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Scanning electron microscopy study of *Cosmocercoides* species an amphibian nematode

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Abstract
The nematological fauna of amphibian species from North Eastern part of India has not received any attention. Amphibians including frogs are prone to suffer from a variety of parasites and diseases like other animals. Thirty species of either sex representing two species (*Rana tigrina, Bufomelano tictus*) of amphibians were collected from various parts of Aizawl, Mizoram during the month of June and July 2016 and searched for presence of any nematodes. *Rana tigrina* and *Bufomelano tictus* were found positive for *Cosmocercoides sp.* The present study describes light microscopy (LM) and scanning electron microscopy (SEM) of *Cosmocercoides* species.

Keywords: Amphibians, *Rana Tigrina*, *Bufomelano tictus*. Nematodes, SEM, Aizawl, Mizoram, India

1. Introduction
Amphibians are distinctive among animals physiologically, morphologically, and medically. Many amphibians are kept or reared for culture, in zoos for public display and also to keep breeding programme for endangered species. Amphibians, particularly frogs (both aquatic and land) besides suffering themselves with different helminthic diseases also serve as intermediate host for life cycle of certain helminthes. Frog meats are also very popular in some South Asian countries. (….). Many diseases of amphibians are closely related to diseases of other ectothermic vertebrates. Frogs may share many pathogens, and in some instances, inter-class transmission (e.g., from fish to amphibian) of some infectious agents occurs. This possibility is an important epidemiological consideration for amphibian disease, and it is essential not to overlook the potential role of other animal classes in disease pathogenesis. One cannot even rule out zoonotic importance of helminthes transmitted by frog. Many types of helminths may infect amphibians. *Clinostomum* –a trematode parasite which metacercarial stages produces grossly visible yellowish lumps under the skin of frogs. Veterinarians and care providers have often adapted diagnostic methods, treatment regimes, preventive measures, and amphibian veterinary care in captive condition. Patterson-Kane *et al*. have reported a nematode of the genus *Strongyloides* in association with a protein-losing enteropathy and death in a captive Cope's gray tree frog (*Hyla chrysoscelis*) [1].
Several species of nematodes have been reported from amphibians by various workers from different parts of world [2-4]. With the exception of few cases, amphibians act as definitive hosts for various species of nematodes. Pough *et al* (2001) [5] pointed out that among helminthes; nematodes are particularly abundant in the digestive tract, lungs and blood vessels of amphibians. Amphibians also harbor annelids, pentastomids and arthropods [6]. In addition to broadening our knowledge over biodiversity and disease, the study of the nematodes of amphibians is crucial as it contributes to our understanding of feeding and other aspects of their biology, and may be useful as biological markers for amphibian populations.
Till date there is very little information pertaining to parasite/s of frog is available from India. However Sarkar and Manna reported occurrence of lung nematode parasites of amphibians from India [7]. In this paper, one species of nematode belonging to the genera *Cosmocercoides sp* in naturally infected frogs is described under LM and SEM study.

2. Materials and methods
2.1 Study area
The present study was conducted in Aizawl district of Mizoram in India.

2.2 Collection of hosts and recovering of nematodes

Adult frogs, *Rana tigrina* and *Bufomelano tictus* were captured by hand using the technique so called visual encounter survey. The live frogs were brought to the laboratory and killed in a chloroform solution. The abdominal cavity of each frog was opened and each visceral organ was carefully examined under stereozoom microscope (Euromex, Holland) for presence of parasite. Any parasite recovered was thoroughly washed in physiological saline before transferring to 70 % alcohol.

2.3 Morphological study

Initial examination of the nematode was carried out under stereozoom (Euromex, Holland). Male and female nematodes ware separated and one male and one female were kept in lactophenol for LM study. On the basis of morphometry the nematode under LM identified under the genus *Cosmocercoides*. For detail surface study one male and female nematodes was further processed for SEM.

2.4 Preparation of sample for SEM.

Both male and female nematodes were fixed in 3% glutaraldehyde. Before dehydration any hindrances like faceal debris, mucus, blood or other body fluids were carefully removed by washing several times with nuclease free water (NFW) several times with the help of a brush. The samples were then fixed at 4°C for 24 hours. The fixed trematodes were washed in phosphate buffered saline (pH – 7.2) three times and then in double distilled water followed by acetone dehydration.

