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# OCCURRENCE OF PROTOZOAN PARASITES FROM CLIMBING PERCH, *ANABAS TESTUDINEUS* FROM OPEN WATERS OF BANGLADESH

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**Abstract:** The study was conducted to identify the protozoan parasites and to determine their occurrence and diversity in climbing perch, *Anabas testudineus* (Bloch, 1972). The sample fish species were collected from mid-October, 2018 to end of the December 2018 from freshwater bodies of Mymensingh, Kishoregonj, Faridpur and Jashore districts. Three species of myxozoa (*Henneguya mystusia, Henneguya qadrii* and *Henneguya acerinae*) and 4species of ciliophora (*Trichodina acuta, Trichodina* spp., *Epistylis lwoffi* and *Amphileptus disciformis*) were identified in *A. testudineus.* Approximately 76.19% of total fish species were infected by at least one of the parasites with average load of 71.38±32.26 per infected host. Myxozoans (97.55%) were clearly dominant group than chiliophorans (2.45%). The highest prevalence of parasitic infection was observed in the fishes of Mymensingh (100%) and lowest in Jashore (40%).The association of parasitic infection of *H. mystusia* (p=0.018), *H. qadrii* (p= 0.00044), *H. acerinae* (p=0.003), *Trichodina acuta* (p= 0.052)and *A. disciformis* (p= 0.023) with study area was statistically significant. Protozoan parasites were most abundant in gills of the hosts. Shannon Diversity Index indicated that hosts were not infested by more parasites and the parasite community was poorly diverged in all study sites but Simpson's Diversity Index showed that, parasites community was moderately diverged in host fish of Mymensingh area and in rest of areas they were poorly diverged.

***Key words:*** Protozoa, Parasite, Infection, Occurrence, Prevalence, *Anabas testudineus*

## INTRODUCTION

Fish is an important component of healthy diet in different countries especially where malnutrition is prevalent such as third world countries. In Bangladesh, fish protein is considered to be the vital source of animal protein. Among the available fish species in Bangladesh, *Anabas testudineus* is one of the vitamin rich, small indigenous, air-breathing fish which is generally known as climbing perch and locally recognized as koi which plays a great economic

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role as edible fish. A number of infectious diseases of fishes are caused by protozoan parasites. Both the ecto and endo-parasitic protozoa have a very important role as being one of the most hazardous threats to fish health. Generally protozoan parasites are the causative agents of various diseases in freshwater fishes causing massive destruction of skin and gill epithelium (Reda 2011). These parasites affect fish populations by causing mortality, reduction in growth, losing of weight, and suppression of reproductive activity (Deshpande and Verma 2015**).** Parasites generally increase in abundance and diversity in more polluted waters which can be an indicatory to the quality of the water (Poulin 1992 and Avenant-Oldewage 2001).

The study on the fish parasites and their frequency and distribution in fishes is very scant in Bangladesh. A considerable number of studies have been done on the metazoan parasites of *A. testudineus* in Bangladesh (Basirullah 1973, Ahmed and Begum 1978, Ahmed 1981, Banerjee and Chandra 1993, Akhter *et al.* 1997and Akhter *et al.*2001, Parveen *et al.* 2006, Ghani *et al.* 2014 and Bhuiyan *et al.* 2014). But a little works has been found related to the study topic (Arthur and Ahmed 2002, Sanaullah 1996, Asmat *et al.* 2003 and Kibria *et al.* 2011).Protozoan parasites have not been studied thoroughly in Bangladesh and only a little knowledge about the distribution, occurrence and control of most of the diseases in natural population of freshwater fish is available. Therefore, it is essential to know the current status of protozoan infestation in the wild fishes of Bangladesh. There has not been enough study on ecto-protozoan parasite of *A. testudineus* in Bangladesh, therefore less information is available. However, the present study was an attempt to build a base line data of protozoan parasites of *A. testudineus* in Bangladesh.

## MATERIAL AND METHODS

*Collection of host sample:* To investigate the protozoan parasites of *Anabas testudineus* in Bangladesh, the study was performed in four different areas of Bangladesh. A total of 21 specimens of wild host fishes were collected alive from the open 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)with the help of fishermen during mid-October, 2018 to end of the December 2018.Sample size of fishes collected from each area was not sharply equal. However, area wise sample size was: Kishoreganj- 5, Mymensingh- 9, Faridpur- 5 and Jashore- 10 fishes.

