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COMPARATIVE STUDY OF PROTOZOAN PARASITE COMMUNITIES BETWEEN *ANABAS TESTUDINEUS* AND *CHANNA PUNCTATUS*

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Abstract

The study was conducted to determine the comparative occurrence of protozoan parasites of two host species- *Anabas testudineus* and *Channa punctatus.* The host fishes were collected from April, 2018 to March, 2019 from freshwater bodies of Mymensingh, Kishoreganj, Faridpur and Jashore districts of Bangladesh. Three species of myxozoa (*Henneguya mystusia, Henneguya qadrii* and *Henneguya acerinae*) and four genera/species of ciliophora (*Trichodina acuta, Trichodina* sp., *Epistylis lwoffi* and *Amphileptus disciformis*) in *A. testudineus*; three genera/species of myxozoa (*Henneguya chaudhuryi, Henneguya bengalensis* and *Myxobolus* sp.), three species of ciliophora (*Trichodina pediculus, Epistylis lwoffi* and *Apisoma piscicolum*) and a few actinosporean stage of myxoza in *C. punctatus* were identified. The prevalence of protozoan infections were found higher in *A. testudineus* (76.19%) compare to *C. punctatus* (51.72%). However, mean intensity was relatively higher in *C. punctatus* (95.93 ± 41.53) than that of *A. testudineus* (71.38 ± 32.26). Myxozoans were clearly dominant group in both hosts. Multiple parasitic infections were higher in *A. testudineus*. The highest parasitic infection was observed in Mymensingh (100%) and lowest in Jashore (40%) in A. *testudineus*, whereas it was highest in fish of Faridpur (100%) and lowest in Mymensingh (33.33%) in *C. punctatus*. Shannon Diversity Indices indicated that the parasite community was poorly diverged in both hosts of all study areas. However, Simpson's Diversity revealed that, parasites community was moderately diverged in both hosts in Mymensingh and in other study areas they were poorly diverged.

Introduction

Fish is one of the principal sources of protein for human being and other animals in the tropics. Being one of the cheapest sources of animal protein, fish plays a major role in the diet of Bangladeshis. There are 265 freshwater fish species belonging to 55 families(1) available at freshwater bodies in Bangladesh. Among the fish species in Bangladesh *Anabas testudineus* and *Channa punctatus* are major contributors to the fisheries sector as

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they are very common as well as fairly popular. They possess a great economical, nutritional and medicinal role in our country along with providing employment for rural people living in the coastal regions. Therefore, it is important to develop a good management practice for disease free fish production.

A. testudineus is a small freshwater indigenous air-breathing fish popularly known as climbing perch which can live without water for days and so were found alive on most of tree tops and hence its name. They remain buried under the mud during dry season. This fish is a column feeder, feed on macrophytic vegetation, shrimps and fish fry and also a larvicidal fish, feeds upon mosquito larvae and hence used to control mosquito larvae[2].

The snake headed fish *C. punctatus* is the most important species of inland fisheries of Bangladesh. It is mud-loving fish and due to its food habit, it can act as an intermediate host for many helminth parasites[3]. As the *C. punctatus* is most popular fish in the country, their abundance is reducing due to over exploitation, environmental stress and the occurrence of various diseases. As a consequence, parasitic infestation may initiate declination in fisheries stock over the time.

In general, fishes are suitable carrier of parasites because they act as an intermediate host of various parasites for the completion of developmental stages of their life cycle. Parasites cause massive destruction of skin, epithelium and gill; thereby affect fish population by growth retardation, weight loss and suppression of reproductive activity even mortality[4]**.** Apart from mortality treatment expenses, growth reduction during and after outbreak of disease cause economic loss and this militates against expansion of aquaculture[5]. However, the report on the fish parasites, their frequency and distribution in fish is very inadequate in Bangladesh. Various metazoan parasites like monogeneans, digeneans, larval cestodes and ectoparasitic crustaceans have been reported in these host fish of Bangladesh[6-10] but report on protozoan parasites are limited and not homogeneous. Moreover, they were not been studied thoroughly in Bangladesh and only a little knowledge about the distribution, occurrence of ecto-protozoan parasite of *A. testudineus* and *C. punctatus* is available[11-14]. Therefore, it is essential to know the current status of protozoan infestation in the wild freshwater fish. In the present study, the relative occurrence of two fish species named *A. testudineus* and *C. punctatus* are studied as being two more popular edible species. The present study was an attempt to build a base line data of protozoan parasites of the two fish species and to control most of the diseases of them in natural population in Bangladesh. Since both host lives in similar habitats comparison of parasite community will reveal the causes behind differences.

