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Scanning electron microscopic observations on sensilla of *Ixodes acutitarsus* recovered from semi-wild cattle *Bos frontalis* Lambert.

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ABSTRACT

Scanning electron microscopic observations on the body surface of the tick Ixodes acutitarsus recovered from semi-wild cattle Bos frontalis revealed the occurrence of fifteen different types of sensory structures. Dorsal side of the body have six types of sensilla like cuticular pit sensilla, sensilla basiconica type I, sensilla basiconica type II, sensilla chaetica type I, sensilla chaetica type II, and bifid sensilla. Whereas, ventral side of the parasite have seven types of sensilla namely sensilla chaetica type I, sensilla chaetica type III, coeloconic sensilla, sensilla trichodea type I, sensilla trichodea type II, sensilla trichodea type III and hair plate sensilla. In the anterior portion of the body, palps are provided with sensilla basiconica type III. In the first pair of legs Hallers organ is provided with four types of sensilla like sensilla chaetica type III, sensilla basiconica type III, multiporous sensilla type I and multiporous sensilla type II. Functional significance of the sensilla in the parasite is discussed.

Keywords scanning electron microscopy, sensilla, *Ixodes acutitarsus*, *Bos frontalis*

INTRODUCTION

Ticks are obligate arthropods which feed on vertebrate blood. They are responsible for transmitting different types of pathogenic micro-organisms like, bacteria, protozoa and also viruses to man and animals causing infectious diseases in tropical and sub tropical countries including India [1]. It has been reported that tick and tick borne diseases causes a major problem on the income of poor farmer in developing countries [2, 3]. These parasitic arthropods are host specific and belong to two groups like endophilic and exophilic [4]. Endophillic types of ticks are found in burrows of their host and feed regularly but exophillic types of ticks are found in exposed areas and these types of ticks are well equipped with different kinds of sensory organs for hunting their hosts [4]. However at present no information is available on occurrence and distributions of sensory structures of an zoonotically important tick like *Ixodes acutitarsus* [5]. *I. acutitarsus* is a haematophagus ectoparasite of domesticated and wild cattle that locate specific site on its host with the help of its different sensory structures, for its survival [6]. Since structure and functions of sensory organs varies from species to species and modified greatly in parasites due to specific adaptive mode of life, detailed investigations are required to elucidate their functional importance in *I. acutitarsus*, an ectoparasite of veterinary significance.

MATERIALS AND METHODS

Ticks (*Ixodes acutitarsus*) were collected from the skin of *Bos frontalis* slaughtered at Ganga market (27°10'N93º62'E) in Arunachal Pradesh, India. The parasites were carefully removed with thumb forceps so that the mouth parts were not left behind during the traction from the host animals. The dry samples were secured horizontally to brass stubs (10 mm dia X 10 mm hgt) with double coated adhesive tape connected via a patch of silver paint to ensure charge conduction. Care was taken to avoid trapped air bubbles. A conductive coating was applied to the samples using JFC 1100 (Jeol) ion-sputter coater. A relatively low vacuum (10^{-3} toor) was established in the sputtering chamber, using gold as the target material. The preparation was examined with scanning electron microscope, JEOL JSM 6360 using the secondary electron emission mode at an accelerating voltage of 20 KV. Tilt control was fixed at 0° C for setting the specimen stage in a horizontal position. WD selector was turned fully anti clockwise to set the working distance at 15 mm. Some of the specimens were processed with the crystal violet method of slifer [7] in order to confirm the porous nature of sensilla.

RESULTS

Stereoscan observations on the surface topography of the tick *I. acutitarsus* revealed the occurrence of 15 different types of sensilla in the dorsal, ventral, dorsolateral side of the body, palps and in the first pair of legs, namely sensilla chaetica type I, sensilla chaetica type II, sensilla chaetica type III, sensilla trichodea type I, sensilla trichodea type II, sensilla trichodea type III, sensilla basiconica type I, sensilla basiconica type II, sensilla basiconica type III, sensilla bifid, multiporous sensilla type I, multiporous sensilla type II, cuticular pit sensilla, hair plate sensilla and coeloconic sensilla.

Cuticular pit sensilla
This type of sensilla are present in the dorsal side, specially in dorsolateral side, near basis capitulli and in the posterior side of *I. acutitarsus*. It measures up to 14.66 µm in diameter (fig.1b).