*Sample preparation:* The fish were examined immediately after collection. The external surfaces of the fish were observed carefully using a magnifying glass. External surface of the fishes were examined and recorded for any abnormalities. Evidences were collected from the body slime, gill slime and blood of host fishes which are the best suited micro-habited for protozoan parasites to get harbor. Smears of body slime, gill slime and blood were made on glass slides on the spot and fixed them in ethanol for further observation in the laboratory. Giemsa's stain technique were used for rapid demonstration of nuclei in ciliates and in microsporidian spores and Klein's dry silver impregnation method were used for staining mobiline peritrichs and other ciliates from the surface of fish. The slides were observed under microscope to note the presence or absence of protozoan parasites. Counts of parasites found in selected organs were recorded. The numbers of observed parasites were counted for statistical analysis and microscopic photographs were captured for the identification of species with the help of 10-megapixel digital camera.

Protozoans were identified according to the description of Lom and Dyková (1992), Sarkar (1985), Eiras (2002), Kalavati and Nandi (2007), Bashě and Abdullah (2010), Kibria *et al.* (2010). Some parasites could not be identified up to species level because these did not match with any of the available published description. Moreover, it seems reasonable to make detail observation where necessary to come to an inference. *Trichodina* sp. was identified up to genus level.

*Calculation:* Prevalence, mean intensity and abundance of infection were determined by following formulae, proposed by Margoles *et al.* (1982) as:

$$\text{Prevalence} = \frac{Total number of hosts infected}{Total number of hosts examined} \times 100$$

$$\text{Intensity} = \frac{Total number of individuals of a particular parasite}{Number of infected hosts by the particular parasite}$$

$$\text{Abundance} = \frac{Total number of individuals of a particular parasite}{Total number of individuals of all parasites in a sample} \times 100$$

Simpson's Diversity Index (Simpson 1949) was used to evaluate for both richness and abundance of parasites within the samples, was counted by the formula: $\mathbf{D} = 1 / C$ where, $C = \sum P i^2$ ($Pi^2 = (Ni/N_T)^2$); here, $Pi$ 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, was calculated by the formula: H = $-\sum_{i=1}^{s} Pi \ln Pi$where, $Pi$ 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 increase diversity as measured by H. The most commonly used index of evenness was 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 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. Significance levels were set at p≤0.05.

## RESULTS AND DISCUSSION

The protozoan parasites were collected from body slime and gills but no parasites were found in blood samples in case of *Anabas testudineus* that were collected from the different study sites. A total of 1142 protozoan individuals were collected from different body parts of 16 infected *A. testudineus* (out of 21 fish examined). Mainly two groups of parasites, Myxozoa and Chiliophora were collected from the host fishes. Of them, 1114 (97.55%) were under Phylum-Myxozoa and 28 (2.45%) were under Phylum Ciliophora. A total of 7 genera/species were encountered where, 3 Myxozoan and 4 Ciliophoran parasites were found (Table 1, Fig. 1).

Table 1. List of protozoan parasites recorded from *Anabas testudineus* during this study

| **Group of the parasites** | **Parasites** | **Sampling area** | **Site of infection** |
|---|---|---|---|
| Myxozoa | *Henneguya acerinae,* Schröder 1906 | Mymensingh, Kishoreganj | Gill |
| | *Henneguya mystusia* Sarkar 1985 | Mymensingh , Jashore Kishoreganj, Faridpur, | Gill, Body slime |
| | *Henneguya qadrii,* Lalitha1965 | Mymensingh | Gill |
| Ciliophora | *Trichodina acuta,* Lom 1961 | Kishoreganj, Faridpur | Gill, Body slime |
| | *Trichodina* sp., Ehrenberg 1838 | Kishoreganj, Jashore | Gill |
| | *Epistylis lwoffi,* Fauré-Fremiet 1943 | Faridpur, Jashore | Body slime |
| | *Amphileptus disciformis,* Chen 1955 | Faridpur | Body slime |