After acetone dehydration, the specimen was dried with liquid CO_2 at its critical point i.e. 31.5°C at 1100 psi. The specimen is then dipped in tetra methyl saline (TMS) for 5-10minutes with two changes at 4 °C. They are then brought to room temperature (25-26 °C) for drying. The samples were mounted on aluminium stubs. Finally they were gold coated in a sputter coat and finally examined under SEM [(JSM-6360-JEOL)] at the North Eastern Hill University (NEHU), Shillong, Meghalaya, India in Sophisticated Analytical Instrument Facilities (SAIF) Laboratory.

3. Results

A total of 30 frogs were examined and 10 were found positive for *Cosmocercoides* sp. The female is larger than male (Fig. 1). The length of the female varies from 4-7 mm while male ranges from 2-3 mm. The mouth bears three lips, an oesophagus which is bulbous posteriorly with a simple intestine (Fig. 2). The female bears a long pointed tail while the male tail is small (Fig. 3 and Fig. 4). The cuticular modifications that are found in the present nematode are cuticular flap, three lips surrounding the mouth; each lip has a cuticular flange overhanging the mouth opening (Fig. 5), somatic papillae (Fig. 6), lateral groove (Fig. 7) and cuticular inflations (Fig. 8). The posterior end of the male possesses pre and post cloacal papillae with prominent cloacal opening (Fig. 9). The female tail is long with sharp spines (Fig. 10).

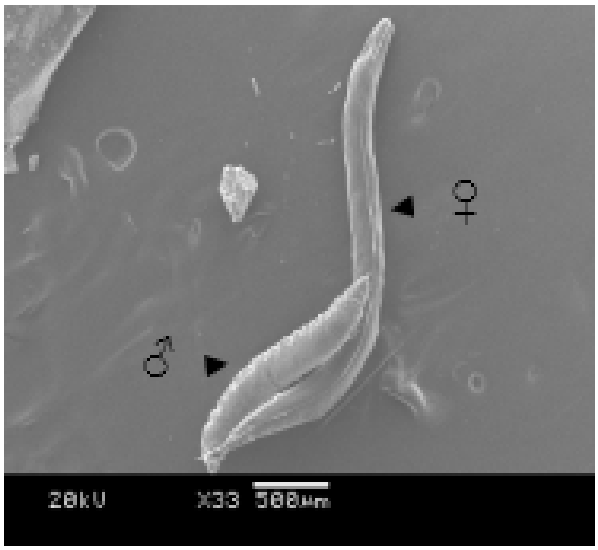

Fig 1: *Cosmocercoides* sp (♂ &♀).

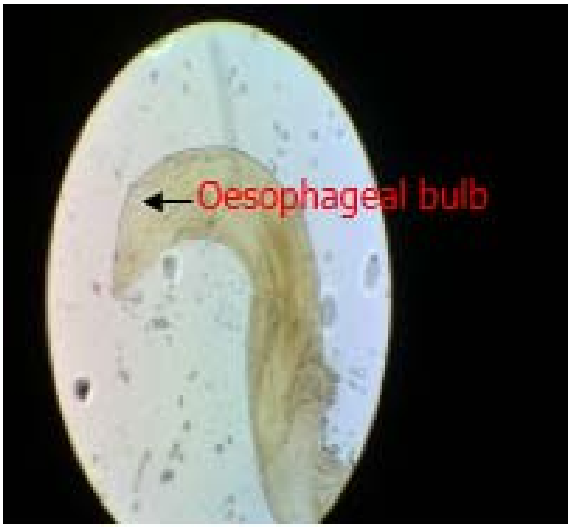

Fig 2: Oesophagus is bulbous posteriorly.

Fig 3: Female with long pointed tail.

Fig 4: Male tail (short).

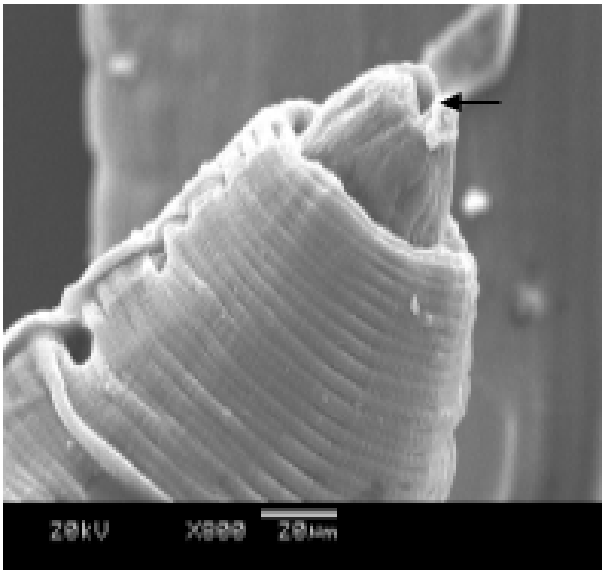

Fig 5: Lips surrounding mouth opening ridges.