Coeloconic sensilla
This type of sensilla are present in the ventral side of the body of the tick. Coeloconic sensilla is a stump like structure, measures 5 µm in length and 2.30 µm in width (fig.2b).

Sensilla chaetica
Three types of sensilla chaetica are observed in different parts of *I. acutitarsus*.

Sensilla chaetica type I: This type of sensilla are present in both the dorsal and ventral side of the body. In the dorsal side sensilla were observed specially in basis capitulli, whereas in the ventral side the sensilla were observed in both sides of the palps. Sensilla chaetica measures 20.5 µm in length and 3.33 µm in width at the base. They have a smooth surface, blunt tip and a base which is found to be not swollen and socketed base (fig.1c and fig.2c).

Sensilla chaetica type II: This type of sensilla are present in the dorsal side of the body having curved body surface with ridges, broad base and pointed tip. It measures 22.64 µm in length and 3.52 µm in width at the base (fig. 1h).

Sensilla chaetica type III: It is a long, straight with smooth body surface and having a pointed tip. This type of sensilla are present in the ventral surface of the anus of the tick and measures 46 µm in length and 4.66 µm in width (fig.2i).

Sensilla basiconica
Three types of sensilla basiconica are observed in dorsal surface, ventral surface and in the Hallers organ of *I. acutitarsus*.

Sensilla basiconica type I: This type of sensilla are present in the dorsal and ventral side, palp and ventral side of basis capitulli. The sensilla measures 33.68 µm in length and 6.84 µm in width at the base. It has smooth body surface, swollen base with blunt tip (fig.1e).

Sensilla basiconica type II: It measures 6.66 µm in length and 5 µm in width, present in the dorsal and ventral side of the palp and also in the ventral surface of the body. It has smooth surface with pointed tip (fig. 1d).

Sensilla basiconica type III: This type of sensilla are socketed, smooth with blunt tip and present in the Hallers organ, and in the anterior portion of the palps. It measures 10 µm in length and 6.46 µm in width (fig. 2d and 3c).

Sensilla trichodea
Three types of sensilla trichodea were observed in the ventral side of *I. acutitarsus.*

Sensilla trichodea type I: This type of sensilla is long, straight, smooth, socketed and tip is pointed. It measures 143 µm in long and 11.36 µm in width at the base and present in coxae 1, 2, 3 and 4 (fig. 2g).

Sensilla trichodea type II: This type of sensilla are present in the ventral surface of the body including the surface of anus. It measures 38.23 µm in length and 4.70 µm in width at the base. This type of sensilla is long, smooth and bent at the edge and tip of the sensilla is pointed (fig.2h).

Sensilla trichodea type III: It measures 29.58 µm in lengths and 5 µm in width at the base. The sensilla are present only in the ventral side of the coxa 1.This type of sensilla is straight, rises from deep pitches, socketed and tip has bifurcation (fig.2e).

Multiporous sensilla

Two types of multiporous sensilla were observed in the dorsal side of the Hallers organ (first pair of legs) (fig. 3d).
Multiporous sensilla type I: This type of sensilla possesses pores whole over the body and it measures 11.11 µm in length and 3.33 µm in width. The tip of the sensilla is blunt (fig.3d and 3e).

Multiporous sensilla type II: This type of sensilla posseses pores whole over the body and it measures 3.92 µm in length and 3 µm in width. The tip of the sensilla is pointed (fig.3f).

Sensilla bifid: This type of sensilla are socketed, splited into two parts up to the middle of its length and present in the dorsolateral surface of the tick. Surface of the sensilla provided with longitudinal ridges. It measures 35 µm in length and 6 µm in width (fig.1f and 1g).

Hair plate sensilla: This type of sensilla is observed all over the body surface like dorsal, ventral, dorsolateral and in legs of *I. acutitarsus*. It measures 53.84 µm in length and 9.61 µm in width. The body surface of the sensilla is smooth and tip is pointed (fig.2f).