*Trichodina acuta* was previously recorded in Bangladesh in *Mystus bleekeri* (Kibria *et al.* 2010) but it was first recorded in a new host in the present study. *Henneguya qadrii* (Lalitha1965) collected from *C. punctatus* and *Henneguya mystusia* (Sarkar 1985, Kumar 2002) collected from *Mystus* sp. were previously recorded in India, however in the present study it was recorded in the new host and locality in Bangladesh. And rest of the parasites was reported as novel in both host and locality in Bangladesh. Till to date, except *Trichodina anabasi* (Asmat *et al.* 2003 and Kibria *et al.*2011) and *Tripartiella* sp. (Arthur and Ahmed 2002) no protozoan parasitic infestations were recorded in *A. testudineus* in Bangladesh.

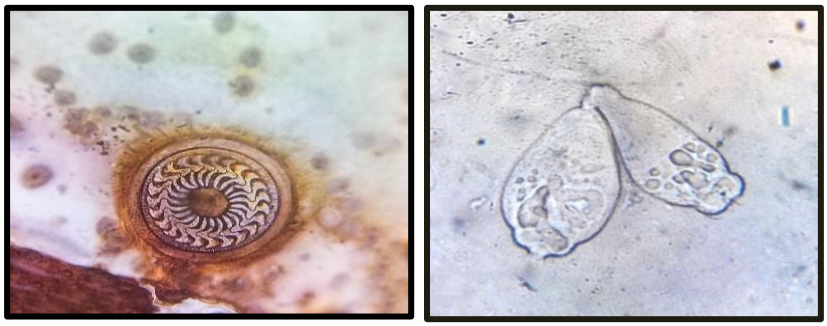
Table 2. Updated list of protozoan parasites from *Anabas testudineus* in Bangladesh, India and Pakistan

| Parasites | Locality | References |
|---|---|---|
| *Henneguya acerinae* **Ω** Δ | Bangladesh | Present study |
| *Henneguya mystusia* **Ω** Δ | India | Sarkar 1985,Kumar 2000, Present study |
| *Henneguya qadrii* Δ | India | Lalitha 1965, Present study |
| *Trichodina acuta* **§ Ω** | Bangladesh | Kibria *et al.* 2010, Present study |
| *Trichodina* sp. | Bangladesh, India and Pakistan | * |
| *Epistylis lwoffi* **Ω** Δ | Bangladesh | Present study |
| *Amphileptus disciformis* **Ω** Δ | Bangladesh | Present study |

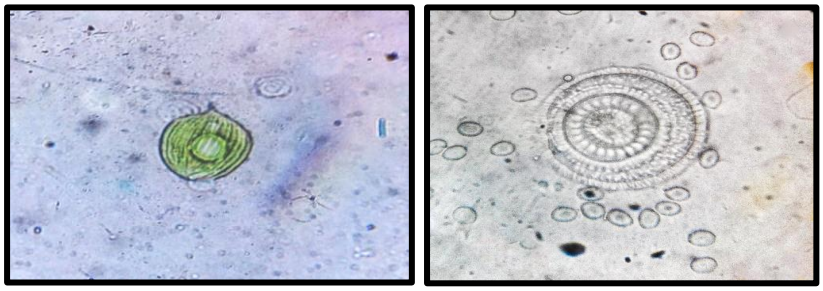
*References of parasites identified up to genus level have not been included in this chart. Ω New host record; Δ New locality record in Bangladesh; § previously recorded in Bangladesh.

Among seven genera/species, the highest prevalence (52.38%) was found in *Henneguya mystusia.* Other parasites having high prevalence's were *Henneguya acerinae* (28.57%), *Henneguya qadrii* (23.81%), *Trichodina acuta* (23.81%) whereas *Epistylis lwoffi* had the lowest prevalence (9.52%) (Fig. 1). The mean intensity was varied from 86.4±60.28 to 1.33 ±0.51 (Fig.1). *Henneguya acerinae* was the most abundant 467(40.89%) parasites and *Epistylis lwoffi* 4(0.35%) and *Trichodina* sp. 4(0.35%) showed the lowest abundance (Fig. 2). In the present study the prevalence of *Henneguya mystusia* was closely similar with the findings of Kumar (2000) who reported that 65% (39 out of 60 fishes) of *H. mystusia* were found in *Aplocheilus lineatus* in India. To the best of our knowledge, no previous record of *Henneguya acerinae* and *H. qadrii are* available in this host as well as in this locality. Kibria *et al.* (2010) reported 46.3% of prevalence of *Trichodina acuta* in total 20 host fishes of *Mystus bleekeri* in Bangladesh. Prevalences were reported as 19.6% (45 out of 230 fishes) and

