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Detection of helminth parasites in commercialized turtles: threats to native Testudines in northeast India

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Abstract

Both biotic and abiotic factors recently constitute a spectacular threat to the freshwater turtles throughout the world including northeast India. However, the high rate of exploitation is meagerly estimated and intervention of scientific approaches is poorly adopted for their proper conservation. The present study examined the commercialized turtles in northeast India and reported the presence of helminth parasites (nematodes and trematodes) in three highly threatened turtles, *Nilssonia gangetica*, *Nilssonia nigrican*, and *Chitra indica*. Both *N. gangetica* and *N. nigricans* have been found to be the first host records of the trematode, *Astiotrema reniferum*. Further, *C. indica* is a new host record for the trematode, *Stunkardia dilymphosa*, which assumed to be host specific suitability due to difference in body size. Additionally, the study highlighted the urgent need of enforcing the veterinary studies, quarantine regulation, and prohibitions of illegal trading of threatened turtles in Tripura state and other parts in India.

Keywords: Testudines, Helminths, Northeast India, Conservation Management

Introduction

Freshwater turtles are one of the oldest organisms on the earth and playing major role in ecosystem services (Kundu *et al*., 2016). However, due to various anthropogenic threats, accidental catch by fishing gear, bush meat crisis, and habitat loss, the population of freshwater turtle are declining everywhere throughout the world including India (van Dijk *et al*., 2000). In the recent past, many conservation agencies have attempted several conservation action plans and awareness program for citizenries to revive the declining population in the wild (Mendiratta *et al*., 2017). Further, it was noticed that, the government and non-government agencies often rescued the confiscated turtles from poachers and merchants (Mendiratta *et al*., 2017). However, the enforcement agencies confronted several challenges while releasing the seized turtles into suitable natural settings (Kundu *et al*., 2018b). Hence, before releasing them back into their suitable wild habitat, it is necessary to evaluate their genetic signature as well as health related issues.

Over the time, the helminth parasites were reported from different freshwater turtle viz., *Lissemys punctata*, *Pangshura sylhetensis*, *Pangshura tecta*, and *Pangshura tentoria* from different geographical regions (Punjab, Uttar Pradesh & Haryana) in India (Thapar, 1933; Bhalerao, 1936; Gupta, 1954; Siddiqi, 1965; Soota & Ghosh, 1977; Ghosh & Srivastava, 1999). Further, various clinical symptoms like lethargy, constant and sideways swimming in water body, hemiplegia, and ulcerative lesions on the carapace were detected in freshwater turtles due to the infection of helminthes (Cardells *et al*., 2014). The trematode eggs also affected the liver, brain, spleen, kidney, myocardium, lung, pancreas, testes, and bladder, and the deposition of the eggs may block small blood vessels within the intestines, causing necrosis and bacteremia (McAllister *et al*., 2015).

The northeastern region of India and its neighboring counties viz., Bangladesh and Myanmar are regarded as one of the treasure trove of freshwater turtle diversity with more than 25 species (Buhlmann *et al*., 2009). Although,

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the abundant turtle diversity has been acknowledged from India; the physiological, ecological, population structure, and interaction with other organisms has appraised in a limited manner. Thus, the present study aimed to determine the presence of helminth worms in the commercialized freshwater turtles collected from markets in northeast India. The study also reports the helminth parasites from the unknown populations of three highly threatened freshwater turtles and presume the possible risk of parasite transmission to the native freshwater turtle populations.

Material and Methods

The survey recorded dozens of freshwater turtles are being traded in most of the fish markets in Agartala, Tripura state in northeast India. We also communicate with the local fisherman communities and dealers to acquire the information of trade route. Total five digestive tracts (esophagus, stomach, small and large intestine) of five host turtles were collected and stored in ethanol containing sterile containers. The digestive tracts were opened along its entire length and examined under a dissection microscope. The nematodes were fixed in 4% formalin, dehydrated in glycerin alcohol and mounted in wax-sealed slides for identification. While, the trematodes were washed, slightly pressed and fixed in Alcohol-Formalin-acetic Acid (AFA), stained with Borax carmine, dehydrated and mounted in DPX mounting medium. Subsequently, the examined helminths were identified and photographed under Olympus BX-53 microscope with an attached DP27 camera. The studied helminths were deposited in National Zoological Collections, Platyhelminths section, ZSI; Kolkata and registration numbers of each specimen were assigned.

