



## Scanning electron microscopy of cuticular sensory structures on the legs of *Micronecta haliploides* (Horvath, 1904) and *Hydrometra greeni* (Kirkaldy, 1898) (Hemiptera: Heteroptera)

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**ABSTRACT:** Structures on the legs of two hemipteran bugs, *Micronecta haliploides* (Horvath, 1904) and *Hydrometra greeni* (Kirkaldy, 1898), belonging to family Micronectidae and Hydrometridae of two infra orders Nepomorpha and Gerromorpha, respectively were investigated using scanning electron microscopy. Both species have a distinctive leg structure bearing specialised cuticular sensory structures. In the study, the sensilla were classified into five basic types: sensilla trichoidea, sensilla basiconidea, sensilla placoidea, porous circular sensilla and sensilla bell mouthed. These sensilla were further differentiated on the basis of shape, size, number, flexibility and type of socket attached. A total of 26 types of sensilla in the legs of these two species were observed. *M. haliploides* showed 18 types of sensory structures and *H. greeni* 8 types. A specific morphological structure of the porous circular sensilla was observed and found to be unique.

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**KEYWORDS:** Aquatic and semiaquatic bugs, sensilla, types, Micronectidae, Hydrometridae

### INTRODUCTION

Insect cuticle provides mechanical support, protection against loss of water, infections and acts as a barrier between insect body and its environment (Imms, 1957; Moussian, 2010). Scanning electron microscope (SEM) is used to study the surface morphology of the biological samples over a large range of magnification (Zhou and Li, 2015), especially for studying the fine structure on the surface of insect epicuticle. The rigid cuticle possesses specialized cuticular structures that respond to vary minute energy change or stimuli like touch, light, temperature, pressure, humidity, chemical and mechanical forces

(Brožek and Bourgoïn, 2013). These are the primary sensory structures of insects that provide information about the internal and external environment. The cuticular structures are of different shapes and sizes, which are classified into four basic types: hair, campaniform, chordotonal and slit sensilla (French and Torkkeli, 2009). Aquatic insects of the order Hemiptera, have successfully adapted themselves in a variety of habitat types that include not only the lotic and lentic systems but also water filled tree holes, bamboo internode cavities and even pitcher plant (Kovac and Yang, 2000). They have both economic and ecological significance in an aquatic ecosystem (Miura and Takahashi, 1988; Papacek, 2001). The freshwater

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systems of north east India are mostly dominated by these true bugs, of Nepomorpha and Gerromorpha (Choudhury and Gupta, 2015; Hazarika and Goswami, 2010; Purkayastha and Gupta, 2015; Saha and Gupta, 2015, 2018; Takhelmayum and Gupta, 2011). *Micronecta haliploides* (Horvath, 1904) (Nepomorpha: Micronectidae) is true aquatic bug that live submerged in water. Not much is known about their feeding habits but are characterised as organic scrapers (Hadicke *et al.*, 2017) and their modified scoop-like fore tarsi (pala) helps them in scrapping. On the other hand, *Hydrometra greeni* (Kirkaldy, 1898) (Gerromorpha: Hydrometridae) is a semiaquatic living on the water surface and possess elongated legs, and known as “classic sit-and-wait predators” as they can wait static for long time until the prey comes closer. It is also a scavenger (Maier, 1977).

Studies on the sensory structures of aquatic and semi aquatic Hemiptera using SEM are mainly restricted to the mouth parts of Nepomorpha (Brožek, 2013) and Gerromorpha (Brožek and Zettel, 2014), antennal sensilla (Nowińska *et al.* 2020) pala (Furth *et al.*, 1978) and abdominal terminalia of Corixidae (Tinerella, 2006), plastron respiration in Naucoridae (Parsons and Hewson, 1974), legs of Gerridae, Hebridae and Veliidae (Nowińska and Brožek, 2017). For Hydrometridae studies were restricted to the anteclypeus and of males and females terminalia (Gapud *et al.*, 2002). A study was undertaken to explore the sensory structures of the legs of *M. haliploides* and *H. greeni* which occupy different habitats with the help SEM.

## MATERIALS AND METHODS

Aquatic bugs, *M. haliploides* and *H. greeni* were collected (during January, 2013 to December 2014) from the two major lentic fresh water systems of Barak Valley region of southern Assam, namely, Bakhri Haor (24° 49' 47.2" N and 92° 36' 51.3" E) located in Hailakandi district and Sonebeel (24° 41' 116" N and 92° 25' 532" E) located in Karimganj district. Identification of samples were carried out under Motic Stereoscopic Zoom Trinocular microscope, using standard keys (Bal and Basu,

2004; Basu *et al.*, 2015; Jehamalar and Chandra, 2014; Nieser, 2002; Yang and Zettle, 2005). Identified samples were fixed for two hours in 2.5 per cent glutaraldehyde buffered with 0.1 molar sodium-cacodylate. Samples were washed in buffer properly and post fixed in one per cent osmiumtetroxide for two hours. This was then followed by dehydration in an ascending concentrations of acetone (50–100%) and drying in critical point drier (Gupta and Gupta, 2004; Barman and Gupta, 2015). The specimens were mounted on aluminium stubs and metalized with gold using a sputter coating device. Then photomicrographs were taken by observing in JSM 6360 Scanning Electron Microscope (JEOL, Japan). From the SEM images, sensory structures on the legs of the two species were classified following studies of Nowińska and Brožek (2017).