Materials and Methods

Collection of host samples: A total of 50 specimens of host fish were collected randomly from four areas of which, 21 fishes were *Anabas testudineus* and 29 fishes were *Channa*

punctatus. Sample size of four areas was not equal. Host fish were collected alive 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) with the help of fishermen from mid of the April 2018 to end of the March 2019.

Sample preparation: The outer surface of fish was examined immediately after capture using a magnifying glass. External surface of the fish was examined and recorded for any abnormalities. Specimens were kept moist during examination by spraying them with a fine mist of water. After collecting the samples, their total length and total weights were measured. Body slime, gill slime and blood of host fish were collected which are the best sources for protozoan parasite. Smears of body slime, gill slime and blood were made on glass slides on the spot of collection and fixed them in ethanol for further microscopic observation in the laboratory.

Giemsa's stain technique was used for rapid demonstration of nuclei in ciliates and spores in microsporidian. Klein's dry silver impregnation method was used for staining mobile peritrichs and other ciliates. The slides were observed under microscope to detect the presence or absence of protozoan parasites. Parasites of selected organ were counted and recorded. The prepared slides were observed under the compound microscope and visualized by 4x, 10x, and 40x lenses for comprehensive morphological details of the protozoan parasites. The number of observed parasites was counted for statistical analysis and microscopic photographs were captured for identification of species with the help of 10-megapixel digital camera.

Protozoans were identified according to the description of Lom and Dyková[15], Kalavati and Nandi[16], Bashě and Abdullah[17] and Kibria *et al.*[18]. Some parasites (*Myxobolus* sp. and *Trichodina* sp.) could not be identified up to species level due to the unavailable sources of articles. So, it seems reasonable to make their detail observation to come to a conclusion.

Calculation: Measurement of prevalence, mean intensity and abundance of infection were calculated according to Margoles *et al.* [19]. Simpson's Diversity Index[20] was used to evaluate both species richness and abundance of parasites found in the samples. Shannon's Diversity index[21] was used to measure the diversity. The most commonly used index of evenness based on the Shannon- Wiener index[22] was used. Margalef Index of Species Richness[23] was used to evaluate the richness of parasites within the samples.

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 $p \leq 0.05$.

Results and Discussion

Comparative study of different aspects of protozoan infestation between *Anabas testudineus* and *Channa punctatus* revealed some differences in almost all aspects of infestation. The protozoan parasites were collected from body slime and gills but no parasites were found in blood samples. Out of 16 infected host (*A. testudineus*) a total of 1142 protozoan parasites were collected from different areas of body (21 fish examined) and a total of 1437 protozoan individuals were collected from different body parts of 15 infected *C. punctatus* (out of 29 fish examined) in the present study. The overall prevalence of *A. testudineus* was higher (76.19%) than *C. punctatus* (51.72%) whereas mean intensity was higher (95.93±41.53) in *C. punctatus* than *A. testudineus* (71.38±32.26). It was settled that, *A. testudineus* was more infected by protozoan fauna than *C. punctatus.* Altogether 13 genera/species and Actinosporean stage of protozoan parasites have been identified from the two host fish (Table 1).

Table 1. Occurrence of protozoan parasites recorded from *Anabas testudineus* and *Channa punctatus*.

