

# Fine Structure of Ventral and Lateral Tarsal Sensilla of the Hard Tick, *Amblyomma americanum*<sup>1,2</sup>

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## ABSTRACT

The sensilla on the ventral, lateral, and dorso-proximal sides of the first tarsi of adult *Amblyomma americanum* (L.) were studied by scanning and transmission electron microscopy. Only a few sensilla have dendrites in the shaft and are thick-walled, with either a single apical slit opening or 8 longitudinal slit grooves along the whole

shaft. Based on their structure, these sensilla are assumed to be chemoreceptors. Sensilla with a single apical opening also possess 2 mechanoreceptive dendrites inserted at the setal base. All other sensilla are assumed to be solely mechanoreceptors since they have basal tubular bodies and no dendrites in the setal shaft.

Ticks wave their first pair of legs in the air, similar to the way insects use their antennae, to detect their environment and orient to hosts (Lees 1948). Sensory receptors occur on the tarsi of the 1st legs of ticks with Haller's organ (composed of a capsule and anterior pit), associated dorsal sensilla and sensilla at the base of the claw being most obvious. The ultrastructure of the above sensilla, as well as those of the palps, have been described in detail in the hard tick, *Amblyomma americanum* (L.), and the soft tick, *Argas arboreus* Kaiser, Hoogstraal, and Kohls, in a series of reports from this laboratory (Foelix and Axtell 1971, 1972, Foelix 1972, Foelix and Chu-Wang 1972, Roshdy et al. 1972, Axtell et al. 1973, Chu-Wang and Axtell 1973).

However, these investigations did not include the remaining setae on the ventral, lateral and dorso-proximal sides of the 1st tarsi. We have examined the fine structure of these setae in *A. americanum*. Since these setae are innervated, it is appropriate to refer to them as sensilla. This report and previous ones from this laboratory provide complete information on all sensilla of the 1st tarsi of *A. americanum* and are a basis for future behavioral and electrophysiological studies. Until such investigations are conducted, suggestions for the functions of these sensilla can be made from the ultrastructural evidence (Thurm 1964, Slifer 1970).

## MATERIALS AND METHODS

Adults of *A. americanum* were either field-collected from Camp Lejeune, N.C., or obtained from the U.S. Department of Agriculture, Livestock Insect Laboratory, Kerrville, Tex.

For scanning electron microscopy (SEM), the ticks were killed in hot water, double-coated with carbon and gold in a vacuum evaporator, and observed in a JEOL JSM-2 scanning electron microscope.

For transmission electron microscopy (TEM), tarsi of the 1st legs were cut from live ticks, fixed in cold 5% glutaraldehyde in sodium cacodylate buffer (Sabatini et al. 1963) for 24 h and post-fixed in 1% OsO<sub>4</sub>

in veronal acetate buffer (Palade 1952) for 2 h. Following dehydration in ethanol the specimens were embedded in hard Epon 812 over propylene oxide. Thin sections were cut on a Reichert Om U2 ultramicrotome with a diamond knife, picked up on formvar-coated grids, and stained in uranyl acetate and lead citrate (each for 15 min). The serial sections were examined in a Siemens Elmiskop 1A at an accelerating voltage of 80 kV.

## RESULTS

*Ventral Sensilla.*—The ventral sensilla are usually arranged in 8 groups, which we designated by consecutive numbers (Fig. 1). Each group consists of 2 setae (1 laterad and 1 mediad) except group 2 which has 4 (Fig. 2) and group 8 which has 3 (Fig. 3). Variation in the number and arrangements of the setae was occasionally observed.

Based on external appearance when viewed by SEM, there are 2 types of sensilla. The 1st type includes all sensilla of groups 1, 6, 7, and 8, and only the medial sensilla of groups 4 and 5. These sensilla are long (ca. 75  $\mu$ ), slightly curved, and make an angle of ca. 35° (10° for the lateral sensillum of group 8) with the long axis of the leg (Fig. 2, 3). A distinct feature found in all sensilla of this type is 2-4 finger-like projections occurring at the setal tip and near the tip region as well (Fig. 2, insert). These sensilla are probably solely mechanoreceptors because each is innervated only by 2 bipolar neurons having the distal "tubular bodies" (Thurm 1964) in the dendrites terminating at the setal base (Fig. 4).