## RESULTS AND DISCUSSION

Legs of *M. haliploides* (Fig. 1a) show numerous sensilla. All the three pairs of fore, mid and hind coxa are covered by thin, pointed, pliable hair-like sensilla trichoidea (ST1) (Fig. 1b). Length of these sensory hairs are much less than 20µm. The fore femur and tibia of this species are bare (Fig. 1c). The pala are devoid of palar pegs and its margin is aligned with thick and long socketed sensilla trichoidea (ST2) (Fig. 1d). These sensilla are also pointed, pliable, hair like but are much longer (more than 50µm in length) with thicker base. These sensilla are supported on collar-like flexible socket at its base. Ventral side of pala (Fig. 1e) shows some hook shaped sensilla trichoidea3 (ST3) and some long non-socketed sensilla trichoidea4 (ST4). ST3 is a curved sensilla, with a hook like structure on its tip. It is almost 10µm in length and is embedded on a flexible socket without a collar. ST4 is a much slender pointed, pliable, hair-like sensilla alongside ST3. It is more than 50µm in length, embedded on a flexible socked without collar. Pala on its dorsal side bears a large, sensilla placoidea (SP) (Fig. 1f). This sensilla is oval shaped depression on the dorsal side of pala.

Mid femur shows scally cuticle with pointed tipped, straight and stout sensilla basiconidea1 (SB1). The sensilla is more than 10µm in length and embedded

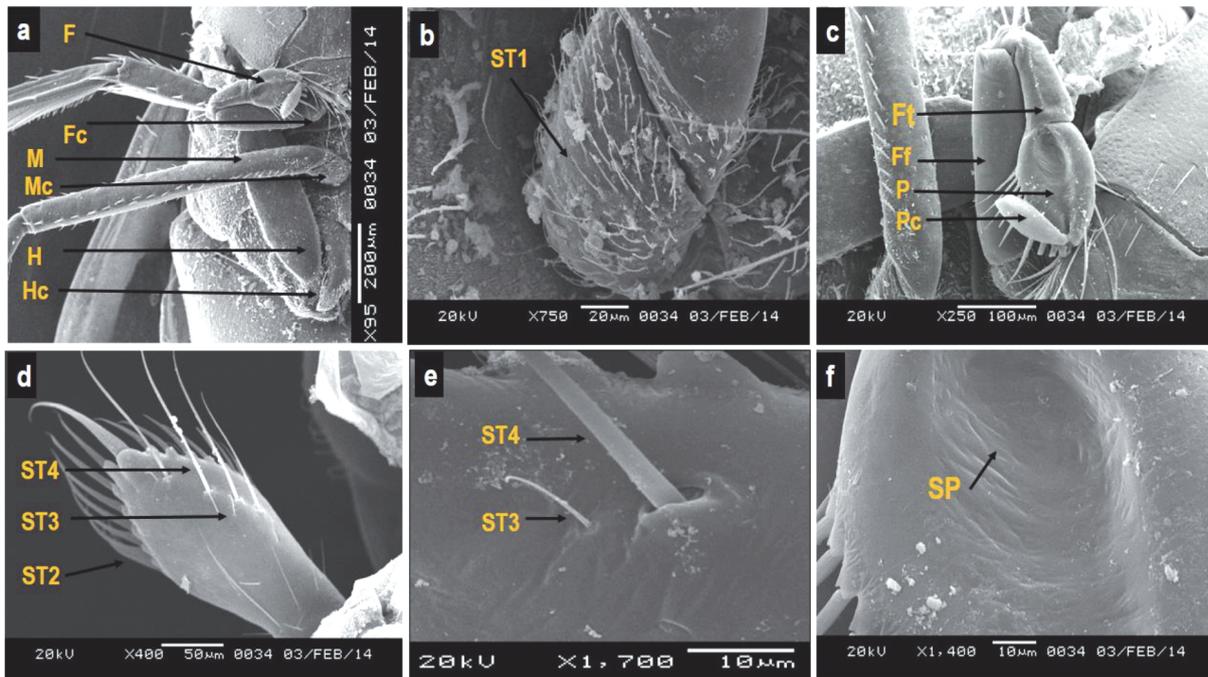


Fig.1 *Microneecta haliploides* :a) Showing fore leg (F), mid leg (M), and hind leg (H) and fore coxa (Fc), mid coxa (Mc) and hind coxa (Hc). b) The fore coxa with Sensilla trichoidea1 (ST1). c) Parts of fore leg showing the fore femur (Ff), fore tibia (Ft), pala (P) and Palar Claw (Pc). d) Showing the ventral side of pala with its margin with sensilla trichoidea2 (ST2). Pala possessing some hook shaped sensilla trichoidea3 (ST3) and some sensilla trichoidea4 (ST4). e) Enlarged view of sensilla ST3 and flexible socket of ST4. f) Enlarged view of sensilla Placoidea (SP) on the dorsal side of the pala.

