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FIRST REPORT ON THE OCCURRENCE OF *MICROPHALLUS INDICUS* IN THE FRESH WATER CRAB, *SARTORIANA SPINIGERA* IN BANGLADESH

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Department of Microbiology and Parasitology,
Sher-e-Bangla Agricultural University,
Sher-e-Bangla Nagar, Dhaka, P.O. Box 1207, Bangladesh
*Corresponding author e-mail: ukmohanta.mipa@sau.edu.bd

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Sartoriana spinigera is an edible freshwater crab species that plays an important role as an intermediate host of *Microphallus indicus* and *Paragonimus westermani. P. westermani*, the lung fluke, has been reported from northeastern India bordering Bangladesh and other parts of India. Nonetheless the existence of *P. westermani* in Bangladesh is yet to investigate. Therefore, the objective of the study was to estimate the prevalence of lung flukes in Bangladesh. During the survey, the metacercariae of food-borne trematodes were investigated from a total of 261 fresh-water crabs collected from different locations in Bangladesh. Only the metacercariae of *M. indicus* were recovered. The overall prevalence of *M. indicus* metacercariae was 26% with intensity of 1–63 per crab. The highest prevalence was recorded from Naogaon district (83.33%), followed by Sirajgonj (57.5%), Rajshahi (40%), Brahmanbaria (16%), Narsingdi (15%), Faridpur (10%), and Sunamgonj (7%). None of the crabs from Rangpur, Thakurgoan, Jashore, Meherpur, Magura, Barguna, Sylhet, Kishorgonj, Cox'sBazar, and Bandarban were infected. Among the water bodies, the highest number of infected crabs was found in the rivers (56%) followed by haor, canal, and ditches (5%). To the best of our knowledge, this study reports *M. indicus* for the first time from Bangladesh.

Keywords: Freshwater crabs, *Sartoriana spinigera*, *Microphallus indicus*, metacercariae, prevalence, Bangladesh

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Crabs are important exportable fishery items and hidden resources of Bangladesh (Ahmed, 1991). Food-borne trematodiasis (FBT) is a neglected tropical disease bearing zoonotic significance. They are transmitted through the consumption of raw or undercooked aquatic foods that harbor the metacercaria affecting humans. More than 40 million people are getting infected and more than 10% of the world's population is at risk of FBT infection (Keiser, Utzinger, 2005, 2009; Sripa et al., 2010; Toledo et al., 2011). The Crustacea-borne trematode infections are caused by fluke parasites belonging to the families Paragonimidae, Microphallidae, Lecithodendriidae, Brachylaimidae etc (Yamaguti, 1971; Anantaraman, Subramoniam, 1976; Janardanan et al., 1987). *Paragonimus westermani* (Kerbert, 1878; Braun, 1899) have been regarded as the most common and widely distributed human pathogen in Asia (Miyazaki, 1974). *Paragonimus heterotremus*, *P. westermani*, *P. miyazakii manipurinus*, and *P. skrjabini* (Chen, 1959) has been reported from Manipur, Nagaland, Arunachol, and Meghalaya states in India (Singh, Singh, 1997; Narain et al., 2003; Singh et al., 2009). Therefore, there is a high chance of their existence in Bangladesh. Hence, the present study was primarily aimed to investigate the occurrence of *Paragonimus* spp. in freshwater crab in different locations in Bangladesh.

Along with *Paragonimus* metacercariae, microphallid metacercepariae have also been reported from crabs in Meghalaya, the northeast India (Goswami et al., 2013). The family Microphallidae is a digenean taxon representing more than 160 species from 28 genera and 10 subfamilies (Bray et al., 2008). They are mostly found in the intestines of vertebrates, mainly birds (Martorelli et al., 2004) such as herring gulls, shore-birds and among mammals, especially rodents (Deblock, 2008). Infective metacercarial stages of microphallid flukes commonly occur in Crustacea (Heard, Overstreet, 1983; Pung et al., 2002) and xiphosuran second intermediate hosts, such as crabs, barnacles, and the king crab (Anantaraman, Subramoniam, 1976). Other *Microphallus* spp. reported in India are *Microphallus nicolli* (Anantaraman, Subramoniam, 1976), *M. dicaecus* (Mukherjee, Ghosh, 1967) and *M. indicus* (Mukherjee, Ghosh, 1967) .