The other ventral sensilla are of the 2nd type. They are short (ca. 53  $\mu$ ), rather blunt tipped, and form an angle of ca. 70° with the long axis of the leg. We recognized 2 subtypes. Each member of the 1st subtype (the middle pair of group 2, all of group 3 and the laterads of groups 4 and 5) is innervated by 2 sets of bipolar neurons,—one set of 2 dendrites with distal tubular bodies terminating at the socket base, and another set of 4 nonbranching dendrites entering into the setal shaft and connecting to the outside environment through a single subterminal slit opening (140 Å wide; 0.5  $\mu$  long) (Fig. 5). These sensilla are probably combined mechano- and chemoreceptors as previously postulated for other similar tarsal sensilla (Foelix and Axtell 1971, Foelix and Chu-Wang 1972, Chu-Wang and Axtell 1973).

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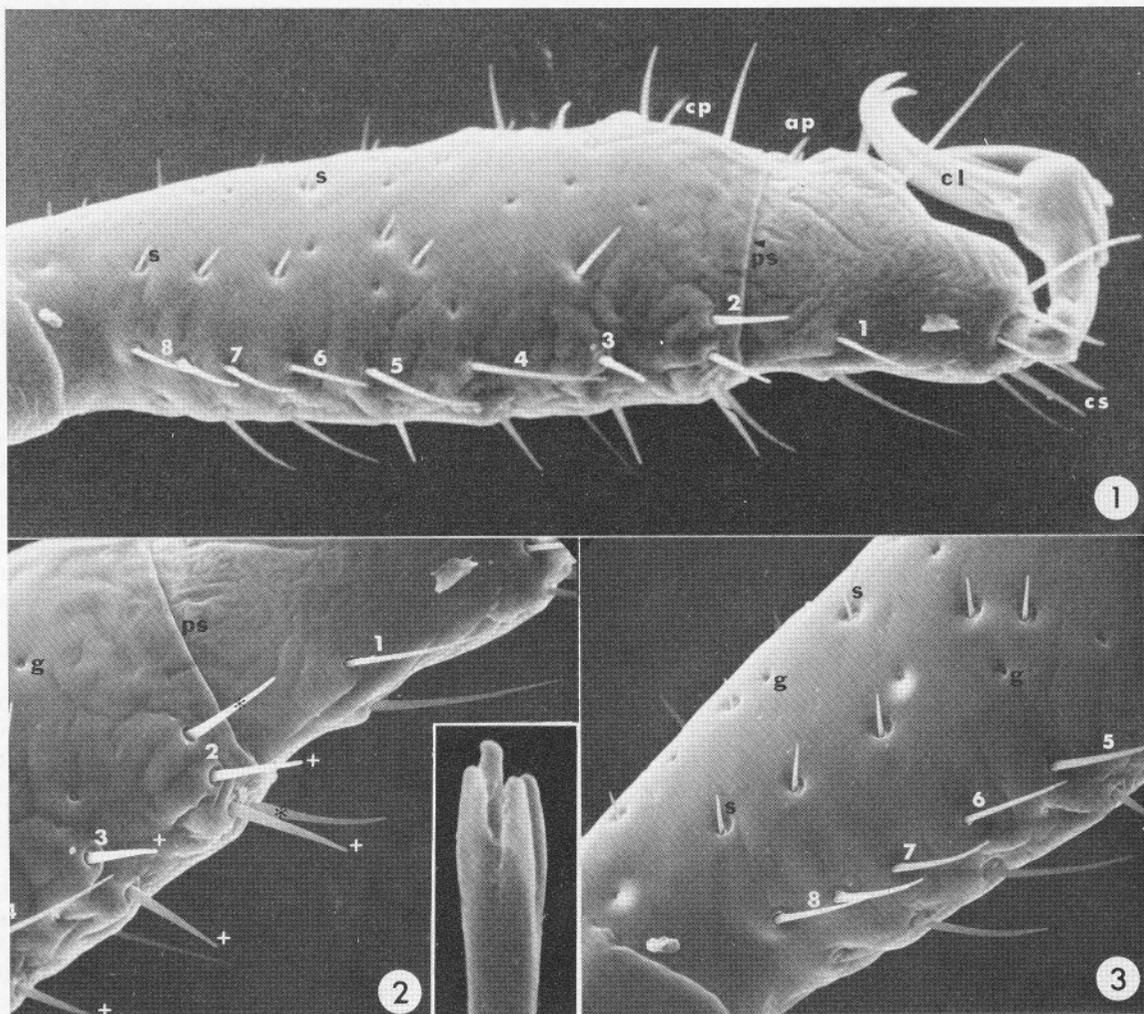