on a thick flexible base without collar. In between these structures, several circular multi-porous sensilla (Cs) are seen (Fig. 2a, b). The sensilla are devoid of any hairy forms and each structure is found to be composed of about 30 to 35 pores and slits. Each sensilla is about 10- 20 $\mu$ m apart from each other. The mid tibia possess thin and long sensilla trichoidea 5 (ST5) with a broad raised socket. Along with it some ribbed, stout, socketed sensilla basiconidea 2 (SB2) uniformly tapered till the tip with broad base (Fig. 2c). Some sensilla SB2 are also found on the intersegmental region of tibia and tarsus (Fig. 2d). On the mid tarsus (Fig. 3a), a column of thick, bifurcated, pointed tipped sensilla basiconidea3 (SB3) are seen embedded on non-flexible socket. A small, curved sensilla trichoidea6 (ST6) similar to ST3 with curved tip embedded on a flexible socket is seen. Unlike ST3, ST6 possess a collar at its base and it is not surrounded by any other sensory structure. On the other side of SB3,

a row of long, very thin hair like sensilla trichoidea7 (ST7) are also seen aligned on the tibia and tarsus (Fig. 3b). These are pliable and are embedded on non-flexible socket. At the base of the claw multi layered folded cuticular structure (FS) resembling a fish gill is observed in a groove. This structure is lined by small thick, tooth-like sensilla basiconidea4 (SB4) (Fig. 3c). Near the base of the claw a single, long, curved sensilla trichoidea3 (ST3) is noted possessing a flexible socket without collar.

Hind femur (Fig. 3d) shows broad based, relatively stout, pointed and non-flexible sensilla trichoidea8 (ST8) with the inner edge aligned by large sized thin sensilla trichoidea7 (ST7). The joint of hind femur and tibia possess small, tooth like stout sensilla basiconidea4 (SB4) and strong, thick, elongated digitiform, sensilla basiconidea5 (SB5). The hind tarsus and tibia are flat, paddle shaped and fringed with various types of sensilla supported by long