Results and Discussion

The study identified the commercialized turtle specimens as the Indian softshell turtle, *Nilssonia gangetica* (IUCN status: Vulnerable), the black soft shell turtle, *Nilssonia nigricans* (IUCN status: Extinct in the Wild) and the Indian narrow-headed softshell turtle, *Chitra indica* (IUCN status: Endangered) based on the carapace and plastron pattern available in previous literature (Das, 1991). The *N. gangetica* and *C. indica* is reported from Pakistan, India, Bangladesh, and Nepal whereas, *N. nigricans* has narrow and scanty distribution in the northeastern region of India (Assam) and Bangladesh (Chittagong) (McCord *et al*., 2003; Kundu *et al*., 2015). Out of two investigated gut contents of *N. gangetica* and *N. nigricans*, one gut of each species was found to be infected by *Astiotrema reniferum*. Total five individuals (Registration Nos. ZSI-W 10270/1 to W 10274/1) was collected from *N. gangetica* gut and 12 individuals (Registration Nos. ZSI-W 10275/1 to W 10286/1) was collected from *N. nigricans* gut (Table 1 and Figure 1A-D). In the recent past, researchers have reported *A. reniferum* from different freshwater fishes (Koiri & Roy, 2017) and amphibians (Chakrabarty & Ghosh, 2010). The *A. reniferum* worm is also recorded from freshwater turtles, including *Batagur kachuga, Lissemys punctata, Chitra indica,* and *Kachuga dhongoka* from different geographical regions in India (Thapar, 1933; Bhalerao, 1936; Gupta, 1954; Siddiqi, 1965; Soota & Ghosh, 1977; Ghosh & Srivastava, 1999). Hence, the present detection of *A. reniferum* from *N. gangetica* and *N. nigricans* is recognized as a new host record of these helminths. The study further detected the effect of nematode species belonging to *Dichelyne* genus in the gut content of *N. gangetica*. However, the nematode could not be identified up to species level due to the shrinkage of body cuticle (Figure 2A-B).

Additionally, the study detected the infection of three trematodes namely, *Stunkardia dilymphosa* (Registration No ZSI-W 10267/1 to W 10269/1) from a single *C. indica* host gut (Table 1 and Figure 3A-B). The *S. dilymphosa* was previously reported from the northern river terrapin, *Batagur baska*, and the Burmese eyed turtle, *Morenia ocellata* (Bhalerao, 1931; Chatterji, 1936). Although the morphological measurements of the examined *S. dilymphosa* were similar to the Indian specimens reported earlier (Mehrotra & Gupta, 1974), but differ from the original description from Rangoon, Myanmar (Bhalerao, 1931). However, the present study assumed that the difference in their size as compared with the original description might be due to the new host specific suitability. Moreover, the study proposed that widespread survey is necessitated to confirm the host specificity of *S. dilymphosa* in *C. indica*.

The present study elucidates the occurrence of endo-parasites in freshwater turtles and evidences the biotic threat in northeast India. However, the intramuscular or

Table 1. Morphometric measurements of two helminth parasites (trematodes) collected from threatened freshwater turtles