FIG. 1.—Scanning electron micrograph of the 1st tarsus of *A. americanum* (ventral-medial view). 1-8, groups of ventral sensilla; ap, anterior pit and cp, capsule of Haller's organ; cl, claws; cs, claw sensilla; ps, pseudosegmentation; s, short, straight sensilla. X 300.

FIG. 2.—Higher magnification of the same specimen as in Fig. 1 showing sensilla groups 1-4. g, opening of the integumental gland; ps, pseudosegmentation; +, sensilla with a subterminal slit opening; \*, sensilla with octagonal slit groove openings in the wall. The other sensilla are mechanoreceptors. X 400. Insert: Tip of a mechanoreceptive sensillum showing the 4 finger-like projections. X 8,000.

FIG. 3.—Higher magnification of the same specimen as in Fig. 1 showing sensilla groups 5-8. All of the sensilla are mechanoreceptors except the lateral one of group 5 (+) which has a subterminal opening. g, opening of the integumental gland; s, short, straight sensilla. X 400.

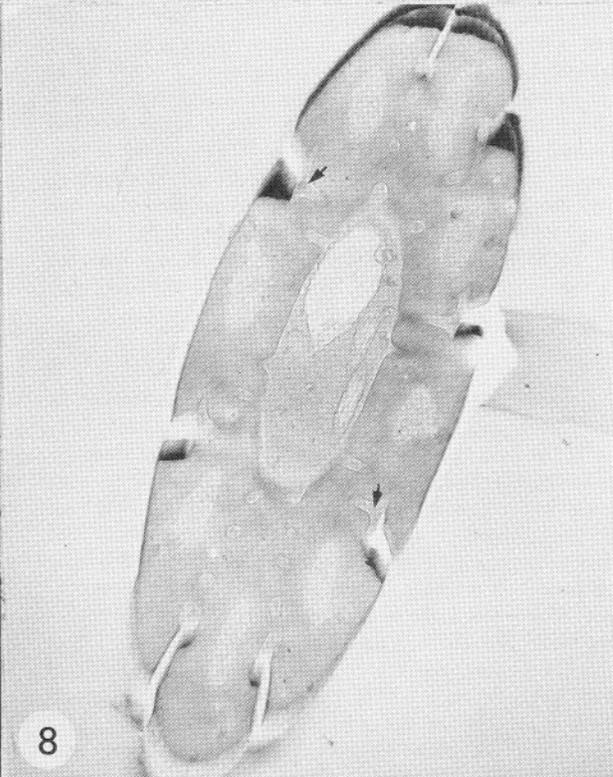
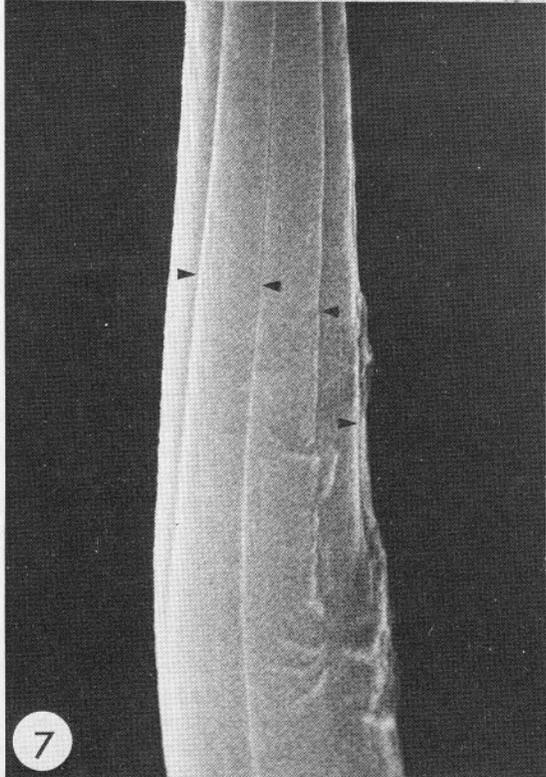
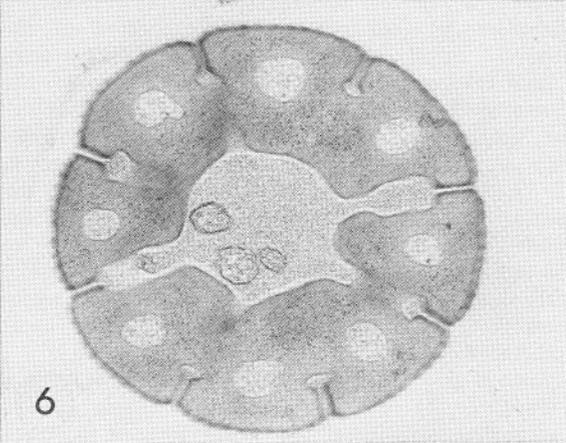
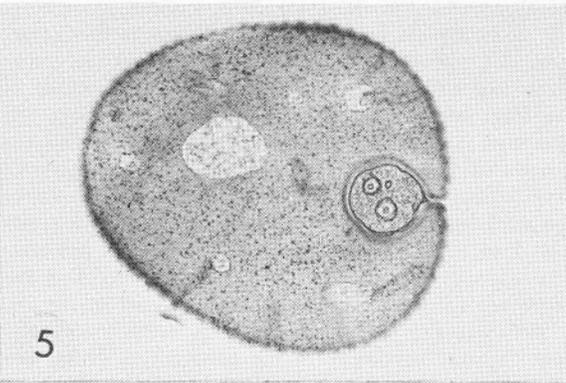
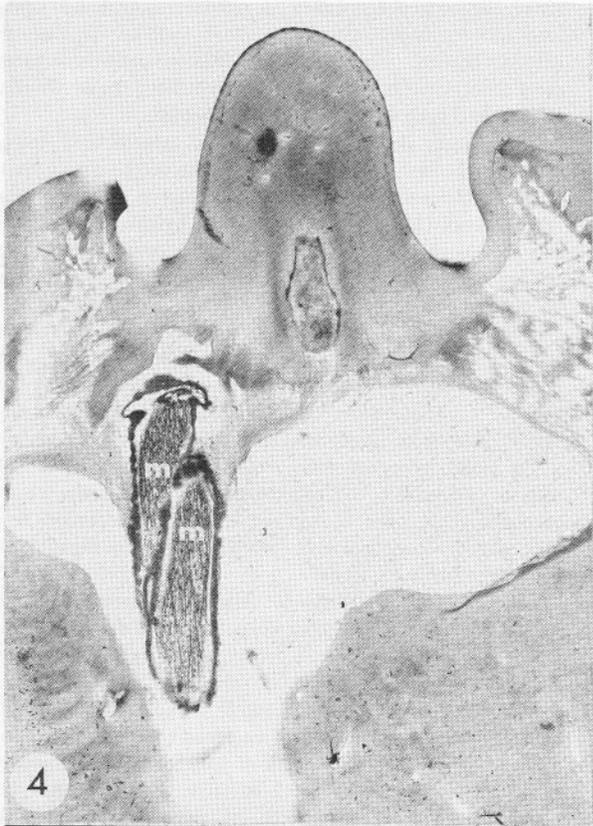
FIG. 4.—Transmission electron micrograph of a mechanoreceptor (oblique-sagittal section). Note 2 mechanoreceptive dendrites (m) terminating at the base. X 10,400.