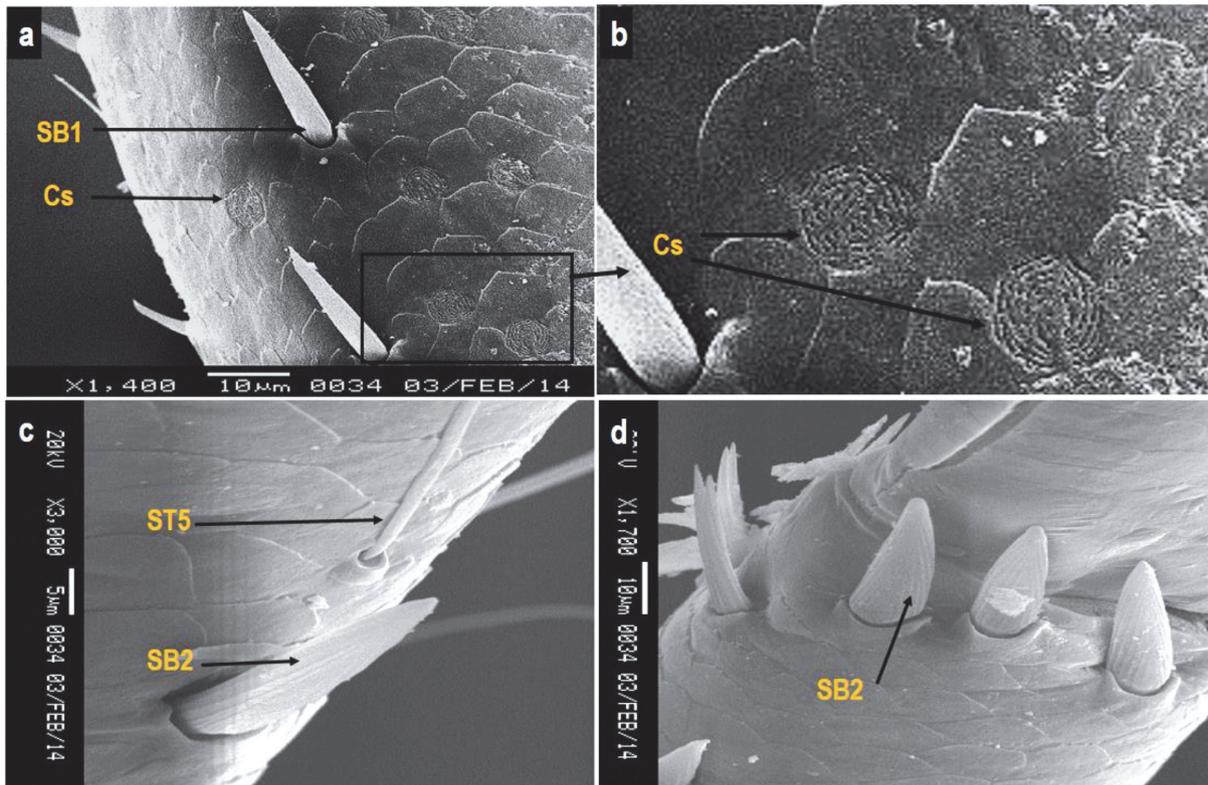


Fig.2 *Micronecta haliploides*: a) Mid femur with sensilla basiconidea1 (SB1) and Circular multi-porous sensilla (Cs) on the scales. b) Enlarged view of Cs. c) Sensilla trichoidea5 (ST5) and flexible socketed sensilla basiconidea2 (SB2) on the tibia of mid-leg. h) More similar sensilla basiconidea (SB2) on the intersegmental area of mid tibia and tarsus.

swimming hairs like sensilla trichoidea7 (ST7) (Fig. 3e). Some paint brush shaped, flat sensilla trichoidea9 (ST9) are found aligned along with the sensilla trichoidea (ST7) and on the dorsal edges ST8 sensilla (Fig. 3f). Intersegmental area of these last two segments are also lined by (SB5) (Fig. 3g). The tip of the hind tarsus also possesses some sensilla basiconidea5 (SB5) surrounding few thin, round, smooth, relatively short digitiform sensilla basiconidea6 (SB6) embedded on well-built socket (Fig. 3h).

The three pairs of legs of *H. greeni* (Fig. 4 and 5), differed in the types of sensilla and their numbers. A thorn like, non flexible socketed sensilla trichoidea10 (ST10) is seen on the bare cuticle of fore, mid and hind coxae. Near to the intersegmental region of coxa and femur a bell mouthed sensilla (BS) is seen. BS is a conical shaped, uni-porous

sensilla. The pit is at the centre enclosed inside an undulating cuticular cone. On the anterior part of the fore femur, some small, thick, sensilla trichoidea11 (ST11) are aligned in a single line (Fig. 4d) whereas on the middle part of fore femur along with the ST11, relatively thinner, spine like sensilla trichoidea12 (ST12) are seen (Fig. 4e). The number and rows of ST12 are found to increase towards the apical part of the femur (Fig. 4f). On the fore tibia (Fig. 4g), density of sensilla trichoidea (ST12) intensifies superimposing the cuticle and the ST11 sensilla trichoidea. The intersegmental area between fore-tibia and tarsus shows an undulating surface (Fig. 4h) that possess relatively thick, spine like sensilla basiconidea7 (SB7). Unlike SB5, this sensilla do not bear a collar on its flexible socket (Fig. 4i). The basal part of the mid femur has sensilla ST11 (Fig. 5 a, b), whereas the apical part has an array of pliable, thin smoothly curved sensilla