Group of the parasites	*Anabas testudineus*			*Channa punctatus*		
	Parasites	Sampling area	Site of infection	Parasites	Sampling area	Site of infection
Myxozoa	*Henneguya acerinae*	Mymensingh, Kishoreganj	Gill	*Henneguya chaudhuryi*	Jashore	Gill
	Henneguya qadrii	Mymensingh	Gill	*Henneguya bengalensis*	Faridpur, Jashore	Gill
	Henneguya mystusia	Mymensingh, Kishoreganj, Faridpur, Jashore	Gill, Body slime	*Myxobolus* sp.	Kishoreganj	Gill
				Actinosporean stage	Mymensingh	Body slime
Ciliophora	*Trichodina acuta*	Kishoreganj, Faridpur	Gill, Body slime	*Trichodina pediculus*	Mymensingh, Faridpur, Jashore	Body slime, Gill
	Trichodina sp.	Kishoreganj, Jashore	Gill	*Epistylis lwoffi*	Faridpur, Jashore	Body slime
	Epistylis lwoffi	Faridpur, Jashore	Body slime	*Apiosoma piscicolum*	Jashore	Body slime
	Amphileptus disciformis	Faridpur	Body slime			

In the present study, *Trichodina acuta* infection in host *A. testudineus* is the first record as a new host in Bangladesh. Although it was previously recorded in Bangladesh in another freshwater species, *Mystus bleekeri*[18]. In *A. testudineus*, myxozoan parasite *Henneguya qadrii* and *Henneguya mystusia* has been first recorded in Bangladesh.

Moreover, till to date, except *Trichodina anabasi*[12,13] and *Tripartiella* sp. no protozoan parasitic infestations were recorded in *A. testudineus* in Bangladesh. Although *Henneguya qadrii*[25] infecting *C. punctatus* and *Henneguya mystusia*[26] infecting *Mystus* sp. were previously recorded in India. The rest three parasites were first recorded in these hosts and also as first locality recorded in Bangladesh. On the other hand, *Trichodina pediculus* was previously recorded in *C. punctatus* in Bangladesh[18,19]. *Henneguya chaudhuryi*[27,28] and *Henneguya bengalensi* [29] collected from *C. punctatus* were previously recorded in this host in India but is newly in Bangladesh. The rest two parasites were the first record infecting these fishes in Bangladesh. *Trichodina cyprinocola, Trichodina pediculus, Trichodina* sp., *Chilodonella* sp., *Ichthyobodo* sp., *Actinophrys* sp., *Ichthyophthirius multifilis*, and *Myxobolus* sp. were recorded from *C. punctatus* in Bangladesh[14,24].

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

Anabas testudineus		*Channa punctatus*	
Parasites	References	Parasites	References
Henneguya acerinae Δ Ω	Present study	*Henneguya chaudhuryi* Δ	Bajpai and Halder, 1982, Chaudhary *et al.* 2017 And Present study
Henneguya mystusia Ω	Kumar 2000 and Present study	*Henneguya bengalensis* Δ	Raychaudhuri and Chakravarty 1970 and Present study
Henneguya qadrii Δ	Lalitha 1965 and Present study	*Myxobolus* sp.	*
Trichodina acuta § Ω	Kibria *et al.* 2010 and Present study	Actinosporean stage Δ Ω	Present study
Trichodina sp.	*	*Trichodina pediculus* §	Deb *et al.* 2015 and Present study
Epistylis lwoffi Δ Ω	Present study	*Epistylis lwoffi* Δ Ω	Present study
Amphileptus disciformis Δ Ω	Present study	*Apisoma piscicolum* Δ Ω	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.

Two groups (myxozoa and chiliophora) of protozoan parasites were recorded from both *A. testudineus* and *C. punctatus* in the present study. In comparison with Chiliophora, Myxozoa was clearly dominant group in both hosts (Fig. 1). Previous record was not found on all the groups of protozoan parasites in both host fishes. Therefore, direct comparison is not possible.