FIG. 5.—Transmission electron micrograph of a group 3 sensillum (cross-section near the tip). Dendrites in the circular lumen are exposed to the outside through a narrow slit opening. Note the membrane structure lining the lumen. X 37,000.

FIG. 6.—Transmission electron micrograph of a lateral sensillum of group 2 (cross section). Slit openings (8) in the wall connect to vase-shaped channels which lead to a central circular lumen containing dendrites. Note the central fluid extending only to the bottom of slit openings. No membrane structure was observed in this type of sensillum (compare with Fig. 5). X 28,000.

FIG. 7.—High magnification scanning electron micrograph of the same type of sensillum as in Fig. 6 showing 4 length-wise grooves indicating the locations (arrows) of the slit openings. X 10,000.

FIG. 8.—Transmission electron micrograph (oblique section) of the same type of sensillum as in Fig. 6 and 7. Note the depth of the slit groove openings, and that the fluid (arrow) in the vase-shaped channels (not showing entire vase-shape at this angle) does not protrude into the slit openings. X 16,000.



The sensilla of the 2nd subtype (the outer pair of group 2, Fig. 2) are innervated by 3 or 6 dendrites which ascend into the setal shaft without branching. The most typical feature of this subtype is the 8 narrow, slit openings (ca.  $0.18 \mu$  long;  $140 \text{ \AA}$  wide) occurring almost equidistant in the circumference of the setal wall (Fig. 6). The location of these slit openings is also evident in scanning electron micrographs of the shaft (Fig. 7), appearing as narrow, longitudinal grooves which extend from shortly above the setal base to near the setal tip. The groove openings lead to "vase-shaped" structures which are continuous with the central circular lumen containing the dendrites (Fig. 6, 8). These vase-shaped structures ( $1900 \text{ \AA}$  in the widest part;  $800 \text{ \AA}$  in the narrowest part) appear randomly in the consecutive cross sections of the shaft. This suggests that the vase-shaped chambers are not continuously connected to the grooves. In all cross-sections examined, there were 8 fluid-filled areas located intermittently between the vase-shaped structures. As previously described for the "medial" (= "4-group") sensilla posterior to Haller's organ (Foelix and Axtell 1971, Axtell et al. 1973), these areas are cylindrical and connect to the central lumen at the base of the shaft. Since the dendrites in the central lumen can communicate to the outside through slit-groove openings in the setal wall, this type of sensilla probably serves as a chemoreceptor (Slifer 1970, Foelix and Axtell 1971, Coons and Axtell 1973).

*Lateral and Dorsal Sensilla.*—A lateral row of 6 (sometimes 7) sensilla found on each side (lateral and medial faces in relation to the body of the tick) of the 1st tarsus is mostly located on the proximal half (Fig. 1, 3). All sensilla are short (ca.  $20 \mu$ ) except for the distal one (sometimes 2) which is longer (ca.  $80 \mu$ ) and set apart from the others. Short, straight setae, essentially of the same size but present in 2 rows of 4–6 setae each, are found on the dorsal surface of the proximal half of the tarsus. Sections of many of these sensilla show that they are innervated by 2 bipolar neurons with tubular bodies at the distal end of the dendrites which are inserted at the base of the shaft. Therefore, all of these sensilla are probably mechanoreceptors.