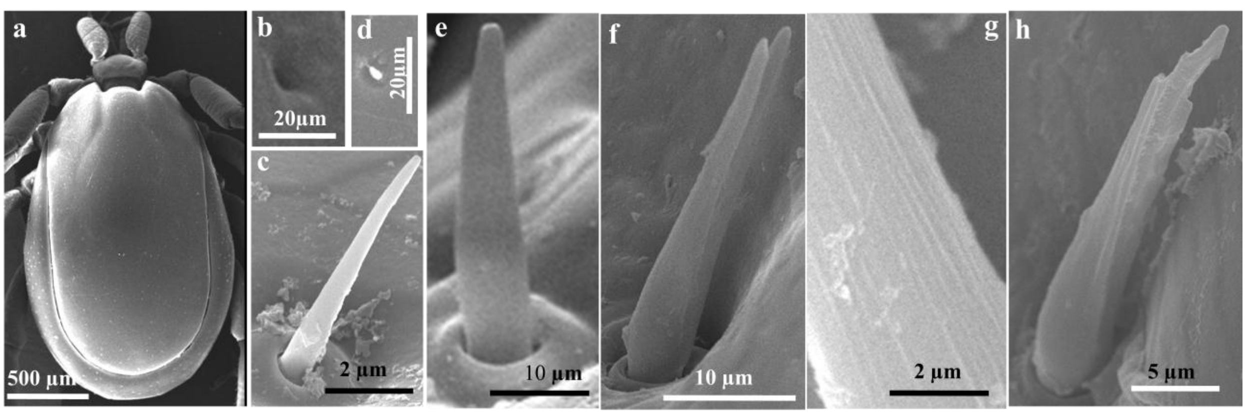

Fig.1 Scanning electron micrographs of *Ixodes acutitarsus* showing different types of sensilla in dorsal side of the body. (a) Dorsal surface of *I. acutitarsus*. (b) Cuticular pit sensilla. (c) Sensilla chaetica type I. (d) Sensilla basiconica type II. (e) Sensilla basiconica type I. (f) Sebsilla bifid. (g) Magnified image of sensilla bifid showing longitudinal ridges in the surface (h) Sensilla chaetica type II.