In *A. testudineus*, multiple species of parasitic infection was found higher than single species of parasitic infection at a time (Fig. 2). On the other hand, in *C. punctatus,* single species of parasitic infection was found higher than multiple species infection at a time (Fig. 2). Moreover, 23.81% and 48.28% hosts were not infected by parasites in *A. testudineus* in *C. punctatus* at a time (Fig. 2). Previous record was not available on multiple infections of protozoan parasites in these host fishes. However, Kaur and Katoch[30] reported that 65.15% of native carp fish had mixed infection of myxozoan species at a time and that result was slightly similar to present study. Multiple infections might be happened due to sharing the same habitat by two hosts.

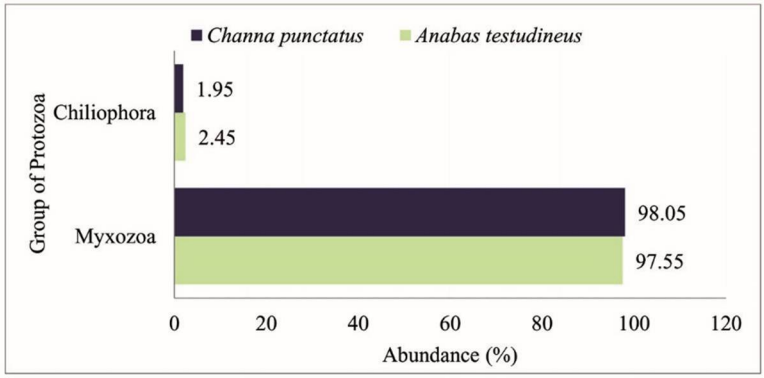

Fig. 1. Comparison of parasitic infestation by Myxozoa and Chiliophora between *A. testudineus* and *C. punctatus*

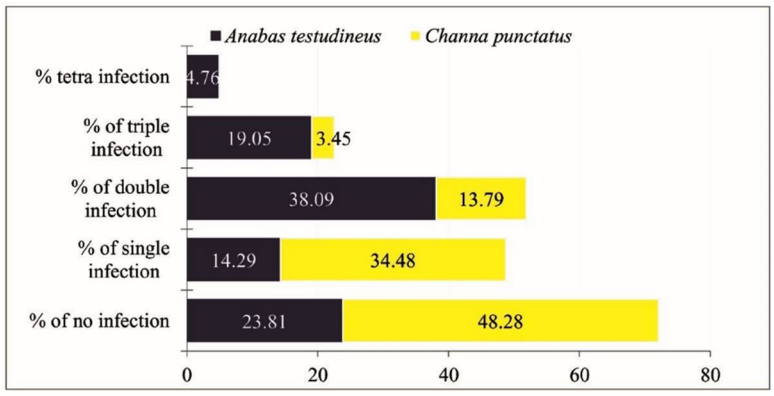

Fig. 2. Multiple infestations of different protozoan parasites in *A. testudineus* comparing with *C. punctatus*.

A total of 7 genera/species of protozoan parasites were found in *A. testudineus* and 6 genera/species and actinosporean stages of protozoan parasites were found in *C. punctatus.* The chiliophora, *Epistylis lwoffi* was common in both *A. testudineus* and *C. punctatus* (Table 3).

Rest of the species of protozoan parasites in both host fishes were host specific. But while considering generic level, *Henneguya* and *Trichodina* were common in both hosts (Table 3). In the present study, the prevalence of *Henneguya mystusia* was closely similar to the findings of Kumar[26] who reported that 65% of *Henneguya mystusia* were found in *Aplocheilus lineatus* in India. Kibria *et al.*[18] reported 46.3% prevalence of *Trichodina acuta* in host fish, *Mystus bleekeri* in Bangladesh. Asmat *et al.*[12] and Kibria *et al.*[13] reported 19.6% and 40.0% prevalence respectively for *Trichodina anabasi* in *A. testudineus* in Bangladesh. To the best of our knowledge, no previous record of *Henneguya acerinae*, *Henneguya qadrii, Amphileptus disciformis and Epistylis lwoffi* has been found in these hosts as well as in the mentioned locality in Bangladesh.

