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Surface ultra structural Studies of an ectoparasite- *Aponomma varanense*, of Indian King Cobra

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Abstract

The objective of the present study is to underscore the ultra-structural morphology of *Aponomma varanense*-a rare tick of reptile. *A. varanense* were collected from an Indian Cobra during post mortem and subsequently processed for both light microscopy and Scanning Electron Microscopy (SEM) studies. Light microscopy study revealed few typical morphological features through which the collected specimen identification was confirmed. Further, SEM study revealed little morphological ultrastructural peculiarity with other ixodid ticks as well difference between male and female *A. varanense* itself. Occurrence of *A. varanense* is reported first time from this part of country and this is the first SEM studies of this hard tick from India.

Keywords: *Aponomma*, morphology, SEM, Cobra, India

1. Introduction

Ectoparasites, like ticks and mites are often found on snakes in the wild but the rate of infections and the effects they have on their hosts are poorly studied^[1]. Although it is rare that ticks occur in large numbers to cause either serious blood loss or other direct injuries but they are responsible for transmission of various vector borne diseases to animals and humans^[2, 3]. As international trade of wild animals are rife in recent years, there is every possibility of high risk of spreading exotic ticks and the associated pathogens in new geographic areas^[4-6]. It is very interesting that during the course of evolution of ticks, the first pair of limbs are modified to form chelicerae for cutting into the skin of the host, the second pair forms the palps, while the third to sixth pair of are the actual legs for walking.

The species of the genus *Aponomma* exclusively occur on reptiles. Tick infestation in snake is common^[7]. Taxonomy of ticks based on morphology and molecular techniques is discussed by various workers^[8] where the genus *Aponomma* is considered as junior synonym of *Amblyomma*. *Aponomma varanense* is one of the most widespread members of the genus *Aponomma* occurring in several countries including India in reptiles^[9]. Limited studies on scanning Electron Microscopy (SEM) of reptilian ticks have been done^[10, 11]. As there is no morphological description of *Aponomma* sp, the present study is undertaken to give an overview of the surface ultra-structure of male and female *Aponomma* sp which have been collected from a poisonous Indian Cobra (*Ophiophagus hannah*).

2. Materials and methods

2.1 Collection of Ticks

An Indian King Cobra (*Ophiophagus hannah*) was found dead on the Aizawl-Sihphir road on 6th June, 2016. The snake was brought to the Department of Parasitology, College of Veterinary Sciences & A.H., Central Agricultural University, Selesih, Aizawl, Mizoram India. After taking all precautionary measure the snake was thoroughly examined for presence of any ectoparasite as well as for endoparasite. On examination 08 (eight) ticks were recovered and all those ticks were immediately transferred to 70% alcohol for further identification on the basis of key provided by Hoogstraal *et al.* (1981)^[12].

2.2 Morphological study

Initially all the ticks were studied under stereozoom (Euromex, Holland) and ticks were identified separately for male and female. One male and another one female ticks were processed and permanent slides were prepared after proper staining. Preliminary

morphological study revealed all these six ticks belongs to the genus *Aponomma*. Further speciation was done on the basis of keys provided by previous workers [12]. To get the surface morphology with more clarity two ticks of each gender were further processed for Scanning electron microscopy (SEM) study.

2.3 Preparation of sample

Both male and female *Aponomma spp* were fixed in 3% glutaraldehyde. Any extracellular debris like mucus, blood or other body fluids and tissue fragments which otherwise hinder the surface to be examined were removed carefully by washing several times in distilled water with a brush. The samples fixed at 4 °C for 24 hours. The fixed ticks were washed in phosphate buffer saline (PH 7.2) three times and then in double distilled water followed by acetone dehydration.

After acetone dehydration, the specimens were dried with liquid carbon dioxide at its critical point i.e. 31.5 °C at 1100 P.S.I. The specimens are then immersed in Tetra methyl saline (TMS) for 5-10 minutes for two changes at 4°C. Then they are brought to room temperature (25-26 °C) to dry. The samples are mounted on aluminium stubs. Ticks were then gold coated in a sputter coat and were examined under SEM [(JSM-6360(JEOL)] at the North-Eastern Hill University (NEHU), Shillong, Meghalaya in Sophisticated Analytical Instrument Facilities (SAIF) Laboratory.

3. Results

Both the male and female *Aponomma* species differ morphologically in their dorsal and ventral surface ultra-structure as described below.