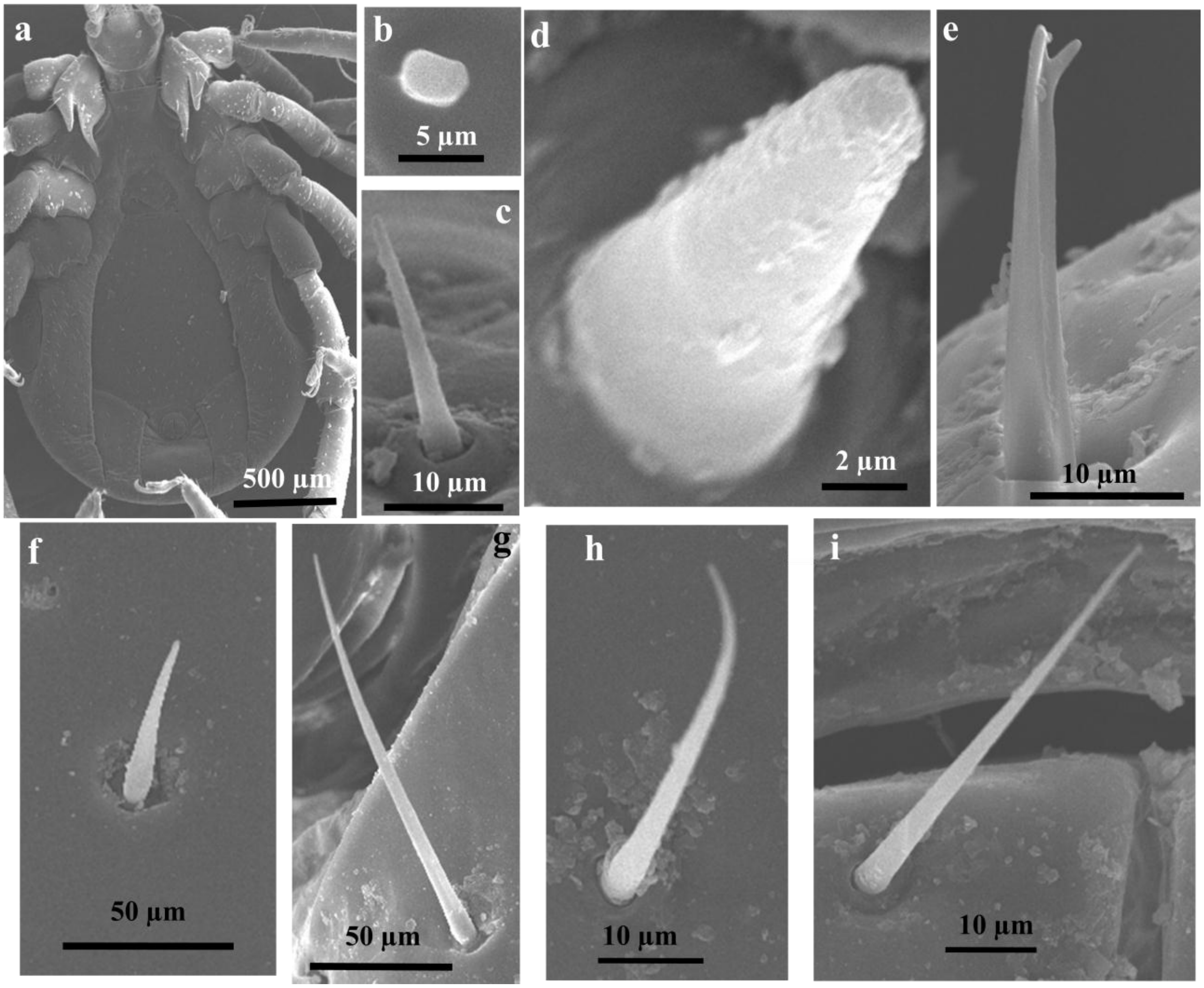

Fig.2 Scanning electron micrographs of *I. acutitarsus* showing different types of sensilla in the ventral side of the body. (a) Ventral surface of *I. acutitarsus*. (b) Coeloconic sensilla. (c) Sensilla chaetica type I. (d) Sensilla basiconica type III. (e) Sensilla trichodea type III. (f) Hair plate sensilla . (g) Sensilla trichodea type I. (h) Sensilla trichodea type II. (I) Sensilla chaetica type III.

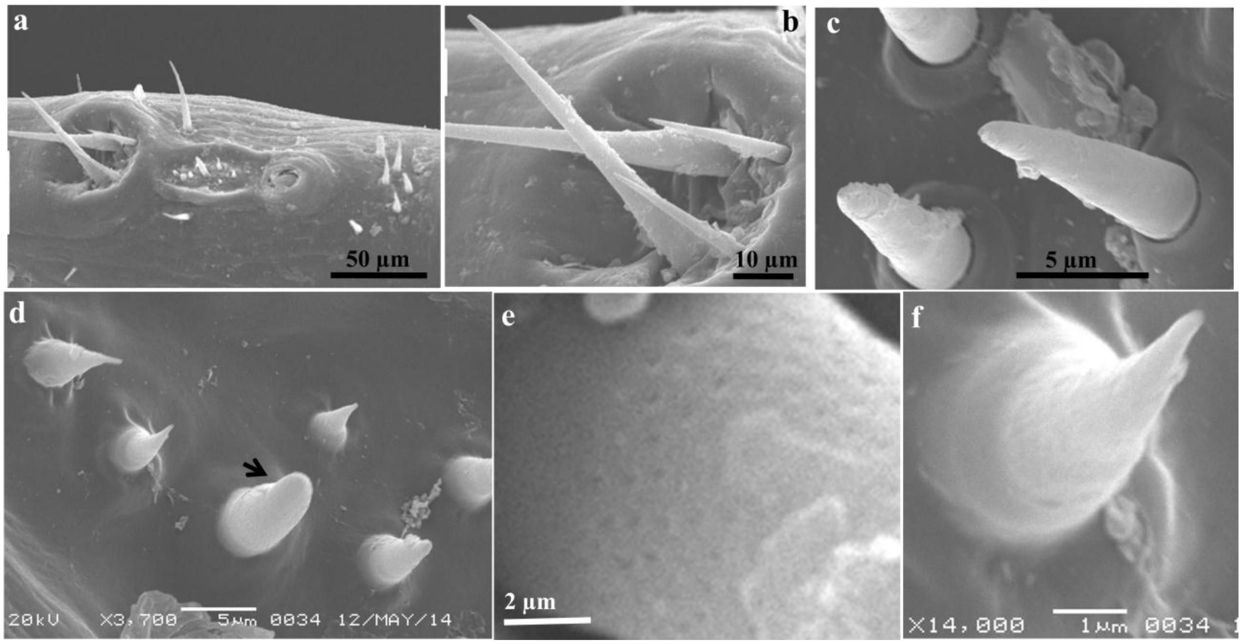

Fig.3 Scanning electron micrograph of Haller's organ of the *I. acutitarsus* showing the presence of different types of sensilla in the first pair of the legs. (a) Hallers organ. (b) Sensilla chaetica type III. (c) Sensilla basiconica type III. (d) Multiporous Sensilla type I with blunt tip (arrow). (e) Enlarged view of multiporous sensilla type I showing pores in the surface of the sensilla. (f) Multiporous sensilla type II with pointed tip.