Collateral information & Morphological data	***Trematodes***		
	Astiotrema reniferum	***Astiotrema reniferum***	***Stunkardia dilymphosa***
Host	*Nilssonia gangetica*	*Nilssonia nigricans*	*Chitra indica*
Site of infection	Small intestine	Small intestine	Small intestine
Body length	1.16-2.95 mm	2.15-8.15 mm	3.7-5.02 mm
Body width	0.34-0.57 mm	0.62-1.18 mm	0.8-0.7 mm
Cuticle	Spinose, More in front of the abdominal sucker (5-20 μm)	Spinose, More in front of the abdominal sucker (8-40 μm)	Smooth
Oral sucker	Terminal, 0.12-0.19 mm x 0.11-0.17 mm	Terminal, 0.10-0.33 x 0.07-0.26 mm	0.41 -0.69 mm x 0.40-0.59 mm
Acetabulum	0.12-0.19 x 0.10-0.17 mm	0.15-0.33 x 0. 11-0.32 mm	0.75-0.98 mm x 0.61-0.69mm
Esophagus	0.09-0.12 x 0.01-0.05 mm	0.11-0.55 mm x 0.01-0.11 mm	Zigzag, 0.23-0.36 mm x 0.18-0.23mm
Pharynx	0.04-0.08 x0.0.4-0.09 mm	0.05 x 0.15 mm x 0.03-0.15mm	Absent
Caecal termination	Hind body	Hind body	Anterior edge of acetabulum
Genital pore	Just in front of abdominal sucker	Just in front of abdominal sucker	Just Prebifurcal
Anterior testis	0.17-045 mm x 0.11-0.35 mm	0.28-1.20 mm x 0.15-0.94 mm	0.37-0.60 mm x 0.22-0.38 mm
Posterior testes	0.17-0.40 x 0.11-0.39 mm	0.27-1.25 x 0.18-1.20 mm	0.36-0.58mm x 0.30-0.48 mm
Ovary	0.10-0.23 x 0.08-0.15 mm	0.14-0.49 x 0.13-0.35 mm	0.11-0.19mm x 0.10-0.14 mm
Eggs	0.02-0.03 x 0.01 mm	0.02-0.05 x 0.009-0.03 mm	0.16-0.17 mm x 0.08-0.09 mm
Excretory pore	Ventral, subterminal	Ventral, subterminal	anterior to acetabulum
Cirrus sac	0.23-0.75 x 0.10-0.18 mm	0.43-1.00 mm x 0.15-0.30 mm	Absent

oral introduction of Praziquantel might be an effective treatment for infected turtles as suggested earlier (Johnson *et al*., 1998). Besides, the northeastern region of India is also known as one of the turtle poaching hubs and every year tons of turtles have been traded for commercialized purposes (Kundu *et al*., 2016). Nevertheless, India and adjacent countries, the consumption of turtle meat is believed to be spiritualist to abstain from sin and get virtuous life by being honest, compassionate, and loving (Kundu *et al*., 2018a). Further, the derived organic material from different body parts of turtles are frequently used by the tribal peoples for various traditional therapeutic purposes (Chen *et al*., 2009). Thus, the turtle meat is lucrative and high demanding in India as well as in other South and South East Asian countries. Moreover, the illegal hunting and illicit international black-market trade imperils many highly threatened turtle species in Southeast Asian countries (Das *et al*., 2012). The fisherman communities often catch this animal during fishing activities from freshwater eco-systems and sold them in commercialized markets with profitable values by outraging the existing wildlife protection laws. This preliminary data suggested that the record of infected turtles in several markets of Tripura state and the on-going trafficking from anonymous population might increase the dispersing risk of the helminth parasites into the native turtle population in northeast India. Furthermore, the man-made abiotic threats are uncontrolled due to the lack of awareness among the local peoples. Thus, to save these threatened turtle species, it is also urgent to pursue repeated awareness programs aimed to the local fisherman's and wildlife traffickers. Additionally, the

study strongly advocated to the state forest departments and wildlife divisions for adopting prompt actions, and ban the on-going commercialized trading of threatened turtle species in Tripura state in northeast India.