3.1 Male

Male tick is dorsoventrally flattened and wider posterior than the anterior portion (Figure-1a). The dorsal surface of the body is covered with hard chitinous shield, called scutum with numerous pores which are randomly arranged (Figure 1b). One pair of cervical groove occurs on the scutum immediately below the emagination line (Figure 1b). The floor of the cervical groove is uneven.

The ventral surface bears one pair ventral plates, surface of which bears some small setae which are arranged in definite orders (Figure 2a). Below the emigration line, a smooth pregenital plate with 3-5 short spines is observed. The surface of the median plate which is located below the genital apparatus is covered with few spines. The surface of the anal plate is rough and the anal groove surrounds the anus posterior. The anal plate is armed with 4-6 rows of symmetrically arranged spines. Genital and median plates are less hairy than accessory adanal plates.

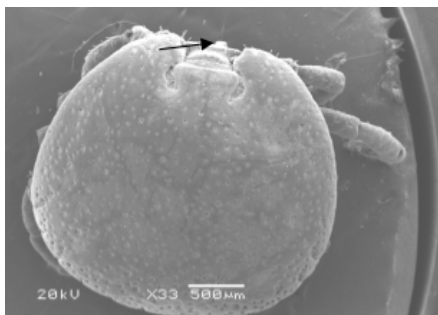


Fig 1a: Dorsal view of male *Aponomma varanense* (mouth parts not visible). Lower arrow in

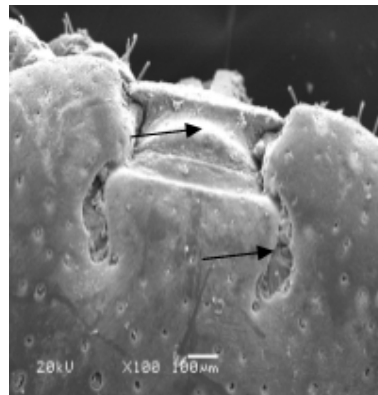


Fig 1b: shows cervical groove with rectangular basis capitulum

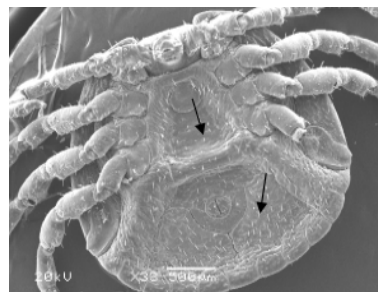


Fig 2a: Male ventral surface showing accessory shield (lower arrow) and genital plates (upper arrow)

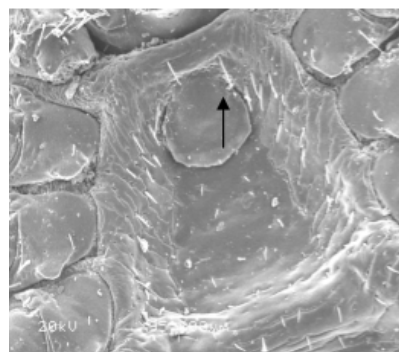


Fig 2b: Male genital organ surrounded by genital plate

3.2 Basis capituli

The basis capitulum which is rectangular in shape bears the mouth parts and not visible from dorsal aspect (Figure 1a). The dorsal surface is smooth with no pores or any spine. Ventral surface is also smooth, have no pores except few spines on either side (Figure 3).

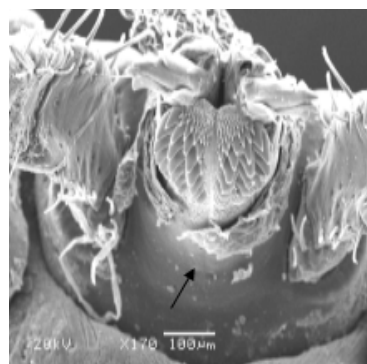


Fig 3: Ventral view of basis capitulum with smooth surface

3.3 Mandible

The ventro-dorsal part of the basis capitulum extends to form the mandible. The passage is broad at base but narrows at the tip. Two sharp projections bordering the groove are observed in the present study. The scales are not visible at the base of the mandible (Figure 4a).



Fig 4a: Mouth parts showing mandibles

3.4 Hypostome

It is a single tooth like structure of the mouth parts of tick. It is longer than mandibles, denticulate and arranged in several rows. The hypostome shows a median toothless line running along the ventral surface. Each coronal surface is smooth and the denticles are having symmetrical in arrangement. The dental formula here is 4/4. Several small teeth are united together at the base that seems like scales (Figure 4b).