#### DISCUSSION

Lees (1948) postulated that the ventral setae of *Ixodes ricinus* L. are tactile. This is probably only partially true according to our ultrastructural findings in *A. americanum*. For example, the middle pair of setae in group 2, group 3, and the lateral setae of groups 4 and 5 are probably both mechano- and chemoreceptors. These functions are suggested by the existence of tubular bodies (Thurm 1964), innervation of the setal shaft and presence of a single opening near the tip of the shaft (Slifer 1970). These sensilla (listed above) closely resemble the proximal sensilla lying behind Haller's organ on the dorsal surface of the tarsus (Foelix and Axtell 1971),—type 2 in the claw sensilla group (Chu-Wang and Axtell 1973), and type A sensilla in the palpal organ

(Foelix and Chu-Wang 1972). The 2 outer ventral sensilla of group 2 are probably only chemoreceptors since they have dendrites in the shaft and slit openings along the length of the shaft wall but possess no tubular bodies. The remaining ventral setae are probably solely mechanoreceptors since they have tubular bodies and lack innervation of the shafts.

The short, straight setae on the lateral and dorsal aspects of the proximal half of the tarsi are apparently mechanoreceptors since they have basal tubular bodies and the dendrites do not invade the shafts. However, it is difficult to conceive that these relatively short setae are mechanoreceptors since one would expect longer shafts for a tactile function. Lees (1948) claimed that these short setae in *Ixodes ricinus* were thermal receptors but he did not present adequate experimental evidence to support that hypothesis. He based his conclusions on experiments in which the ticks continued to respond to temperature after Haller's organ was covered but this did not rule out the possibility of receptors other than the short, straight setae. As far as we know, there is no reliable way to interpret a thermoreceptive function from ultrastructural evidence.

Some typical features of the tick chemoreceptive sensilla are quite different from those of typical insect sensilla (Slifer 1970): 1, relatively few occur in the tick; 2, the single pore of the thick-walled chemoreceptor in the tick ( $140 \text{ \AA}$  wide in slit opening type) is smaller than those found in most insects ( $0.1\text{--}2.0 \mu$  diam); 3, in the type with 8 longitudinal slit openings (a type not reported in insects), although the openings extend the length of the shaft, the vase-shaped channels (connecting the central lumen and slit openings) are only randomly connected to the groove openings; 4, the fluid surrounding the dendrites fills the central lumen and the vase-shaped channels whereas the actual slit is void of fluid (Fig. 5, 6, 8); 5, in the plugged pore type (a type not reported in insects) found in Haller's organ and nearby sensilla (Foelix and Axtell 1971, 1972), although the ovoid opening ( $0.1 \mu$ ) is relatively large, the pore canal is plugged by a lens-shaped body. Perhaps these features are devices for reducing water loss in the tick, but evidence for this is not available.

It is especially interesting that all the tick sensilla have a distinct socket (except the sensilla inside the capsule) in contrast to some insect chemoreceptive sensilla that are not socketed. Tick sensilla which have presumably different functions do not have distinctly different setal tips as are usually found in insect sensilla (e.g., extremely sharp-tipped setae serving as mechanoreceptors and round-tipped ones as chemoreceptors). One should be very cautious in assigning a function to a seta simply on the basis of shape from a scanning electron micrograph.

Completion of this ultrastructural analysis of the sensilla of the 1st tarsus of *A. americanum* provides a basis for behavioral and electro-physiological investigations to elucidate the functions of the various types of receptors. Hopefully this species can serve as a model for the study of other hard ticks. It is

reasonably clear, from the previously reported morphological evidence, that the capsule of Haller's organ contains sensilla that are olfactory chemoreceptors and that the anterior pit sensilla of Haller's organ are of 4 types with probably different functions, including, presumably, humidity detection as postulated by Lees (1948). Other tarsal sensilla are mechanoreceptors, contact chemoreceptors, or a combination of the 2 capabilities. Ticks clearly respond to temperature gradients and it is not known which sensilla respond to that stimulus. The close positioning of many of these sensilla, and the morphological evidence for dual functions in many, necessitate cautious and precise experimental procedures to demonstrate functions.

With the tarsal sensilla of *A. americanum* investigated in depth, one may speculate with extreme caution on the sensory functions of setae viewed by SEM in other species of ticks (e.g., Wooley 1972, Nosek and Sixl 1972) but correlated observations by transmission electron microscopy are highly desirable. Studies are in progress to compare other tick species with the hard tick, *A. americanum*, as well as to the soft tick, *Argas arboreus*.

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