Freshwater crabs play an important role in transmitting metacercariae to the final host. Poor and marginal people of Bangladesh eat freshwater crabs seasonally, and the tribal people eat raw, uncooked, or pickled crabs regularly. As the metacercariae are distributed in northeast India, their prevalence in Bangladesh would not be surprising. Unfortunately, no attempt has been made yet to investigate the crab-borne trematode infection in Bangladesh. Therefore, the present study aimed to test whether trematode metacercariae occur in fresh-water edible crab species, the potential intermediate hosts for digenetic flukes, in selected localities of Bangladesh.

MATERIALS AND METHODS

Sampling area and crab-catching

A total of 261 crabs were collected from different geographic areas of Bangladesh, namely: Bandarban, Sylhet, Sunamgonj, Kishorgonj, Rajshahi, Rangpur, Naogaon, Thakurgoan, Sirajgonj, Barguna, Cox's Bazar, Brahmanbaria, Narsingdi, Meherpur, Jashore, Magura and Faridpur districts. For further studies, the specimens were either preserved in 70% ethanol in a plastic container or kept in the bucket full of water.

Crab identification

For the identification of freshwater crabs, morphological characteristics of each specimen were studied in detail. The color, shape, size of the whole body, the carapace brown chillete color; the texture and shape of the carapace were considered for the crab identification. Species identification was based on by keys and description of Wood-Mason (1871).

Collection and preservation of metacercariae

After removing the carapace and the internal organs, the body and leg muscles were blended in 10.0 mL acid-pepsin (1g pepsin and 1 liter of 0.7% hydrochloric acid (HCl); 1 : 1000) using the blender. Acid-pepsin solution (Artificial gastric juice) was used to facilitate the digestion of the host tissue. Blended crab tissues were incubated at 37°C overnight (Tandon et al., 2007). The subsequent digest was filtered through a wire sieve (1 mm mesh), allowed to sediment, and washed several times. After the removal of the supernatant, the sediment was placed into Petridishes and observed under a binocular microscope. Then, the metacercariae were collected, and counted the number of metacercariae to assess the intensity, and preserved in 70% ethanol at 4°C for further morphological identification.

Whole mounting

The newly collected fresh metacercariae were placed on a glass slide. After adding a few drops of PBS solution, the slide was placed under binocular microscope. The outer shell of the metacercaria was broken down with the help of a needle to remove the pyriform-shaped larva. The larva was straightened, and then the coverslip was placed on it and tied with a thread. The slides were kept in a coplin jar containing 70% alcohol at least overnight, and the flattened specimens were processed for whole mounts. For staining, specimens were transferred to 50% ethanol and distilled water for an hour each. Then specimens were placed in hematoxylin solution for 24 hours. The excessive stain was removed by acid alcohol (3% HCl). The stained specimen was dehydrated with ascending grades of alcohol (70–100%), cleared in xylene and mounted with Canada balsam.

RESULTS

Morphological description of metacercariae

The excysted metacercariae were morphologically identified as *M. indicus* based on the keys and description of Mukherjee and Ghosh (1967). Metacercariae when removed from crabs round (Fig. 1*A*), cysts elliptical with a prominent thick wall composed of two layers. Outer layer thick but transparent, and inner layer thin. Vitelline glandular cells clearly visible in two opposite poles of encysted metacercariae (Fig. 1*B*).

On whole mounts, the body is pyriform and minute. The oral sucker is subterminal; ventral sucker post-equatorial (Fig. 1*D*). Oesophagus is long, bifurcating in mid body region. Intestinal caeca are short, pretesticular and preacetabular (Fig. 1*D*). Testes are symmetrical, one on either side of ventral sucker; cirrus present, curved at level of caecal bifurcation; cirrus is well-developed. Ovary located on the right side of the ventral sucker or slightly overlapping right testis (Fig. 1*D*). Vitelline glandular cells are arranged in two groups, one with seven lobes right, other with six lobes left (Fig. 1*D*). Excretory vesicle is V-shaped (Fig. 1*C*).