Fig 4b: Hypostome with 4/4 arrangements of teeth

3.5 Appendages

Four pairs of legs are attached at the ventro-lateral aspect of the body. Each consists of coxa at the base, then trochanter, femur, patella, tibia metatarsus and tarsus. Cox I and II are flat and have no spurs while Coxa III and IV bear spur like structure (Figure 5a). Each segment bears few spines both on dorsal and ventral side. Each of the segments is attached by a soft articulation membrane. Each tarsus bears a pretarsus stalk like structure with a pair of curved pointed claws (Figure 5b). The base of each claw is originated from a special structure, called pulvillus, attached with pretarsus.



Fig 5a: Showing haller's organ on 1st left leg



Fig 5b: Leg with curve pointed-claws

3.6 Spiracles

One pair of Spiracles is present below the fourth coxa. Each spiracle is comma shaped. The upper surface shows a cuticular elevation. The margin of spiracle is distinct and surface is smooth and hairless (Figure 6).



Fig 6: Picture showing comma shaped spiracle (upper arrow) with marginal festoon (lower arrow)

3.7 Haller's organ

It is a sensory organ located on the terminal segment of the first pair of legs (Figure 5a)

3.8 Genital organ

It is located in the midline immediately below the basis capituli and between the second pair of coxa. The lateral surface of the genital organ is striated with few grooves and bears some short spines. The male genital orifice is more or less oval in shape without any appendages (Figure 2b).

3.9 Anus

It is located within the anal plate and is below the genital organ. Two valves guard the anus. Five bristles of each valve project outward. Anal valves are surrounded by a prominent circular anal margin. Posteriorly the anal groove surrounds the anus (Figure 7).

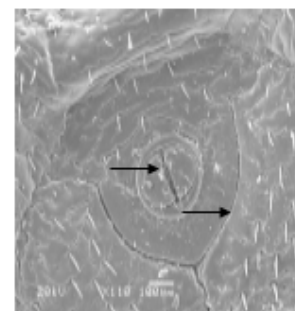


Fig 7: Male anus (upper arrow) with anal groove (lower arrow)

3.10 Festoons

The posterior part of the body is notched forming festoons, which are 11 in numbers. Few spines are visible in most of the festoons (Figure 6).

3.11 Females

The adult female *Aponomma varanense* is more or less similar in size to that of the male tick and dorsoventrally flattened (Figure 8). The position of basis capitulum is sub ventral like male and the posterior border is deeply notched with 11 numbers of festoons. Unlike male, the dorsal surface of basis capituli of female bears two porose areas each of which are interrupted with large number of minute pores (Figure 9). The scutum covers one third of the total body surface with numerous pores on its dorsal surface (Figure 10). There is one pair of elongated comma shaped cervical groove on the surface of the scutum. Below the scutum, median, postero-lateral and lateral groove are visible (Figure 8). The dorsal surface of the body also bears several spines. The ventral surface is devoid of any plates except the anal and genital plate (Figure 11). Mandibular structure is similar to male. Some nodular structure is visible on the inner surface of mandible. Hypostome is similar to male with symmetric dental arrangement. The dental formula is 4/4. At the base of the hypostome, several small teeth cluster together to form scale like structure.

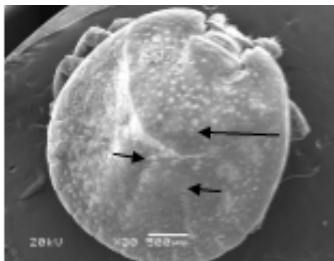


Fig 8: Dorsal view of female *Aponomma varanense* with prominent scutum (upper arrow) and median (lower left arrow) and lateral grooves (lower right arrow)

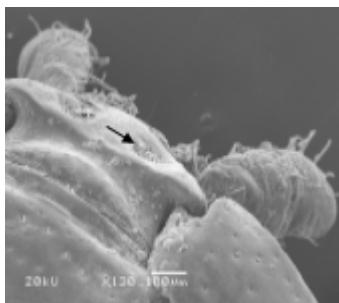


Fig 9: Basis capitulum of female with two porose areas (arrow pointed)

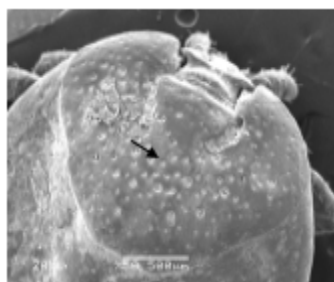


Fig 10: Scutum of female tick with numerous pores

3.12 Spiracle

The spiracle of female is slightly smaller than that of male. The ventro-larval surface surrounding spiracles show fine folds.

