan infestation also varied in different organs of fish body. During the study, the highest prevalence (44.83%) was found in gill slime of *C. punctatus* than that of the body slime (41.38%) (Fig. 2). This could be explained as the gills are the centre of filter feeding and the site of gaseous exchange. No parasites were found in blood sample of *C. punctatus* during this investigation. Emere and Egbe (2006) reported highest load of protozoan parasites in the gill of host species *Synodontis clarias* and Nyaku *et al.* (2007) reported highest load of protozoan parasites in the gills of

Auchenoglanis occidentalis, *Oreochromis niloticus* and *Bagrus bajad* in River Benue. According to Roberts and Somerville (1982), the sieving ability of the gill rakers might help to trap some organisms and this could be attributed to the presence of the protozoan parasites on that site.

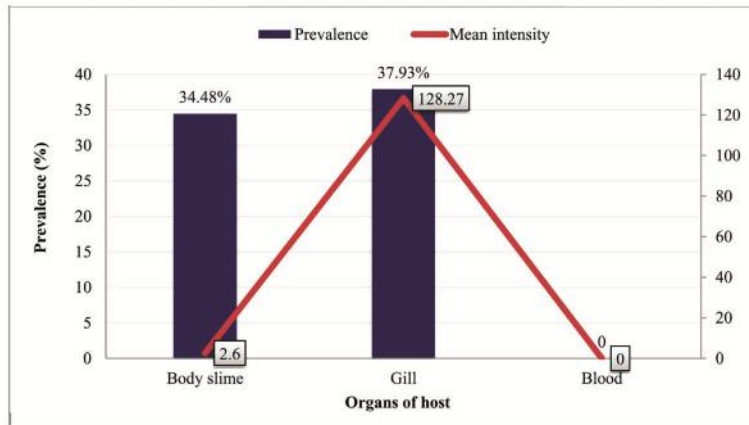


Fig. 2. Prevalence and mean intensity of parasitic infestation in organs of *C. punctatus*.

During the study, the highest prevalence (%) of parasitic infection of *C. punctatus* observed in Faridpur (100%) district which was followed by Jashore (50%), Kishoregonj (40%) and Mymensingh (33.33%) district, respectively (Fig. 3). Fisher's Exact test showed that the association of parasitic infestation with study areas was not statistically significant ($p=0.118$, since $p\leq 0.05$) in *C. punctatus*. The highest mean intensity was found in samples collected from Jashore district (173.8 ± 60.44) and lowest mean intensity (2.67 ± 0.58) was found in Mymensingh district (Fig. 3).

In the present investigation, *Trichodina pediculus* was recorded in three study areas with highest prevalence in Faridpur (60%) and lowest in Jashore (20%) (Table 4). *Henneguya bengalensis* and *Epistylis lwoffii* were recorded in two study areas where highest prevalence of both parasitic species were found in Faridpur and rest of the four species were found only in one study area (Table 4). Fisher's Exact test showed that the association of parasitic infestation of *Henneguya bengalensis* and *Myxobolus* sp. with study areas was statistically significant ($p=0.024$ and 0.049 , whereas $p\leq 0.05$) in *C. punctatus*. Infections of rest of parasite species with samples sites were not statistically significant.

The site wise comparison of richness value showed that Jashore had the highest (0.550) species richness in *C. punctatus* (Table 5). The lowest value was observed in Mymensingh (0.138) district (Table 5). Evenness of parasitic distribution in Mymensingh showed moderately higher value (0.955) indicated that community structure was well constructed by evenly distribution of all parasite species that was well diverged (Table 5). In Jashore (0.465) and Faridpur (0.152) had poor evenness value which meant that parasite community was poorly constructed and low diverged (Table 5). In Kishoreganj area parasite community showed no evenness, richness and diversity in *C. punctatus* because there was found only one protozoan parasite (Table 5).