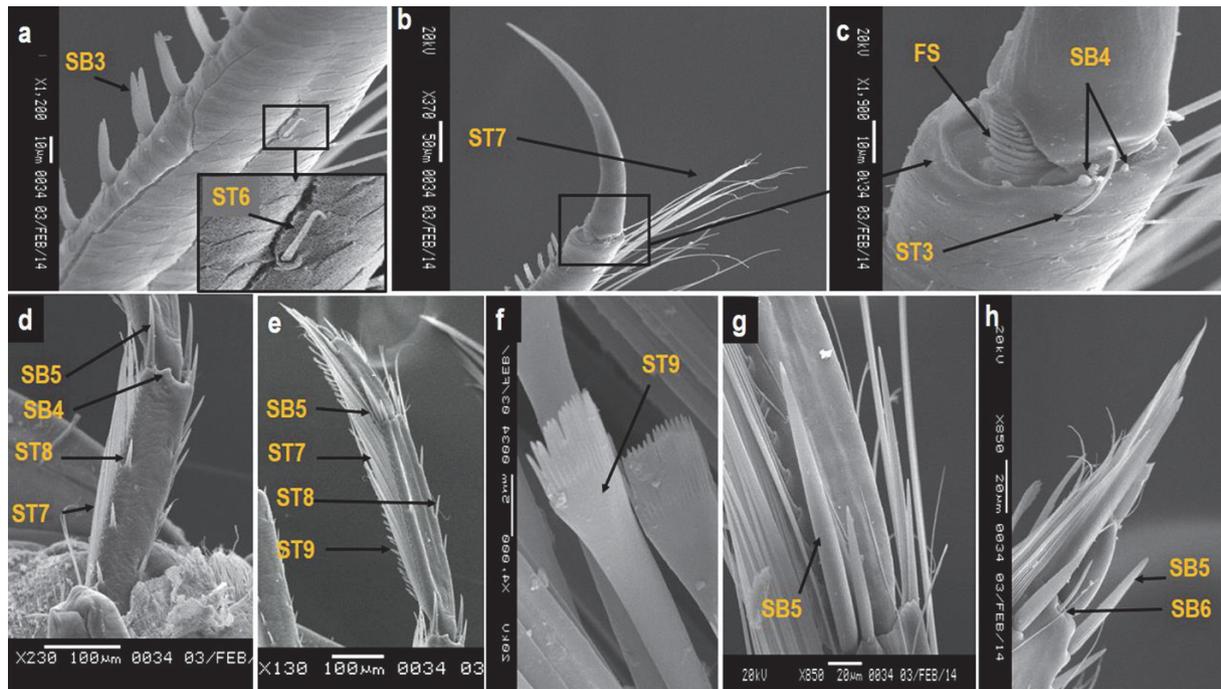


Fig. 3 *Micronecta haliploides*: a) Row of bifurcated sensilla basiconea3 (SB3) and a single hook shaped sensilla trichoidea6 (ST6) with collar at its flexible socket is seen on the mid tarsus. b) Long, thin non socketed sensilla trichoidea7 (ST7) and folded structure on the base of the claw/ long digitalis. c) Enlarged view of the folded structure (FS) with its base possessing broad based stout tooth like sensilla basiconea4 (SB4) and a hook shaped sensilla trichoidea3 (ST3) without collar on its flexible base. d) Hind femur with sensilla trichoidea8 (ST8) and long swimming hairs like sensilla trichoidea7 (ST7) aligned on the edges. Sensilla basiconea4 (SB4) and sensilla basiconea5 (SB5) on the apical portion of the hind femur. e) Hind tibia with sensilla trichoidea8 (ST8) and flat brush shaped sensilla trichoidea9 (ST9) and sensilla trichoidea7 (ST7). f) Enlarged view of sensilla trichoidea9 (ST9). g) The intersegmental region between the hind tibia and tarsus showing thick socketed sensilla basiconea5 (SB5). h) Sensilla basiconea5 (SB5) and sensilla basiconea6 (SB6) at the base of the claw.

trichoidea13 (ST13) on a non-flexible socket (Fig. 5c). The curvature of these ST13, is away from the cuticle. Femur and tarsus of mid leg is also fully covered by ST13 but the intersegmental area between them shows bare undulating surface (Fig. 5d). The intersegmental area between tibia and tarsus of mid leg, is surrounded by relatively thick row of sensilla trichoidea14 (ST14) (Fig. 5e). Length of these sensilla ranged from 20- 60 $\mu$ m. These sensilla are arranged in a group of about 20- 25 sensilla aligned on non-flexible socketed base.

The basal portion of the hind femur shows presence of both sensilla ST11 and ST12 (Fig. 5f) that increases towards the apical part. The pattern of sensilla in the intersegmental area of hind femur

and tibia is similar to the intersegmental region of the mid femur and tibia. Very few sensilla basiconea (SB5) are seen in one or two places among the ST13 sensilla trichoidea covering the apical part of hind femur and basal part of hind tibia. In between these two, the undulating joint part is bare (Fig. 5g). Again, on the hind leg tarsus and tibia, sensilla ST14 are seen as in the intersegmental region between tibia and tarsus of mid leg (Fig. 5h). Hind tarsus is covered by non-flexible socketed sensilla trichoidea15 (ST15) (Fig. 5i). Unlike ST13, the curvature of these fine curved sensilla is towards the cuticle.

The sensory structures on the leg appendages of *M. haliploides* and *H. greeni* showed variation in size