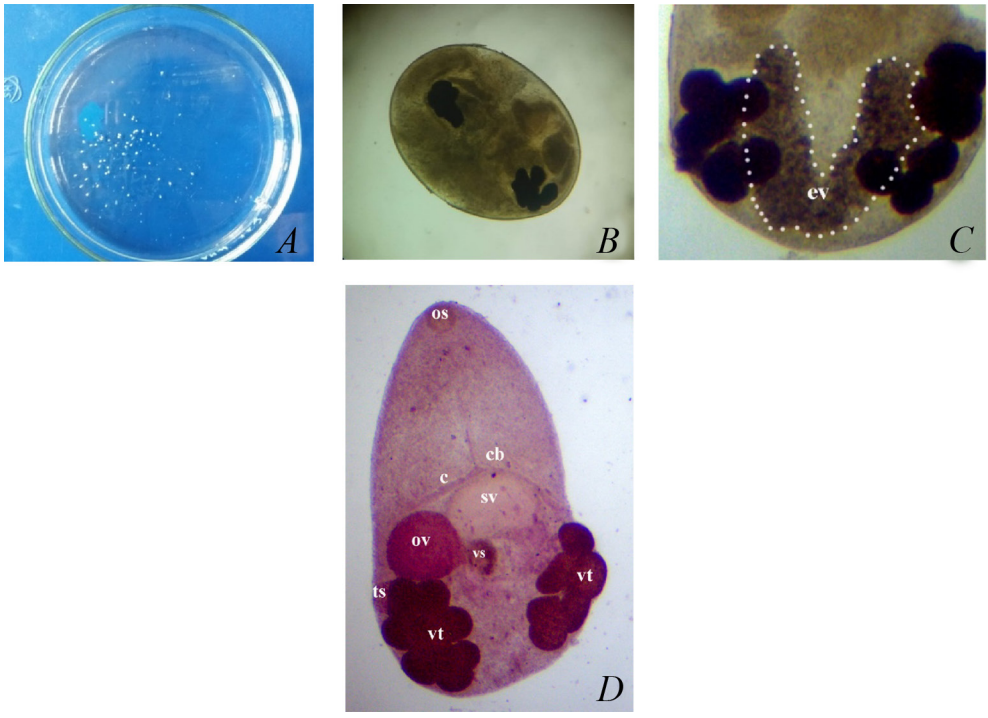

Figure 1. Different body parts of the larval stage of *Microphallus indicus*. *A* – freshly harvested metacercariae of *M. indicus* from infected crabs, *B* – encysted Metacercariae pressed with glass slide (4X), *C* – excretory vesicle 'V' shaped (10X), *D* – excysted metacercariae with whole mount (Ventral view). Ovary located on right side of ventral sucker. Vitelline glandular cell in right 8 and in left 7 (10X). os – oral sucker, cb – caecal bifurcation c – caeca, sv – seminal vesicle, vs – ventral sucker, ov – ovary, ts – testes, vt – vitellaria, ev – excretory vesicle.

Identification of metacercariae

These morphological characters of the metacercariae were in accordance with those described by Mukherjee and Ghosh (1967); and Goswami et al. (2013), so they were identified as *M. indicus.* Unlike *M. indicus*, *M. nicolli* metacercariae have a Y-shaped excretory vesicle a 3-layer spherical cyst (Anantaraman, Subramoniam, 1976), and *M. dicaecus* have multi-layer: outer layer thin; inner layer thick; ovoid cysted with V-shaped excretory pore (Mukherjee, Ghosh, 1967).

Prevalence of microphalid metacercariae

Among the 261 crabs collected, none were found to harbor the metacercariae of *Paragonimus* spp. in any of the areas studied, and 66 (26%) were found to harbor microphalid metacercariae (Table 1). However, the highest metacercarial infection prevalence (83.33%) was observed in the crabs collected from Naogaon, followed by Sirajgonj (57.5%), Rajshahi (40%), Brahmanbaria (16%), Narsingdi (15%), Faridpur (10%), and Sunamgonj (7%). None of the crabs collected from Rangpur, Thakurgoan, Jashore, Meherpur, Magura, Barguna, Sylhet, Kishorgonj, Cox's Bazar and Bandarban were infected (Table 1). The highest intensity of metacercariae was found in crabs from Naogaon (1–63 metacercariae per crab), followed by Narsingdi (4–60 per crab), Sirajgaonj (1–33 per crab), Rajshahi (1–12 per crab), Brahmanbaria (2–6 per crab), Faridpur (0–5 per crab), and Sunamgonj (0–5 per crab), respectively.