DISCUSSION

The present study reveals the occurrence of different types of sensilla on the dorsal side, ventral side and dorsolataral parts of the body and on the legs of *Ixodes acutitarsus*. The function of different types of sensilla and the significance of distribution pattern of most of them can be interpreted using the data presented by different authors in other parasitic ticks and insects. Ultrastructural features of surface architecture, types and distribution of sensilla as observed in the present study are grossly in conformity with those reported from other parasitic ticks, flea and insects [8-12].

Cuticular pit sensilla: This type of sensilla were also reported from honey bee, spider wasp, *Grphium sarpedon* and *Chironomus samoensis* and attributed to perform the function of temperature, humidity and carbon dioxide receptors [13-16]. Similar to the present observations, Ronghang and Roy [17] also observed the cuticular pits in the dorsal and dorsolateral sides of *Rhipicephalus sanguineus* recovered from the same host *Bos frontalis*, however, occurrence of the sensilla in the capitulli is a new record in *I. acutitarsus*. Cuticular pits are regarded as opening to epidermal glands, which secretes an oily substance that coats the surface of parasitic flea [18].

Coeloconic sensilla: This is the first report of the sensillla from the ventral side of the body. Earlier, several workers reported the sensilla from dorsal side of several parasitic and non parasitic insects and were reported to be thermo-hygro perceptive and or olfacto receptive [19-21]. Odor receptive function of the sensilum is also proposed by several workers [22, 23]. Recently, Ronghang and Roy [17] reported four sensilla in horizontal position in the dorsal surface of the parasitic tick *R. sanguineus*.

Sensilla chaetica: Sensilla chaetica are generally known as mechanoreceptors or as contact chemo receptors because of their origin from a socket [24-27]. Three types of sensilla chaetica were observed in the tick with their restricted distribution for each type in different parts of the tick. Four types of sensilla chaetica with similar types of distribution was also observed in *R. sanguineus* collected from the same host *B. frontalis* [17].

Sensilla basiconica: This type of sensilla are usually shorter in length with thin body wall. They perform the function of chemoreceptions [28-29], however, involvement of this sensilum in olfection and pheromone reception has also been advocated [30, 31]. Tectile receptive function of the sensilum, particularly by parasitic flea and tick was also proposed where the parasites used the receptors to select suitable site in host skin for feeding [12, 32].

Table. 1 Comparison of morphological features, distribution and functions of different kinds of sensilla present in *I. acutitarsus*.