*Henneguya acerinae* (40X) *H. mystusia* (40X) *H. qadrii* (100X)



*Trichodina acuta* (100X) *Epistylis lwoffi*(40X)



*Amhileptus disciformis*(100X) *Trichodina* sp. (100X)

**Fig. 1.** Photomicrograph of protozoan parasites recorded from *Anabas testudineus*.

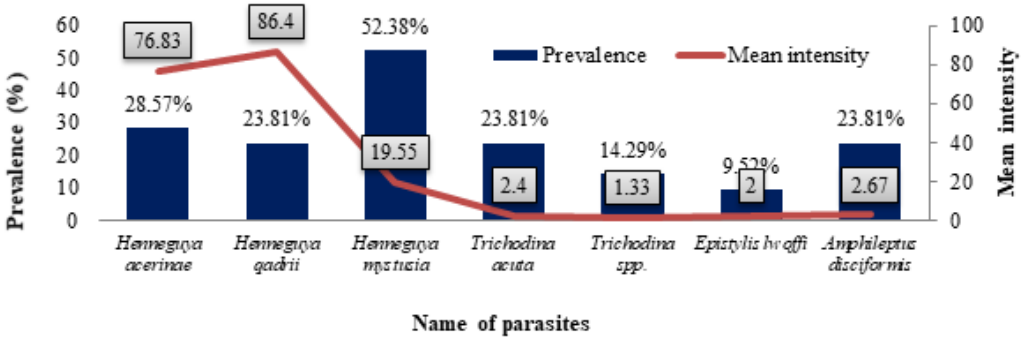



**Fig. 2.** Prevalence and mean intensity of different species of protozoan parasites in *Anabas testudineus.*

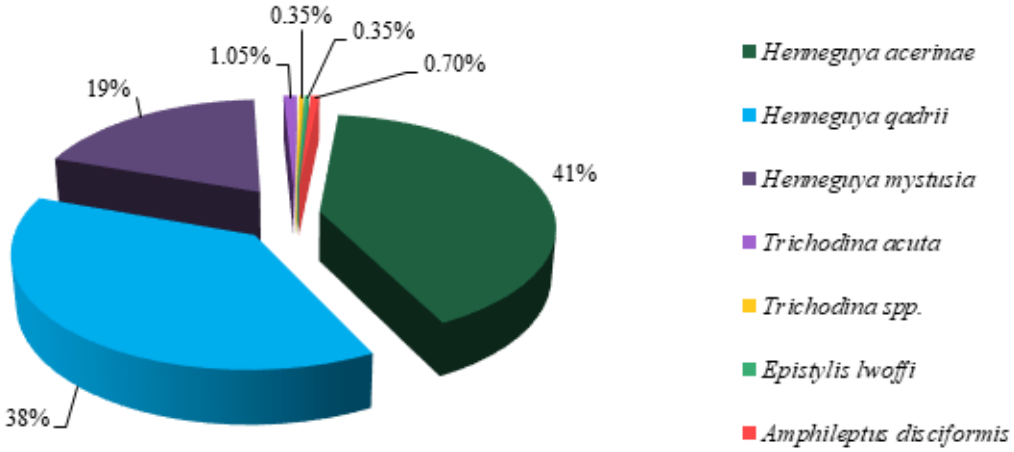



**Fig. 3.** Abundance of species of protozoan parasites in *Anabas testudineus*

40.0% (10 out of 25 fishes) for *Trichodina anabasi* in *A. testudineus* according to Asmat *et al.* 2003 and Kibria *et al.* 2011, respectively.

