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Incidence of *Ophiotaenia* spp (Family: Protocephalidae) in different species of snakes in Mizoram, India

Gautam Patra, Basanta Saikia, Subhamoy Ghosh, Parthasarathi Behera and Madhurendra Bachan

Abstract
The main aim of this study was to report the occurrence of tapeworm *Ophiotaenia* spp in various species of snakes by conventional faecal sample examination as well as molecular confirmation by polymerase chain reaction (PCR). A total of 65 snakes from both poisonous and non-poisonous were examined over a period of one year from July, 2016 to August, 2017 from different parts of Mizoram. Some tapeworms were also collected during post mortem (PM) examination and permanent slides were made. Three faecal samples were found positive for tapeworm eggs, while two rat snakes belonging to the family Colubridae were found positive for tapeworm s during PM. Scolex could not be recovered from intestine due to a time gap between death and PM examination. It was not possible to determine the *Ophiotaenia* species due to the non recovery of scolex.

Keywords: Tapeworms; *Ophiotaenia*; snakes; PCR; Mizoram; India

1. Introduction
Snakes belonging to the reptile play a significant role in the ecosystem. Like other reptiles, snakes also harbour different trematodes, cestodes and nematodes [1-4]. In zoos, snakes are often confined together and the resultant stress causes alteration of host parasite relationship. Sometimes snakes can be responsible for transmission of various diseases to animals and human.

Ophiotaenia sp is the second most important genus of protocephalidean cestodes [5]. The genus *Ophiotaenia* is characterized by globose or somewhat tetragonal head with no rostellum. No hooks or spines. Suckers are circular or oval, with margins entire. Necks are usually long. Testes are in two long lateral fields anterior to the ovary. Vagina anterior or posterior to cirrus pouch. Ovary is bilobed, flattened. Parenchyma with fine meshes and musculature is broken.

Tapeworms of the order Protocephalidea are parasites of fishes, amphibians and reptiles and the genus *Ophiotaenia* falls under this order. Tapeworms are diagnosed on the basis of morphological features: scolex, external segmentation, variation of reproductive organs, position and number of genital pore etc. Mariaux (1998) [6] have defined numbers of synapomorphis for this order Protocephalidea on the basis of molecular data.

Although a diverse species of reptiles are found in Mizoram, India, there is no systematic information about the endoparasitic fauna of reptiles and their role of transmission of veterinary and zoonotic disease. The objective of this paper was to report the occurrence of *Ophiotaenia* sp in snakes on the basis of morphology and molecular technique.

2. Materials and Methods
2.1 Place of study
The study was carried out in different districts of Mizoram, India from July, 2016 to August, 2017. In addition, few snakes either run over by vehicle or killed by local people were also included or killed by local people were also included. A total of 65 snakes of four families namely Pythonidae, Viperidae, Elapidae and Colubridae were examined.

2.2 Collection and examination of faecal sample
Fresh faecal samples (n=300) were collected from captive snakes as far as possible. Direct smear, floatation and sedimentation techniques were used for faecal examination as per standard procedure [7-9]. Faecal samples of dead snakes were examined during PM examination.

2.3 Post-mortem examination
At least 35 dead snakes brought to the Department of Parasitology during the whole study period were examined by PM. Snakes were cut open after taking utmost precaution and viscera were thoroughly examined and parasites recovered were washed in distilled water and kept in 70% alcohol for further study. A portion of mature segment from the recovered tapeworm was also processed and the permanent slide was prepared after proper staining.

2.4 Extraction of genomic DNA
DNA was extracted from the segment of tapeworm by using a commercial kit (DNeasy® Blood and Tissue kit, Qiagen) following manufacturer's protocol with slight modification. Briefly approximately 225mg of tapeworm segment was triturated with buffer and proteinase K was added to the mixture and incubated at 56 °C for complete lysis. After adding ethanol (96-100%). The mixture was transferred into a spin column and centrifuged. After washing with buffer twice, the elution buffer was added to the column membrane and incubated for 1 minute at room temperature. Finally, the column was centrifuged and titrate was collected and kept in -20 °C. PCR was performed specific oligonucleotide primers to amplify the mitochondrial 18S rRNA gene fragment.

3. Result
Out of 65 snakes examined, 1 poisonous and 2 non-poisonous snakes were found positive for *Ophiotaenia* species. The species was identified on the basis of tapeworm mature segment (fig. 1) and egg structure (fig. 2). Rat snake was also found infected with *Ophiotaenia* sp during PM examination. Mature proglottids were found wider than longer and the internal longitudinal musculatures were well developed. The size of the specimen varies from 5-18cm, upto 1.5mm wider, flattened dorso-ventrally, with last proglottids very long and almost spherical in transverse section. Immature and mature proglottids wider than long to longer than wide; pre-gravid and gravid proglottids longer than wide.

Eggs were spherical, with thin, hyaline collapsed outer envelope, inner envelope consisting of two-layered embryophore with 3 pairs of embryonic hooks and measured 10-100µm in diameter. The morphologic and morphometric data allowed to speculate that the tapeworm involved in the parasitism was a species of the genus *Ophiotaenia*.