Table 3. Overall prevalence and mean intensity of different species of protozoan parasites in *A. testudineus* and *C. punctatus.*

Anabas testudineus			*Channa punctatus*		
Name of parasites	Prevalence (%)	Mean Intensity (± SD)	Name of parasites	Prevalence (%)	Mean Intensity (±SD)
Myxozoa					
Henneguya acerinae	28.57	76.83 ± 53.55	*Henneguya chaudhuryi*	6.90	240.0 ± 62.81
Henneguya qadrii	23.81	86.40 ± 60.28	*Henneguya bengalensis*	13.79	197.75 ± 85.33
Henneguya mystusia	52.38	19.55 ± 21.51	*Myxobolus* sp.	6.90	67.5 ± 22.36
			Actinosporea stage	6.90	1.5 ± 0.41
Chiliophora					
Trichodina acuta	23.81	2.40 ± 1.29	*Trichodina pediculus*	24.14	2.00 ± 1.02
Trichodina sp.	14.29	1.33 ± 0.51	*Epistylis lwoffi*	10.34	3.30 ± 1.11
Epistylis lwoffi	9.52	2.0 ± 0.68	*Apisoma piscicolum*	3.45	4.0 ± 0.74
Amphileptus disciformis	14.29	2.67 ± 1.02			

Chaudhary *et al.*[28] reported higher percentage (59.3%) of *C. punctatus* infection by *Henneguya chaudhuryi* in India which was relatively higher than that of found in this study. Deshpande and Verma[31] reported that 28.1% of *Channa striatus* were found to be infected with *Myxobolus* sp. in India which was higher than found in the present study. Deb *et al.*[24] reported that 3.33% of *C. punctatus* were infected with *Trichodina pediculus* and 33.33% of *C. punctatus* were infected with *Trichodina cyprinocola* in their study. Some 32.50% of *Trichodina* sp.[14] was found in *C. punctatus* in Bangladesh. Trichodinids were neither host nor site specific[32]. Prevalence was found 8.33% of *Apiosoma* sp.[33] that was previously recorded in Bangladesh from skin of *Cirrhinus reba* but no infection status has been found in *C. punctatus* previously. *Apisoma piscicolum* showed a diverse range of host variability and has a cosmopolitan distribution in Europe, Asia and South Africa[34].

Mixed infections of *Epistylis lwoffi* and *Apiosoma piscicola* were found in the fry of *Salvelinus fontinalisin* in Canada[35]. Similar findings had been also recorded in the present study.

Table 4. Richness, evenness and diversity of the parasite communities of *A. testudineus* and *C. punctatus.*

Characteristics	*Anabas testudineus*	*Channa punctatus*
Number of fish examined	21	29
% of fish infected	76.19	58.62
No. of parasites collected	1142	1437
No. of parasite species	7	7
Species evenness	0.641	0.526
Species of Richness 'R'	0.852	0.825
Shannon Diversity Index (H)	1.249	1.026
Simpson's Diversity Index (D)	0.655	0.577

A. testudineus had comparatively higher value (0.852) of parasite richness than *C. punctatus* (0.825. Evenness of parasite distribution showed moderate value in both *A. testudineus* (0.641) and *C. punctatus* (0.526) which indicated that protozoan parasite community was distributed moderately in their host and not well diverged (Table 4).

Shannon Diversity Index (H= 1.249 and 1.026) of protozoan fauna in *A. testudineus* and *C. punctatus*, respectively showed that parasite community was poorly diverged and host fishes were not infested by more protozoan parasite. But Simpson's Diversity Index (D= 0.655 and 0.577) of protozoan fauna in *A. testudineus* and *C. punctatus* respectively indicated that parasite community was moderately diverged in both hosts (Table 4).