Prevalence of *Trichodina heterodentata* (Asmat 2004) was found 12.4% (24 out of 194 fishes) and prevalence of *Trichodina* sp. were found 40% (Theerawoot 2008) at *A. testudineus* in Bangladesh and Thailand respectively. *Amphileptus sp.* was recorded from the gills of *Clarias gariepinus* (El-Tantawy and El-Sherbiny2010) previously. Around 18% of *Amphileptus* sp. was obtained from the gills and skin of 61 host fishes of *Lates niloticus* (El –Tantawy 2016).

In the present study, multiple species of parasitic infection was found higher than single species of parasites infection at a time in *A. testudineus* (Fig. 4). However, 23.81% of host fishes had no infection (Fig. 4). No previous record was available on multiple infections of protozoan parasites in these host fish.

However, Kaur and Katoch (2016) reported 65.15% (418 out of 1380 fishes) of native carp fish had mixed infection of Myxozoan species at a time and that result was slightly similar to this study. Multiple infections might be occurring due to sharing the same habitat by the host.

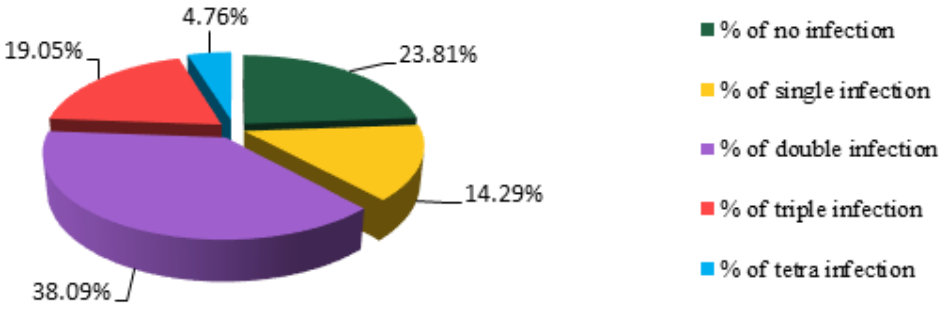



**Fig. 4.** Relative proportion of mixed infection by protozoan parasites in *Anabas testudineus*

The occurrence of protozoan infestation varies in different organs of fish body. The heavy load of parasites was found in the gills compared to the other parts of the body resulting impaired respiratory function even death (Omeji 2011). It was revealed from the present study that gills were harbored by highest number of protozoan parasites than body slime in host fish (Table 3). This could be due to the reason that, gills are the center of filter feeding food behavior of fish and are the sites of gaseous exchange to breath. This observation supported the study conducted by Mandal *et al.* (2016) that reported parasites were prevalent at a highest rate in gill (37%) of *A. testudineus* followed by outer body layer (35%) and intestine (28%) in India. According to Roberts and Somerville (1982), the sieving ability of the gill rakers may help to trap some organisms, and this could be attributed to the presence of the protozoan parasites there.

**Table 3. Prevalence and mean intensity of parasitic infestation in infected organs of *A. testudineus***

| Infected organ | No. of host | | Prevalence (%) | Parasites collected | Mean intensity (±SD) |
|---|---|---|---|---|---|
| | Examined | Infected | | | |
| Body slime | 21 | 12 | 57.14 | 32 | 2.67±0.77 |
| Gill | 21 | 15 | 71.43 | 1110 | 74.00±32.41 |
| Blood | 21 | - | - | - | - |

The result showed that the highest prevalence (%) of parasitic infection of *A. testudineus* was observed in the sample fish collected from Mymensingh (100%) followed by Kishoregonj (80%), Faridpur (80%) and Jashore (40%)(Fig. 5).Fisher's Exact test showed that the association of parasitic infestation with study areas was not statistically significant (p= 0.141, since p≤0.05) in *A. testudineus.* The highest mean intensity was also found in Mymensingh (151.67±56.22) and lowest in Jashore sample (15.00±4.24)(Fig. 5).