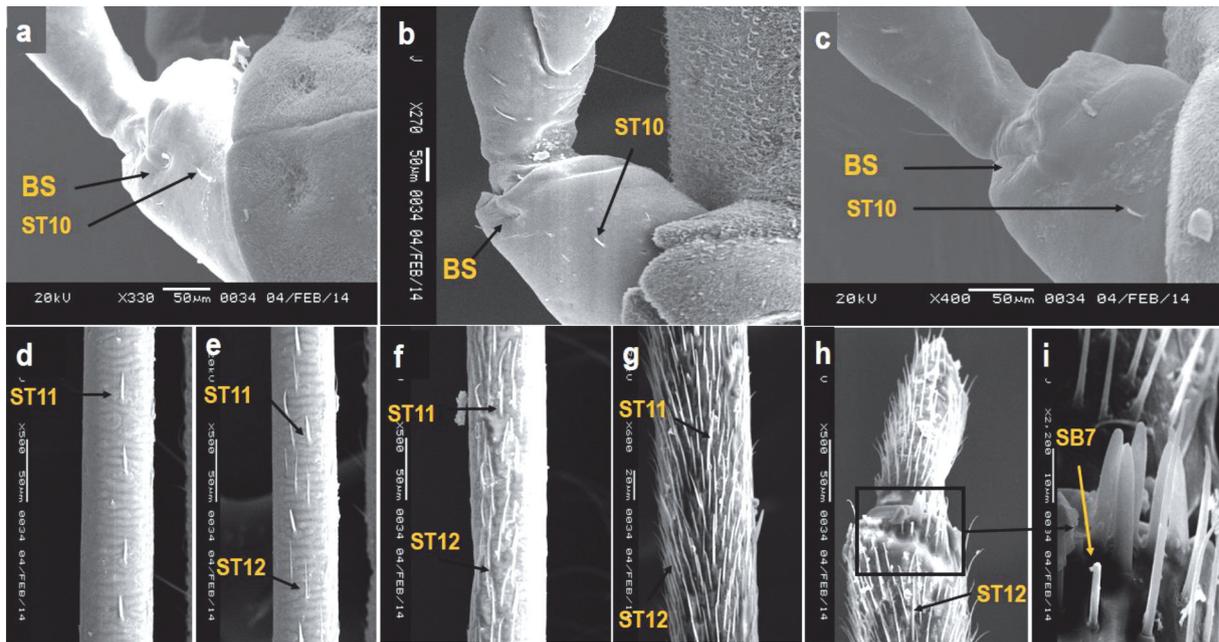


Fig. 4 *Hydrometra greeni*: a) Fore coxa, b) mid coxa and c) hind coxa, with a bell mouthed porous sensilla (BS) and a thorn like sensilla trichoidea10 (ST10). d) Base of the fore femur with thick sensilla trichoidea11 (ST11); e) Middle part of fore femur showing ST11 and a thin spine like rows of Sensilla trichoidea12 (ST12). f) Apical part of fore femur. g) Fore tibia covered by sensilla ST11 and ST12. h) The intersegmental area between fore tibia and tarsus is covered with long sensilla trichoidea2 (ST12). i) Enlarged view of the intersegmental portion showing relatively thick, spine like sensilla basiconidea7 (SB7) supported on a flexible socket on an undulating surface.

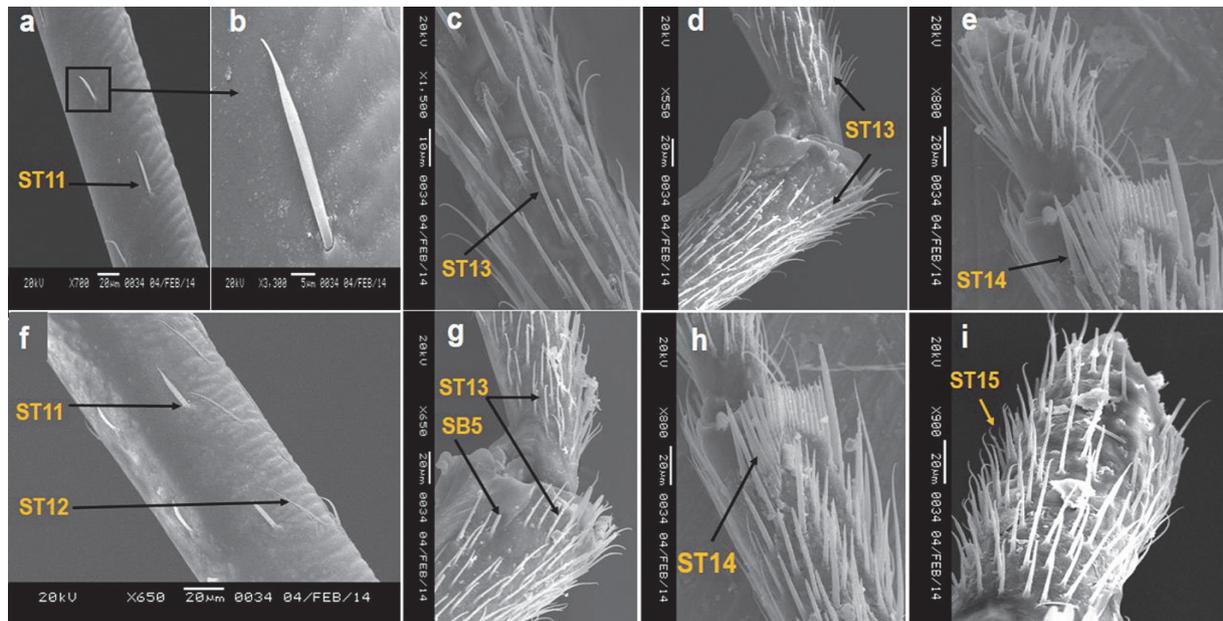


Fig. 5 *Hydrometra greeni*: a) Basal part of mid femur showing sensilla trichoidea ST11. b) Enlarged view of ST11 sensilla trichoidea. c) Apical part of mid femur with sensilla trichoidea13 (ST13). d) Sensilla (ST13) on mid-femur and tarsus and the undulating surface on the intersegmental area. e) Intersegmental area between mid-tibia and tarsus shows spine like sensilla trichoidea14 (ST14). f) Basal part of hind femur showing both ST11 and ST12. g) The intersegmental area between hind tibia and hind tarsus showing sensilla basiconidea5 (SB5) along with sensilla ST13 on the apical portion of femur. h) The intersegmental area between hind-tibia and hind tarsus is covered by sensilla trichoidea (ST14). i) Hind tarsus covered by sensilla trichoidea15 (ST15).