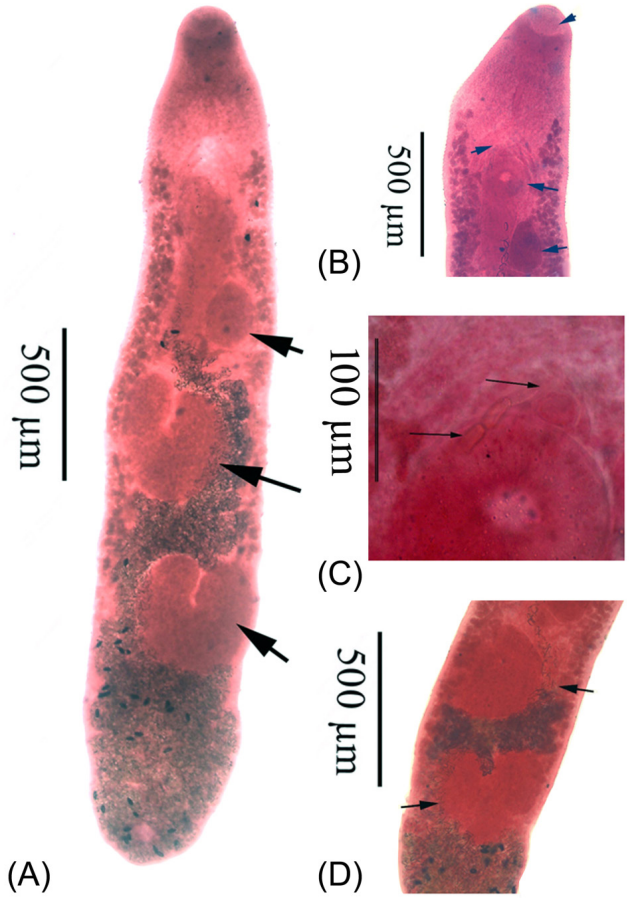

Figure 1. *Astiotrema reniferum*: **(A)** entire, ventral view (arrow marks anterior to posterior denoted ovary, anterior testis, and posterior testes), **(B)** Anterior end, ventral view (arrow marks anterior to posterior denoted oral sucker, gonopore, acetabulum, and ovary), **(C)** Gonopore, and egg are marked by arrow anterior to posterior, and **(D)** Testes, ventral view.

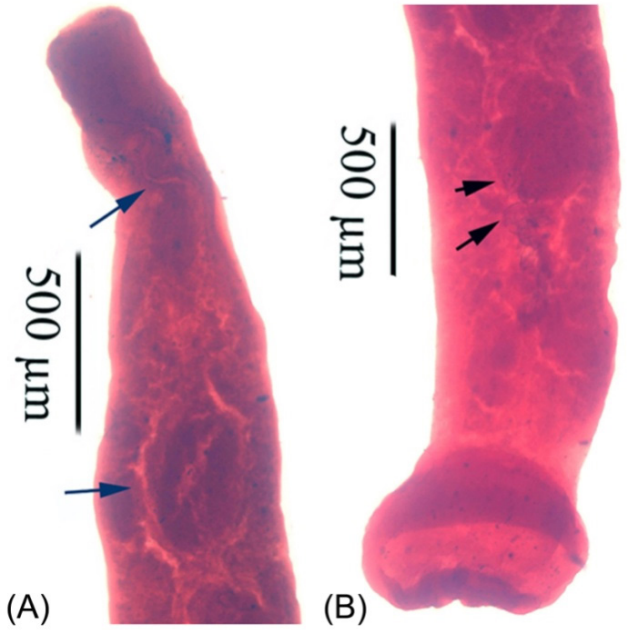

Figure 2. *Stunkardia dilymphosa*: **(A)** Anterior end, ventral view (arrow marks anterior to posterior denoted esophagus, and anterior testis), and **(B)** Posterior end, ventral view (arrow marks anterior to posterior denoted posterior testis, and eggs).

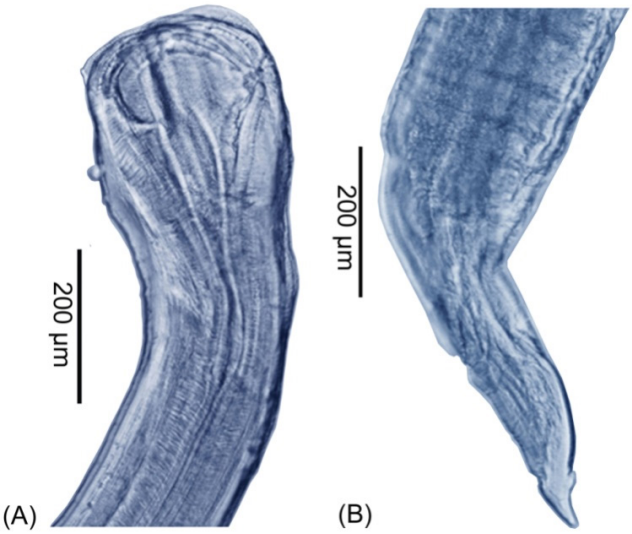

Figure 3. *Dichelyne* species: **(A)** Esophagus, lateral view, and **(B)** Tail, lateral view.

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