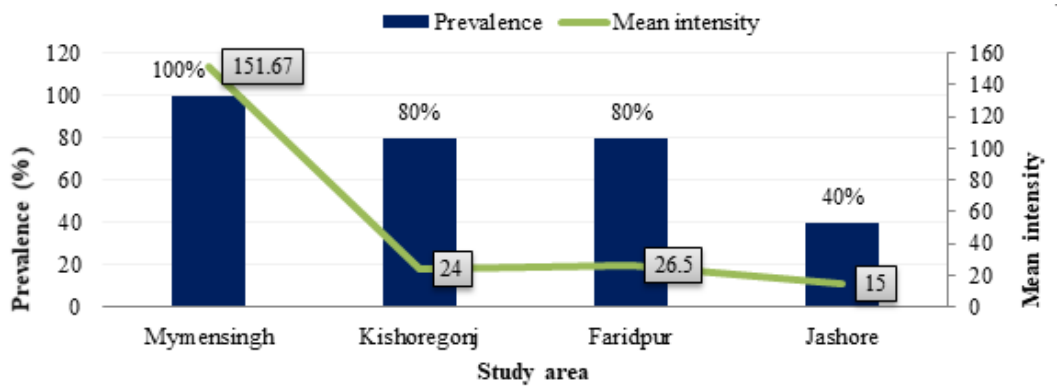



**Fig. 5.** Prevalence and mean intensity of protozoan parasites of *Anabas testudineus* in study areas

In this investigation, *Henneguya mystusia* was found in all the four study area where the highest prevalence was exhibited in Mymensingh (100%) and lowest in Kishoregonj (20%) and Faridpur (20%) (Fig. 6).Fisher's Exact test showed that the association of parasitic infection of *Henneguya mystusia* with study area was statistically significant (p=0.018, since p≤0.05) in *A. testudineus.*

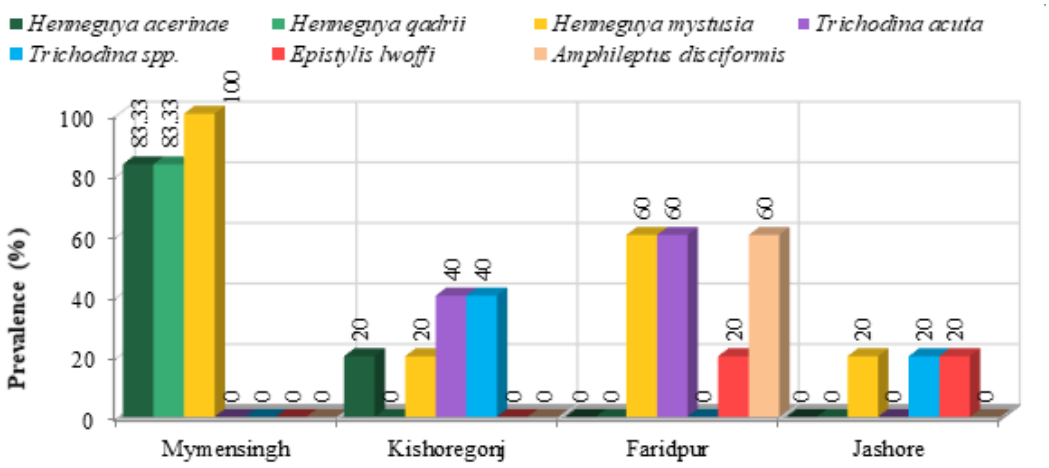



**Fig. 6.** Prevalence of Protozoan parasites in *Anabas testudineus* in different study areas

*Henneguya acerinae, Trichodina acuta, Trichodina* spp. and *Epistylis lwoffi* were found in only two study area.The association of parasitic infection of *Henneguya acerinae* and *Trichodina acuta* with study area was statistically significant (p= 0.003 and p= 0.052 respectively, whereas p≤0.05) and *Trichodina* spp. and *Epistylis lwoffi* was not statistically significant (since, p≥0.05). *Henneguya qadrii* and *Amphileptus disciformis* were found in only one study area and their parasitic association with study area was statistically significant (p= 0.000442 and p= 0.023 respectively, whereas p≤0.05).