and shape among the two species and in different segments of their legs. Based on size, shape, pattern, their number and cuticular attachment (socketed or non-socketed), 26 types of sensilla were marked in these two species (Table 1). They are basically of 5 types as: sensilla trichoidea, sensilla basiconidea, sensilla placoidea, some porous circular sensilla and sensilla bell mouthed. Sensilla trichoidea (ST) are long, slender, hair-like structures with thin distal tip. Sensilla basiconidea (SB) are relatively shorter, stouter digitiforms with broad base and blunt distal tips. Both ST and SB are found buried inside depressions on the cuticle that can either be flexible socketed or non-flexible socketed and with collar or without collar. Sensilla placoidea (SP) are concave depressions. Sensilla bell mouthed is a funnel shaped, wavy cuticular fold with a pit (Nowińska and Brożek, 2017).

Legs of *M. haliploides* are mainly comprised of sensilla trichoidea followed by sensilla basiconidea. More or less similar sensilla ST1 found on the coxa of this species were also reported in *Anisops sp.* (Gupta, 2008). Sensilla trichoidea are known to function as a mechanoreceptor (Nowińska and Brożek, 2017). Sensilla placoidea (SP) on the dorsal side of the pala *M. haliploides* shows smooth surface. A study on antennomeres of adult females of *Cotesia sesamiae* and *Cotesia flavipes* reported sensilla placoidea as a sponge-like surface indicating that the structure is porous (Obonyo *et al.* 2011). Sensilla placoidea functions as an olfactory receptor (Gao *et al.*, 2007; Nowińska and Brożek, 2017; Obonyo *et al.*, 2011).

The multi-porous circular sensilla (Cs) structures on the mid femur have a specific morphological structure. It is devoid of any hairy forms and each sensilla is found to be composed of numerous pores and slits. Each sensilla are about 10µm distant apart from each other. Multiporous sensilla are characterised as chemoreceptors and functions as olfactory sensilla (Nowińska and Brożek, 2017). The folded structure on the base of the hind claw or the long digitalis of *M. haliploides* resembles the stridulatory file reported on the abdomen of *Micronecta burillu* (Bailey, 1983). Stridulation is well known among the Micronectidae (Reid *et al.*,

2017; King, 1999). Lawson and Chu (1971) reported similar structures on the wings of Hemiptera-Homoptera bug, *Galgupha ovalis*.

In *M. haliploides*, sensilla ST3 (found on the ventral side of pala and base of the claw) is quite similar to the sensilla ST6 (found on the mid tarsus of *M. haliploides*) unlike the presence of a collar on the socket of ST6. Similar sensilla is also reported in *Amemboa cristata*, *Amemboa brevifasciata* and *Onychotrechus esakii* (Nowińska and Brożek, 2017) as densely and uniformly distributed sensilla on the first and third antennomeres whereas in the legs of *M. haliploides* it is noted singly and no other cuticular structure or sensilla was found surrounding it. Sensilla possessing a flexible socket are predicted to function as mechanoreceptors, and they receive stimuli by being touched, moved or deformed (Nowińska and Brożek, 2017). The position of sensilla ST9 found on the tibia and tarsus of hind leg, in the midst of swimming hairs and their characteristic shape as flat brushes implies that these sensilla might function as mechanoreceptors for sensing the pressure or temperature of the water and may accomplish a mechanical role in either propelling or halting the insect in water current. Mechanoreceptors have the ability to perceive external stimuli during locomotion, oviposition and feeding (Nowińska and Brożek, 2021).

*H. greeni* on the other hand shows different types and numbers of cuticular structures on its legs. The bell mouthed porous sensilla (BS) on its coxa is also reported as a new sensilla on the antennae of *Aquarius paludum*, another Gerromorpha bug belonging to the family Gerridae (Nowińska and Brożek, 2017). The presence of such characteristic morphological structures on selective Gerromorpha bugs signifies their unique function that need further study. The gradual increase in the sensory structures from the base of femur to the apex of the tarsus of *H. greeni* is also reported in Gerridae, *Halobates germanus*, and *Aquarius elongates* (Perez-Goodwyn, 2009). Such structures provide support and liberty in locomotion on water surface. The densely spread cuticular structures provide hydrophobicity and distribute its body weight over

Table 1 List of sensory structures with morphplogy on the legs of  
*Micronecta haliploides* and *Hydrometra greeni*