Table 1. Prevalence and burden of microphallid metacercariae in freshwater crabs collected from 17 districts in Bangladesh

Location	Number of crabs		Prevalence, %	Intensity	Abundance (Mean±SD)	F value
	Examined	Infected				
Sirajgonj	40	23	57.5	1-33	4.78±7.27	
Naogoan	36	30	83.33	1-63	13.43±14.01	
Rajshahi	10	4	40	1-12	2.0±0.0	
Rangpur	10	0	0	-	-	
Thakurgoan	10	0	0	-	-	
Jashore	10	0	0	-	-	
Faridpur	10	1	10	0-5	0.5±1.58	
Meherpur	10	0	0	-	-	*5.70
Magura	10	0	0	-	-	
Barguna	10	0	0	-	-	
Brahmanbaria	25	4	16	2-6	0.6±1.52	
Narsingdi	20	3	15	4-60	3.75±1.29	
Sylhet	15	0	0	-	-	
Sunamgonj	15	1	7	0-5	0.5±1.29	
Kishorgonj	10	0	0	-	-	
Cox'sBazar	10	0	0	-	-	
Bandarban	10	0	0	-	-	
Total	**261**	**66**	**26**			

SD = Standard Deviation. * indicates that statistically 1% level of significance.

The highest prevalence was found in rivers (56%), followed by haor, canal, and ditches (5%) (Table 2). None of the crabs collected from the ponds were found to harbor metacercaria.

Table 2. Prevalence and burden of metacercariae in freshwater crabs according to types of waterbodies

Location	Number **of crabs**		Prevalence, %	Intensity
	Examined	**Infected**		
River	121	61	56	1-63
Haor, canal and ditches	100	5	5	1-33
Ponds	40	0	0	-

DISCUSSION

Paragonimus westermani is widely distributed in south and southeast Asian country such as Japan (Miyazaki, 1991), Korea (Miyazaki, 1991), China (Miyazaki, 1991), Vietnam (Doanh et al., 2009), Thailand (Sugiyama et al., 2007), and Sri Lanka (Devi et al., 2010). *P. westermani* are prevalent in the northeast India namely, Nagaland, Arunachol, and Manipur (Narain et al., 2003; Tandon et al., 2007; Devi et al., 2010). Despite of bordering Bangladesh in the east, there was no previous study of *P. westermani* in Bangladesh. In this study, the recovered metacercariae were not morphologically matched with *P. westermani.* In addition, recovered metacercariae morphologically identified to *M. indicus* described by Mukherjee and Ghosh (1967). Although the reasons behind the absence of *P. westermani* are Bangladesh are not clear, the differences in the food habit of most of the people in the country might contribute to the unavailability of the parasites. In the present study, a total of 261 trapped crabs were examined, and identified as *S. spinigera*. The occurrence of *M. indicus* was reported from crabs (*Barytelphusa lugubris*, *Potamiscus manipuriensis*) in northeast India namely, Assam, Meghalaya, Manipur (Athokpam, Tandon, 2013; Goswami et al., 2013), while *M. nicolli* was recovered from Madras, South India (Anantaraman, Subramoniam, 1976). Therefore, the existence of microphallid metacercariae in freshwater crabs in Bangladesh was anticipated. In addition, microphallid metacercariae have also been reported from sand crabs and brackish-water prawns near the south-eastern coast of the Indian subcontinent (Anantaraman, Subramoniam, 1976; Jayasree et al., 2001). A comparison of the morphological features of *M. indicus* (Mukherjee, Ghosh, 1967) described so far from crustacean hosts in India reveals a close similarity to the metacercarial stage recovered in this study. The present study showed that the microphalid metacercaria is distributed in different districts of Bangladesh. Metacercariae of *M. indicus* were also recovered from *Barytelphusa lugubris* and *Potamiscus manipuriensis* in Meghalaya and Assam, and Manipur, respectively (Goswami et al., 2013). In contrast, *Sartoriana spinigera* are abundantly found in the mud soil, and wetlands of Bangladesh, Pakistan, and India (Assam, Meghalaya, Manipur, Arunachal, Bihar, West Bengal). Therefore, they can act as second intermediate hosts of the microphallids in Bangladesh. This study gives an overview on the metacercarial infection of freshwater crabs in Bangladesh. In this study, the overall prevalence of *M. indicus* was 26%, where the intensity from 1 to 63 per crab. The overall

prevalence of microphalid is higher than that reported in Assam (15.70%, Goswami et al., 2013), and Manipur (15%, Athokpam, Tandon, 2013) in India. On the contrary, this prevalence is much lower than the prevalence (91.05%) with extremely high intensity (285/crab) reported from Meghalaya (Goswami et al., 2013) in India. In addition, the intensity of metacercariae of our study is lower than the findings of previous study (Goswami et al., 2013). The variation in the prevalence might be due to the variations in sampling size, different geographic locations, species of intermediate host, and availability of final host.