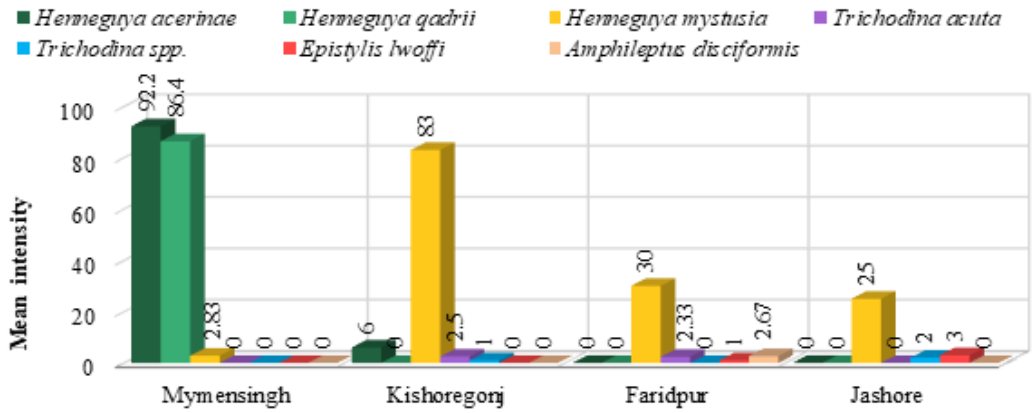



**Fig. 7.** Mean intensity of different species of protozoan parasites of *Anabas testudineus* in study areas.

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 available data on the water quality of sample collection areas. During the study, water quality of the water bodies was not recorded. Therefore, the reasons caused the difference of distribution of parasites in sample collecting areas could not be exactly described. According to Banerjee and Bandyopadhyay (2010) water quality has a great impact on the abundance of fish pathogens and their ability to survive on host. Accumulation of toxic substances and water eutrophication with algae blooms contribute to the poor water quality that acts as stress factor in increasing fish susceptibility to parasites and stimulates an unbalanced state of the host-parasite-environment system (Coutant 1998).

The site specific comparison of richness value showed that Kishoreganj had the highest (0.66) parasite richness in *A. testudineus* and the lowest value was observed in Mymensingh (0.29). Evenness of parasite distribution in Mymensingh showed moderately higher value (0.703)which meant that community structure was well constructed and well diverged in Mymensingh. Jashore area showed moderate value implying that community structure was moderately distributed and not evenly diverged. Faridpur (0.402) and Kishoreganj (0.385) had low evenness value which implied that parasite community structure was not evenly distributed of all parasite species and poorly diverged (Table 4).

Table 4: Comparison of the richness, evenness and diversity of the parasite communities of different sampling areas in *Anabas testudineus*

| **Characteristics** | **Mymansingh** | **Kishoreganj** | **Faridpur** | **Jashore** |
|---|---|---|---|---|
| Number of fish examined | 6 | 5 | 5 | 5 |
| % of fish infected | 100 | 80 | 80 | 40 |
| No. of parasites collected | 910 | 96 | 106 | 30 |
| No. of parasite species | 3 | 4 | 4 | 3 |
| Species Evenness | 0.703 | 0.385 | 0.402 | 0.512 |
| Species of Richness 'R' | 0.29 | 0.66 | 0.64 | 0.59 |
| Shannon Diversity Index, H | 0.773 | 0.533 | 0.557 | 0.563 |
| Simpson's Diversity Index,D | 0.518 | 0.248 | 0.272 | 0.301 |

Shannon Diversity Index, H=0.773, 0.533, 0.557, 0.563 in Mymensingh, Kishoreganj, Faridpur and Jashore site respectively, these indicated that the sample fishes were not infested with more parasites and the parasite community were poorly diverged. In contrast, Simpson's Diversity Index, D=0.518, 0.248,

0.272 & 0.301 in Mymensingh, Kishoreganj, Faridpur and Jashore site respectively indicated that in Mymensingh fish samples were infected with parasites community which was moderately diverged and rest of the sites were not infected with more parasites and the parasite community was poorly diverged (Table 4).

Since the host fish play an important role as food fish, assessing the parasitic infestation is necessary to limit further damage. A primary database of protozoan parasites of *A. testudineus* has been established by the present study which will be supportive for further extension regarding this spectrum in future. For more specification, an extensive study including larger and diverse sample size might be undertaken.

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