Host fishes, *A. testudineus* and *C. punctatus* were collected from four selected study areas and found to be infected by various protozoan parasites. In case of *A. testudineus*, samples of Mymensingh district was found to be severely infected among all the districts; whereas in *C. punctatus,* fishes of Faridpur district were found to be more infected than all other districts (Table 5). It was concluded that, *A. testudineus* and *C. punctatus* of Faridpur area were found to be more infected than rest of the study sites during the study period. The association of parasitic infestation with study areas was not statistically significant in case of both *A. testudineus* (p= 0.141, since $p \leq 0.05$) and *C. puntatus* (p= 0.118, since $p \leq 0.05$) species. The myxozoan parasite, *Henneguya mystusia* was found in all the study sites in *A. testudineus*. On the other hand, the chiliophoran parasite, *Epistylis lwoffi* was only found in Faridpur and Jashore samples in both hosts.

During the present study, protozoan parasites exhibited variation in species composition, prevalence and mean intensity in both 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 the host fish. Banerjee and Bandyopadhyay[36] reported that, water quality has a great impact on the abundance of fish pathogens and their ability to survive on host.

Table 5. Infestation by protozoan parasites in different study areas in *Anabas testudineus* and *Channa punctatus*.

Study area	*Anabas testudineus*		*Channa punctatus*	
	Prevalence (%)	Mean intensity (±SD)	Prevalence (%)	Mean intensity (±SD)
Mymensingh	100	151.67±56.22	33.33	2.67±0.58
Kishoregonj	80	24.00±14.01	40	67.5±20.37
Faridpur	80	26.50±10.31	100	85.00±47.26
Jashore	40	15.00±4.24	50	173.8±60.44

After analyzing the richness, evenness and diversity of parasite communities of different sampling sites of both hosts, the site wise comparison of richness value between two hosts showed that, samples collected from Kishoreganj district had the highest (0.657) parasite richness value in *A. testudineus* whereas samples collected from Jashore had the highest (0.550) species richness in *C. punctatus* (Table 6).

Table 6. Richness and evenness of the parasite communities of different sampling areas between *Anabas testudineus* and *Channa punctatus*.

Study area	Richness		Evenness	
	A. testudineus	*C. punctatus*	*A. testudineus*	*C. punctatus*
Mymensingh	0.294	0.138	0.955	0.703
Kishoregonj	0.657	0.00	0.385	0.00
Faridpur	0.643	0.275	0.152	0.402
Jashore	0.588	0.550	0.465	0.512

Evenness of parasite distribution in samples of both hosts were collected from Mymensingh district and showed moderately higher value, which meant that parasite communitiy structure was well constructed and well diverged (Table 6).

Shannon Diversity Index (H) indicated that samples of both hosts and study sites were not infested by more parasites and the parasite communities were poorly diverged (Table 7). In contrast Simpson's Diversity Index (D), indicated that fish samples of *A.*

testudineus collected from Mymensingh were infected by parasites community which was moderately diverged and rest of the sites were not infected by more parasites and the parasite communities were poorly diverged (Table 7). In case of *C. punctatus,* parasite communities were moderately diverged in Mymensingh and Jashore (Table 7). Whereas, fish samples collected from Kishoreganj had no parasites diversity and fish samples collected from Faridpur was not infected by more parasites and the parasite community was poorly diverged (Table 7). Direct comparison was not drawn due to lack of available previous findings.

Table 7. Shannon Diversity Index (H) and Simpson's diversity Index (D) of the parasite communities of the different sampling areas in *Anabas testudineus* and *Channa punctatus*.

Study area	Shannon Diversity Index (H)		Simpson's diversity Index (D)	
	A. testudineus	*C. punctatus*	*A. testudineus*	*C. punctatus*
Mymensingh	0.773	0.662	0.518	0.536
Kishoregonj	0.533	0.00	0.248	0.00
Faridpur	0.557	0.167	0.272	0.064
Jashore	0.563	0.748	0.301	0.504

Since the host fishes play an important role as popular edible fish in Bangladesh, assessing the parasitic infestation is necessary to limit their further damage. A primary database of comparative analysis between protozoan parasites of *C. punctatus* and *A. testudineus* has been established by the present work which will be helpful for further study with a broader extent including more study areas and larger sample size.

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