Two palps arise from each side of the base of the hypostome. Surface bears sensory hairs. Tip of the pulp is well organized a rounded head like structure with sensory bristles projecting outward from a comma shaped depression (Figure 11).

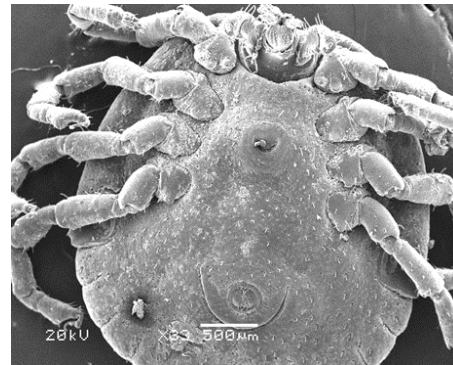


Fig 11: Ventral surface of female showing basis capituli with palp and without any accessory shield

4. Discussion

Host parasite interactions play a vital role in population dynamics and even to the verge of extinction. These interactions also ensure healthy ecosystem and in stabilizing food webs [13, 14]. A parasite whether ecto or endo may cause mechanical injury including inflammatory or immune responses or simply sap the nutrition from the host. Reptiles harbor different parasites ranging from protozoan to arthropods. Among these, ticks are the most common arthropods that parasitise reptiles. *A. varanense* was first reported from rock python and cobra in captivity by earlier workers [15]. The present study was intended to describe surface ultra-structural morphology of *A. varanense* under SEM. This is the first report of ultra-structural description of *Aponomma varanense* from India. It is still unclear whether other species of snakes are refractory or susceptible and there might be ecological or evolutionary reasons for this type of host specificity. This study would help to obtain an accurate identification of this species.

There are some important morphological structures which were difficult to observe by light microscopy (LM) but are clearly visible under SEM. The present study is carried out according to available SEM studies on other ticks observed by other workers [10, 11, 15]. Numerous punctuations are uniformly distributed throughout the scutal surface of *A. varanense* and *Amblyomma gervaisi*. The most distinguishing feature observed in *A. varanense* is the position of mouth parts which are sub ventral contrasting to other hard ticks. All the characteristics of scutum, coxae and palpal articles in this study agree with that are observed by LM. Festoons are presented in both sexes and in ornate as in *A. gervaisi*.

The basis capituli is rectangular in shape as in *Amblyomma varium* [17] while spatulate in *Amblyomma gervaisi* [15] and in *Amblyomma geobelone*. In male *Amblyomma geochelone*, basis capituli is ornate dorsally with pairs and few pointed spine not found in male *A. varanense*. The dental formula on the hypostome of *A. varanense* is 4/4 as in *A. goeldii*, while it is 3/3 in *A. gervaisi*. The hypostome shows a median toothless line in the present species as in *A. gervaise* but not found in *A. fescum*. The structure of palps is more or less similar to that of

A. gervaisi, though palp head is less hairy in *A. varanense* as in *A. fescum*.

The male genital organ, anus, Haller's organ and spiracle of *A. varanense* are almost similar to that of *A. fescum* and *A. gervaisi*, although, the spiracle plate of *A. varanense* is slightly comma shaped with anterior broad end and tapering posterior. The Haller's organ of the present species is somewhat oval shaped than that of *A. gervaisi*. According to general description, the shape of Haller's organ varies in *Ixodes* spp from oval, elliptical, triangular, or roughly rectangular, but more or less rounded in *A. varium* [16].

The genital organs of both male and female are well developed. The genital plate of female *A. varanense* is roughly rounded as in female *A. gervaisi*. The male genital organ of *A. geochetone* is broadly U- shaped, oval in *A. gervaise* but rounded in the present species. The anal plate is oval in shape in male while in female it is circular in shape and has a posterior margin.

It is worth mentioning that whether *Amblyomma* and *Aponomma* are synonymous or not is still uncertain. No bibliographic information available about the surface structure of *Aponomma* species. Based on the above discussion it can be seen that different species of ticks infesting reptiles may show variation in their surface morphology. Such information would be crucial for inter disciplinary cooperation among specialist and on a wider level, improve the wildlife inspection process. Such studies are also mandatory because many of them are vectors of Rickettsia and Lyme disease [18].

4.1 Conflict of interest statement

We declare that we have no conflict of interest.

5. Acknowledgements

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