Name of sensilla	Length (L) and Width (W) of sensilla (µm)	Distributions	Characters	Functions	References
1. Sensilla chaetica type I	L=20.5 µm W= 3.33 µm	Dorsolateral and venterolateral side of the palps, and dorsal and ventral side of the body.	Smooth surface, base not swollen, socket present and blunt tip.	Chemoreceptor and mechanoreceptor.	[24-27]
2. Sensilla chaetica type II	L= 22.64 µm W= 3.52 µm	Dorsal side of the body.	Curved body surface with ridges, broad base and pointed tip.	Chemo receptors and mechanoreceptors.	[24-27]
3. Sensilla chaetica type III	L= 46 µm W= 4.66 µm	Ventral surface of the anus.	Straight body, smooth surface, broad base and pointed tip.	Chemo receptor and mechanoreceptor.	[24-27]
4. Cuticular pit sensilla	W=14.66 µm	Dorso-lateral side near basis capituli.	Round in shape and presence of deep holes.	Humidity, temperature and CO_2 receptors.	[13-17]
5. Sensilla basiconica type I	L= 33.68 µm W= 6.84 µm	Dorsal and ventral side of the palp, ventral side of basis capitulli.	Smooth surface, swollen base and blunt tip.	Olfactory functions and host locations.	[28-32]
6. Sensilla basiconica type II	L= 6.66 µm W= 5 µm	Dorsal and ventral side of the palps, ventral body surface.	Smooth surface, pointed tip, base not swollen.	Olfactory functions and host locations.	[28-32]
7. Sensilla basiconica type III	L= 10 µm W= 6.46 µm	Hallers organ and in the anterior portion of the plaps.	Smooth surface, blunt tip and broad base with socket.	Olfactory functions and host locations.	[28-32]
8. Sensilla traichodea type I	L= 143 µm W= 11.36 µm	Ventral side of the tick specially in coxae 1,2 3 and 4.	Straight, long and Smooth, arise from a broad base, pointed tip.	Mechano reception or contact chemoreceptors.	[12,16]
9. Sensilla traicodea type II	L= 38. 23 µm W= 4.70 µm	Ventral side of the body, surface of the anus.	Smooth, well socketed pointed tip, bent at the edge of the sensilla.	Olfaction or chemoreception.	[12,16]
10. Sensilla traichodea type III	L= 29. 58 µm W= 5 µm	Ventral side of the body in coxae 1.	Straight, rises from deep pitches, socketed and tip has 2-4 types of bifurcations.	Mechanoreception.	[12,16]
11. Multi porous sensilla type I	L=11.11 µm W= 3.33µm	First pairs of leg (Hallers organ).	Whole body of the sensilla provided with pores, blunt tip.	Olfactory receptors, mate locations, possibly for detecting female pheromones.	[10, 16, 33-35].
12. Multiporous sensilla type II	L= 3.92 µm W= 3 µm	First pairs of leg (Hallers organ).	Whole body of the sensilla provided with pores, pointed tip.	Olfactory receptors, mate locations, possibly for detecting female pheromones.	[10, 16, 33-35].
13. Sensilla bifid	L= 35 µm W= 6 µm	Dorsolateral side of the body.	Straight with longitudinal ridges on the surface, socketed, bifurcation started from the base.	Protective function.	[36, 37]
14. Hair plate sensilla	L=53.84 µm W= 9.61 µm	All over the body surface including in legs,	Smooth body surface, pointed tip.	Proprioceptive position detectors.	[22, 23]
15. Ceoloconic sensilla.	L=5 µm W= 2.30 µm	Ventral side of the body surface.	Sensilla has stump like structure, arises from deep pitches.	Thermo hygroperception and odor reception.	[9, 10, 22]

Sensilla trichodea: Sensilla trichodea are known as mechanoreceptors or contact chemoreceptors are reported from different parasitic arthropods [12]. They are distinguished from other sensilla in being of relatively greater length and in having thicker walls. Out of three types of sensilla trichodea as observed in the present study sensilla trichodea I and III have flexible base i.e., socketed, confirming their mechanoreceptive role. Sensilla trichodea type II with non flexible base appears to be chemoreceptive in function. Distribution of the sensilla in ventral side of the tick suggests the importance of the sensilla in perceiving chemical information from its host [16]. Extensive length of the sensilla in comparison to other sensilla observed in the tick indicates that it may help the insect in sensing its microhabitat from a distance.

Multiporous sensilla: Two types of multiporous sensilla i.e., blunt and pointed are present in the legs of the tick. Similar to our observations, Sridharan et al., [10] recorded the presence of porous and pointed sensilla on the legs of the haematophagous tick *Argas persicus* and established its olfactory role through ultrastructural studies. Olfactory sensilla having pores throughout its surface appear to be an adaptive advantage in the blood feeding tick to locate its host compared to other ectoparasites [16]. Gustatory role of porous sensilla are also advocated by some workers where location of feeding resources and assessing the chemical nature of food was suggested [33]. Probable role of porous sensilla to detect the female sex hormones for locating mate was also proposed by different workers [34, 35].

Hair plate sensilla: This type of grouped sensilla are present through the body and perform the function of proprioception to detect position. Simillar type of grouped sensilla are also reported from the fly *Chironomous samoensis* where the sensilla are present in raw perpendicular to the joint [16].

Bifid sensilla: This type of sensilla are present in the dorsolateral side of *I. acutitarsus* and perform the function of protection [37]. Similar types of sensilla were recorded from red palm weevil (*Rhynchophorus ferrugineus*) and (*R. palmarum*) from lateral side of the body, middle abdominal segment and from antennal club of the body [36, 37].

CONCLUSION

Observations made on the surface topography of various types of sensilla in different locations of the tick *Ixodes acutitarsus* collected from the semi-wild cattle *Bos frontalis* suggest the importance of sensory system in determining the physiological behavior of the parasite. However, electro-physiological and transmission electron microscopic information are required to verify the hypothetical conclusion made on functional significance of some of the sensilla.

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