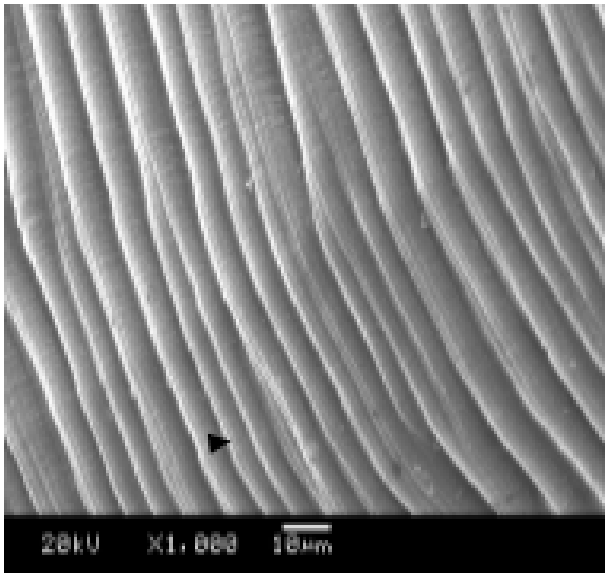

Fig 6: Somatic papillae and transverse.

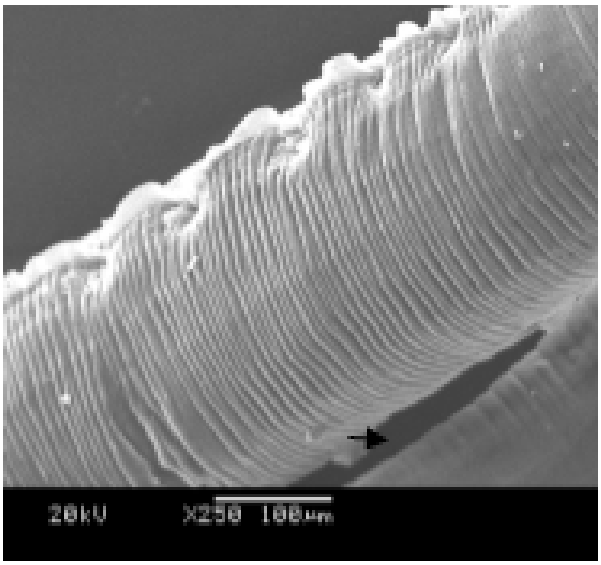

Fig 7: Lateral groove.

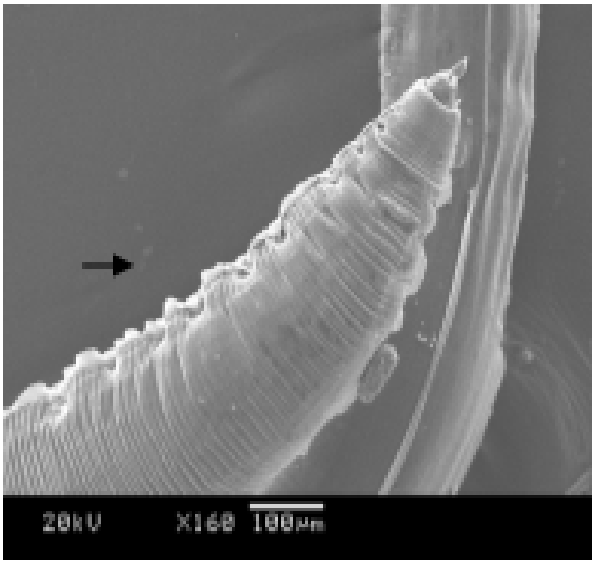

Fig 8: Cuticular inflations.