Sl no	Structures	Morphology	Position on the specimen leg
1	Sensilla trichoidea 1 (ST1)	Pliable, hair like, length less than 20µm	Fore, mid and hind coxae of <i>M. haliploides</i>
2	Sensilla trichoidea 2 (ST2)	Pointed, pliable, hair like, almost 50µm in length, embedded on a flexible socked with collar.	On Palar margin of <i>M. haliploides</i>
3	Sensilla trichoidea 3 (ST3)	Curved sensilla, with a hook like structure on its tip, embedded on flexible socket without collar.	Ventral side of pala and near the base of the mid leg claw of <i>M. haliploides</i>
4	Sensilla trichoidea 4 (ST4)	Pointed, pliable, hair like. More than 50µm in length, embedded on a flexible socked without collar alongside of ST3.	Ventral side of pala of <i>M. haliploides</i>
5	Sensilla trichoidea 5 (ST5)	Long slender sensilla with raised flexible socket.	Mid tibia of <i>M. haliploides</i>
6	Sensilla trichoidea 6 (ST6)	Small sensilla with curved tip embedded on a flexible sockets with collar.	Mid tarsus of <i>M. haliploides</i>
7	Sensilla trichoidea 7 (ST7)	Row of long, very thin hair like, pliable sensilla, embedded on non-flexible socket.	Mid tarsus, mid tibia and entire Hind leg of <i>M. haliploides</i>
8	Sensilla trichoidea 8 (ST8)	Broad based, relatively stout, pointed and non-flexible.	Hind femur of <i>M. haliploides</i>
9	Sensilla trichoidea 9 (ST9)	Paint brush shaped, flat.	Along with sensilla ST7 in <i>M. haliploides</i>
10	Sensilla trichoidea 10 (ST10)	Thorn like non flexible socketed.	Fore, mid and hind coxae of <i>H. greeni</i>
11	Sensilla trichoidea 11 (ST11)	Small, slender but thick, non-flexible socketed.	Fore femur of <i>H. greeni</i>
12	Sensilla trichoidea 12 (ST12)	Relatively thinner than ST11, spine like, non-flexible socketed.	Fore femur of <i>H. greeni</i>
13	Sensilla trichoidea 13 (ST13)	Array of pliable, thin smoothly curved, non-flexible socket.	Mid and hind femur of <i>H. greeni</i>
14	Sensilla trichoidea 14 (ST14)	It is a group of sensilla aligned at nonflexible socketed base, length of these sensilla ranged from 20- 60µm.	Intersegmental area of tibia and tarsus of mid and hind leg of <i>H. greeni</i>
15	Sensilla trichoidea 15 (ST15)	Array of pliable, thin smoothly curved, non-flexible socket. These are curved towards the cuticle.	Hind tarsus and tibia
16	Sensilla basiconidea 1 (SB1)	Pointed tipped, straight, stout, 10µm in length, thick flexible base without collar.	Mid femur of <i>M. haliploides</i>
17	Sensilla basiconidea 2 (SB2)	Ribbed, stout, uniformly tapered tip with broad base and flexible socketed.	Mid tibia of <i>M. haliploides</i>
18	Sensilla basiconidea 3 (SB3)	Thick, bifurcated pointed tipped sensilla	Mid tarsus of <i>M. haliploides</i>
19	Sensilla basiconidea 4 (SB4)	Small thick, tooth-like.	Base of mid leg claw of <i>M. haliploides</i>
20	Sensilla basiconidea 5 (SB5)	Strong, thick, elongated, well-built flexible socket.	Base of mid leg claw and Intersegmental areas of mid and hind legs of <i>M. haliploides</i>

Sl no	Structures	Morphology	Position on the specimen leg
21	Sensilla basiconidea 6 (SB6)	Round, thin, relatively short digitiform, on well-built flexible socket.	Tip of the hind tarsus of <i>M. haliploides</i>
22	Sensilla basiconidea 7 (SB7)	Round, thin, relatively short digitiform, without collar.	Intersegmental area between fore-tibia and tarsus of <i>H. greeni</i>
23	Sensilla Placoidea (SP)	Concave depressions .	Dorsal side of pala of <i>M. haliploides</i>
24	Circular multi-porous sensilla (CS)	Consists of about 30 to 35 slits in a circular form.	Mid femur of <i>M. haliploides</i>
25	Folded cuticular structure (FS)	Multi-layered, resembles stridulatory organs.	Base of mid leg claw of <i>M. haliploides</i>
26	Bell mouthed sensilla (BS)	Conical shaped, porous sensilla, pit enclosed inside an undulating cuticular cone.	Fore, mid and hind coxae of <i>H. greeni</i>

a large area of the surface film for support on the water surface (Andersen, 1989).

### ACKNOWLEDGEMENTS

The authors are thankful to the Head, SAIF, North-Eastern Hill University (NEHU) Shillong, Meghalaya for Scanning Electron Microscopy of the samples and to the Head of the Department of Ecology and Environmental Science, Assam University, Silchar, for providing laboratory facilities. Authors also convey their gratitude to the Department of Science and technology - Science & Engineering Research Board (DST-SERB) for financial support. The authors declare no conflict of interest in this manuscript.

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(Received August 07, 2021; revised ms accepted October 31, 2021; printed December 31, 2021)