An approximately 1280bp of the ITS 2 region of mitochondrial 18S rRNA of *Ophiotaenia* was amplified from the collected tapeworm for molecular identification. Amplified genomic DNA was checked by gel electrophoresis with 1.5% agarose gel and seen in UV trans-illuminator system.

4. Discussion
The fauna of reptiles in Mizoram is vast but till date there is no report of protocephalidean cestodes occurring in snakes from this region. This lack of reports reflects a low sampling efforts and shortcomings of parasitological studies. *Ophiotaenia* spp are differentiated from closely related species on the basis of morphology. However, it is difficult and sometimes impossible to identify correctly based on morphological observation. Hence molecular confirmation is necessary in such cases. Due to non-recovery of scolex and non-differentiation of internal organs even after proper staining made it more complicated to correctly identify the genus. Identification of the correct genus was made possible by PCR. In the present investigation, the recovered tapeworm showed wider proglottid than longer with well-developed musculature. A similar observation was also made by previous workers [5, 10, 11]. Zehnder and Mariux (1999) [12] used two rDNA sequences to infer phylogenetic relationship among the Eucestoda, order Protocephalidea and concluded that the molecular ribosomal gene provided a better resolution of relations among Protocephalidea. The phylogeny of the order Protocephalidea has also been studied at generic and subfamiliar level by other workers [13, 14].

Protocephalids are mainly parasites of fishes but a number of species are also found in amphibians and reptiles. Because of the non-recovery of scolex and fragmented nature of our cestode species, we were unable to assign them to a species. However, the typical morphology of the mature proglottid and the egg structure together with amplification of 1280bp gene fragment by PCR allowed the assignment of the specimens to *Ophiotaenia* group. This is in agreement with Goldberg *et al.* (2006) [15] who also reported *Ophiotaenia* infecting *Norops fuscoauratus* from Brazil on the basis of morphology.

In the present study, generic diagnosis of *Ophiotaenia* spp under the subfamily Protocephalinae [16] is amended on the basis of some morphological features and molecular data. The positive PCR confirmed that the recovered tapeworm belongs to the genus *Ophiotaenia*. However, identifying at species level based on morphology and positive PCR reaction are not sufficient and speciation is only possible by complete sequencing of full length of the targeted ITS2 region.

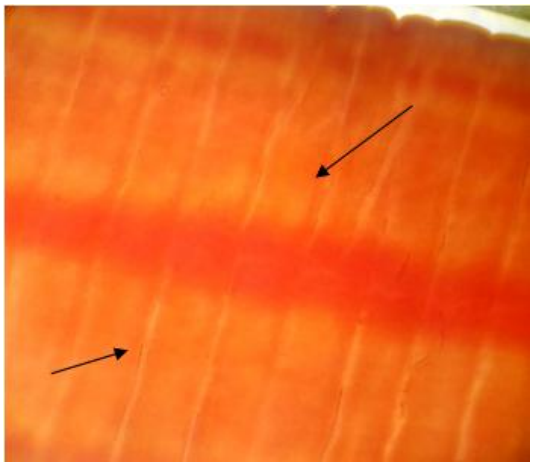

Fig 1: Mature segments of *Ophiotaenia* sp under light microscope

Fig 2: Egg morphology of *Ophiotaenia* sp (Under X10)

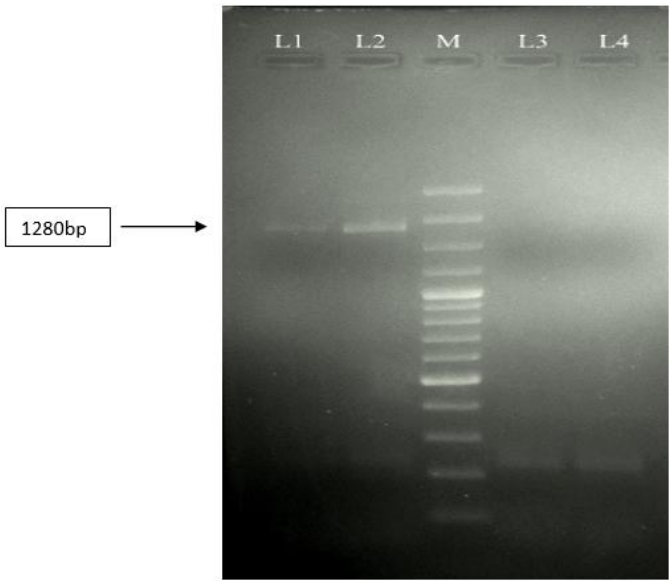

Fig 3: PCR amplification of *Ophiotaenia* sp (approx. 1280bp) in 1.2% agarose
L1, L2 – positive amplification
L3, L4 – Negative
M – 100bp DNA ladder

5. Conclusion

This is the first report of *Ophiotaenia* spp infecting snakes in Mizoram, India. Based on this study, it is quite clear this species is rare in snakes with both prevalence and intensity low. Molecular data corroborated the findings at the morphological level.

6. Conflict of interest statement

We declare that we have no conflict of interest.

7. Acknowledgements

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