In this study, the northern parts of Bangladesh showed the highest metacercarial infection, which might be due to availability of intermediate crab hosts and final hosts. These northern regions of Bangladesh are predominantly river based, with year round water availability. So, the crabs and snails inhabit these areas all the year round. AEZ-3, AEZ-4 and AEZ-5 containing northern parts of Bangladesh, namely Naogaon, Rajshahi, Natore, Pabna, and Sirajgonj, conveys common rivers and beels (Chalanbeel), a large water body. Moreover, a large population of wild ducks inhabit these areas, which might transmit eggs to the intermediate hosts via feces.

The present study showed the highest prevalence of microphalid metacercariae in the crabs trapped in rivers (56%) followed by haor, canals, and ditches (5%), and ponds (0%). The intermediate snail host, crayfishes, and crabs can share the habitats in both in Bangladesh and India due to some common rivers. Due to the availability of food and water all over the year, crabs get a good environment in the river where they can complete their life cycle easily. Then, the intermediate hosts, crabs, are predated by the final host like shore birds, herring gulls, jackals, wild ducks, turtles etc. Moreover, water fowl migrate in Bangladesh during winter season which can play an important role in dispersing parasites. Due to the lack of final host at pond and no connection with infected source, there are probably no infections in the ponds.

CONCLUSION

This study investigates the prevalence of *M. indicus* metacercariae in freshwater crabs from different locations in Bangladesh for the first time. However, this study could not detect any metacercariae of *P. westermani* in any of the water bodies examined in Bangladesh. Nevertheless, molecular study of the freshwater crab-borne metacercariae in Bangladesh may provide a more comprehensive information.

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ETHICS APPROVAL AND CONSENT TO PARTICIPATE

All the protocols were performed in studies involving animals were in accordance with the ethical standards of the institution or practice.

CONFLICTS OF INTEREST

The authors have declared that they have no conflicts of interest to this research work

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ПЕРВОЕ СООБЩЕНИЕ ОБ ОБНАРУЖЕНИИ *MICROPHALLUS INDICUS* В ПРЕСНОВОДНОМ КРАБЕ *SARTORIANA SPINIGERA* В БАНГЛАДЕШ

Аль Васеф, Ануп Кумар Адхикари, С. М. Абдуллах, Удай Кумар Моханта

Ключевые слова: пресноводные крабы, *Sartoriana spinigera*, *Microphallus indicus*, метацеркарии, распространенность, Бангладеш

РЕЗЮМЕ

Sartoriana spinigera – это промысловый пресноводный краб, который играет существенную роль в качестве промежуточного хозяина *Microphallus indicus* и *Paragonimus westermani.* Легочная трематода *P. westermani* ранее отмечена в северо-восточной Индии, граничащей с Бангладеш, и в других районах Индии. Мы исследовали 261 экз. пресноводных крабов из разных районов Бангладеш. Обнаружены только метацеркарии *M. indicus*, общая зараженность которыми составили 26% при интенсивности 1–63 личинок в одном крабе. Это первое обнаружение *M. indicus* в Бангладеш. Наибольшее количество зараженных крабов найдено в округе Naogaon (83.33%), меньше их было в Sirajgonj (57.5%), Rajshahi (40%), Brahmanbaria (16%), Narsingdi (15%), Faridpur (10%) и Sunamgonj (7%). Ни один из крабов, собранных в Rangpur, Thakurgoan, Jashore, Meherpur, Magura, Barguna, Sylhet, Kishorgonj, Cox'sBazar и Bandarban, не был заражен трематодами. Наиболее зараженными метацеркариями *M. indicus* оказались крабы из рек (56%), значительно меньше зараженность особей в водно-болотных системах (хаорах), каналах и канавах (5%).