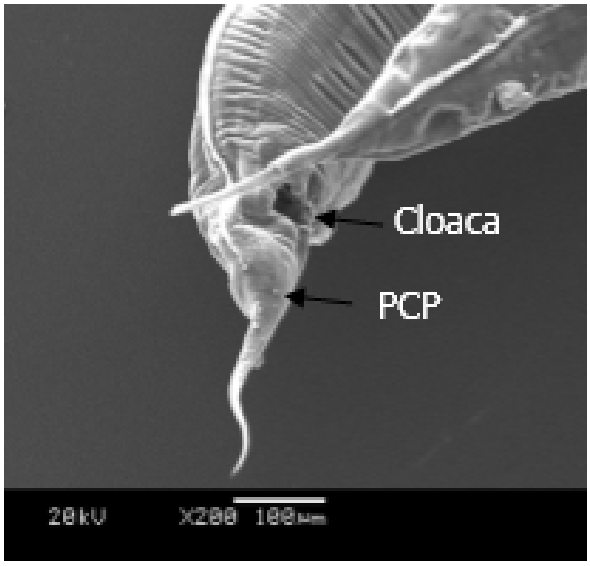

Fig 9: Pre and post cloacal papillae with prominent cloacal opening (Male tail).

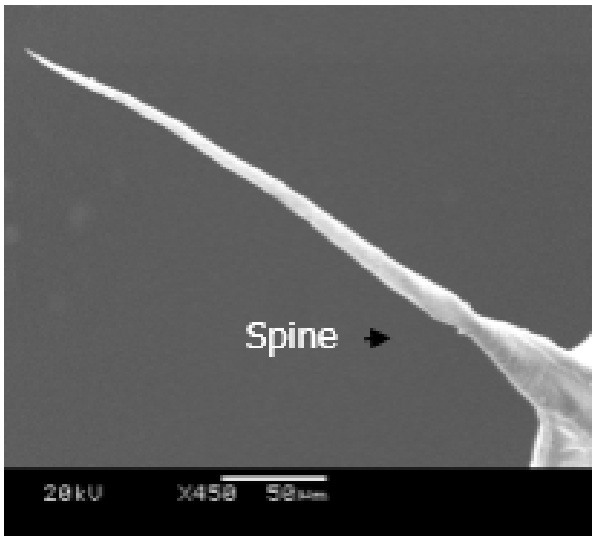

Fig 10: Female tail with sharp. spines

4. Discussion

It is vital to study all morphological aspects of nematodes under light microscope in the context of their systematic classification. The scanning electron microscopy is an additional tool for the detail study of nematodes. The SEM could be used to separate morphologically identical specimens, validate species and delineate differences between populations or races [8, 9]. *Cosmocercoides* is one of the most commonly found nematodes in frogs. Even though, few numbers of frogs were examined during the present study, the incidence of infection is consistent with other amphibian surveys. Although *Cosmocercoides sp* are capable of developing in several species of frogs, they are more restricted only one or two species of frogs and rarely in other genera.

In the present study, all nematodes were confined in the small intestine. Campbell *et al* (1999) [10] pointed out that food in the stomach have not broken down completely and food passing through the lower end colon is generally less abundant of desired nutrients than within the small intestine. Lower parasite burdens within the stomach and large intestine may reflect these facts.

Out of 30 frogs examined, only one frog carried the nematode in the stomach and two frogs harboured in the large intestine. No significant difference was observed regarding the intensity of infection in male and female frogs. The parasite of the genus *Aplectana* falls under the family cosmocercoididae and has merited insufficient attention due to limited literature in scientific descriptions. Species of *Aplectana* are often found within the intestine of amphibian hosts [9]. These worms interfere nutrient absorption within the intestine. Though the present species share some morphological similarities with *Aplectana* species but further validation is necessary before confirming the present species as *Aplectana sp.*

Cuticular modifications are a common feature on the surface of the many nematodes. SEM was used to study the surface tegument of *Cosmocercoides* sp. Variations on the cuticular structures have been reported by many other investigators [11-14]. The features observed along the body surface of the present species include: cuticular annulations, cuticular flange, longitudinal and transverse ridges, cuticular inflation and spination.

Based on the above observation it can be concluded that nematode infection is quite common in frogs. The morphology carried out by LM and SEM of the presently recovered *Cosmocercoides* species will provide valuable information to the biologist for systematic classification and evolutionary relationship between frogs and nematodes.

5. References

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