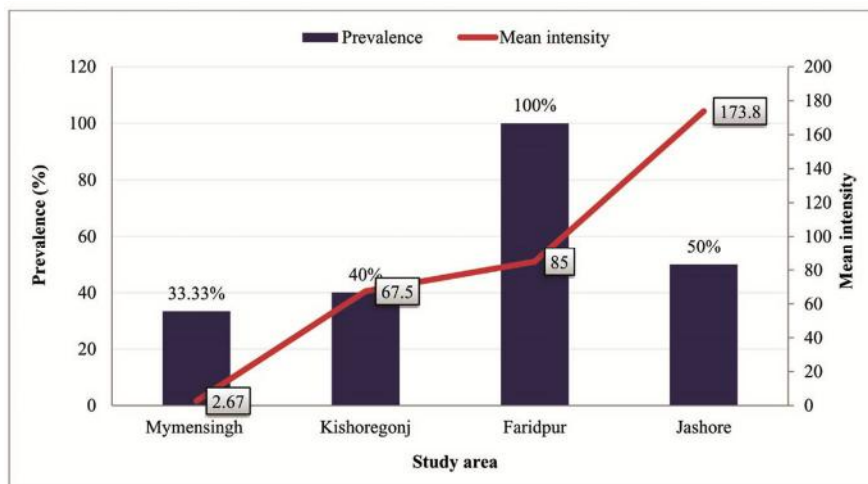


Fig. 3. Prevalence (%) and mean intensity of protozoan parasites of *C. punctatus* in study areas.

Shannon diversity index, $H=0.662, 0.00, 0.167$ and 0.748 in Mymensingh, Kishoreganj, Faridpur and Jashore site respectively, indicated that the sample fishes were not infested by more parasites and the parasite community were poorly diverged (Table 5). On the contrary, Simpson's diversity index, $D=0.536, 0.00, 0.064$ and 0.504 in Mymensingh, Kishoreganj, Faridpur and Jashore site respectively, indicated that parasite community were moderately diverse in sample fishes of Mymensingh and Jashore (Table 5). Whereas, fish sample collected from Kishoreganj district had no diversity and fish samples collected from Faridpur were not infested by more parasites and parasites community were poorly diverged (Table 5).

Table 4. Prevalence and mean intensity of different species of protozoan parasites in *C. punctatus* in study areas.

Name of parasites	Mymensingh		Kishoregonj		Faridpur		Jashore	
	Prevalence (%)	Mean intensity (\pm Sd)	Prevalence (%)	Mean intensity (\pm Sd)	Prevalence (%)	Mean intensity (\pm Sd)	Prevalence (%)	Mean intensity (\pm Sd)
<i>Henneguya chauthuryi</i>	-	-	-	-	-	-	20	240 (\pm 102.93)
<i>Henneguya bengalensis</i>	-	-	-	-	60	137 (\pm 108.71)	10	380 (\pm 120.17)
<i>Myxobolus</i> sp.	-	-	40	67.5 (\pm 52.39)	-	-	-	-
Actinospor- ean stage	22.22	1.5 (\pm 0.71)	-	-	-	-	-	-
<i>Trichodina pediculus</i>	22.22	2.5 (\pm 1.33)	-	-	60	2 (\pm 1.30)	20	1.5 (\pm 0.67)
<i>Epistylis lwoffi</i>	-	-	-	-	40	4 (\pm 2.30)	10	2 (\pm 0.63)
<i>Apisoma piscicolum</i>	-	-	-	-	-	-	10	4 (\pm 1.26)

Table 5. Comparison of the richness, evenness and diversity of the parasite communities of the different sampling areas in *C. punctatus*.

Characteristics	Mymansingh	Kishoreganj	Faridpur	Jashore
Number of fish examined	09	05	05	10
% of fish infected	55.56	40	100	50
No. of parasites collected	10	155	556	894
No. of parasite species	02	01	03	05
Species evenness	0.955	0.00	0.152	0.465
Species of richness 'R'	0.138	0.00	0.275	0.550
Shannon diversity index, H	0.662	0.00	0.167	0.748
Simpson's diversity index, D	0.536	0.00	0.064	0.504

During the study, protozoan parasites exhibited variation in composition, prevalence and mean intensity in host, which might be dependent upon the factors such as parasite biology, host size, feeding habits and habitat of the host, water quality, metabolic state and weak immune system of fish. There was no previous comparative data and the cause of diversity of protozoan parasites of *C. punctatus* in the study areas. So direct comparison of the present study was not possible. There was no available data on the water quality of sample collection areas. Banerjee and Bandyopadhyay (2010) reported that water quality has a great impact on the abundance of fish pathogens and their ability to survive on host. Therefore, the reasons caused the difference of distribution of parasites in sample collecting areas could not be exactly described.

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