*Integrative Acarology. Proceedings of the 6th European Congress*
*M. Bertrand, S. Kreiter, K.D. McCoy, A. Migeon, M. Navajas, M.-S. Tixier, L. Vial (Eds.)*
*European Association of Acarolgists, 2008*

# FUNCTIONAL MORPHOLOGY OF MECHANORECEPTORS IN ASTIGMATIC MITES

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## Abstract

Although the mechanoreceptor organs of astigmatic mites are used extensively as taxonomic characters, knowledge of their fine structure is minimal. Mechanoreceptors include setiform sensilla and cupules, and light microscopy, scanning electron microscopy and transmission electron microscopy were used to investigate their morphology. Setiform sensilla are movable and arise from a socket of thin procuticle. The setal base is anchored in the socket by radiating suspension fibers that connect the socket to a thin layer of more heavily sclerotized cuticle surrounding the setal base. Two dendrites terminating in tubular bodies are associated with the sensillum. Four pairs of cupules are found on the lateral margin of the opisthosoma. The cupule consists of a thin layer of cuticle (covering membrane) that overlies a "cup-like" depression within the remaining cuticle. A layer of densely-packed, thick fibers radiate dorsally through the "cup", attaching to its sides and centrally associated with a vacuole-like structure. A dendrite containing a single tubular body enters the cup through an opening in the basal cuticle and connects with the vacuole-like structure rather than with the covering membrane. It is postulated that distortions of the covering membrane are relayed through the fibers and thereby compress the vacuole-like structure which in turn deforms the tubular body and stimulates the dendrite. The cupules are thought to be cuticular receptors that register strains in the cuticle caused by gravity, vibrations, movements of the mite, and/or internal changes in pressure.

## Key-words

Cupule, sensillum, seta, slit sense organ, tubular body

## Introduction

Although the functional morphology of mechanoreceptors has been extensively investigated in insects and spiders (see McIver 1975, Barth & Blickhan 1984, Keil 1997, 1998, Barth 2002), they have received comparatively little attention in the Acari with the possible exception of ticks (order Ixodida). Since ticks are larger in size than most other mites as well as extremely important in disease transmission and direct damage to both humans and livestock, they are the most intensively studied of the Acari and therefore provide the most detailed knowledge of acarine sensory structures (Coons & Alberti 1999, de Lillo *et al.* 2004). In comparison, a relatively few studies have been conducted on species in the orders Mesostigmata, Prostigmata and Oribatida, and almost none on species in the order Astigmata. The present paper provides a description of the no pore setiform sensilla (np sensilla) of three species of astigmatic mites and the idiosomal cupules of one species. Since the np sensilla of arthropods are relatively well known and those of astigmatic mites show only minor differences, they are only briefly discussed. The idiosomal cupules, however, differ quite radically from the slit sense organs described for other arachnids, including those of other mites,

and are therefore taken up in more detail.

## Methods and Materials

Specimens of *Hericia janehenleyi* (Family Algophagidae) were collected from sap flux on oak trees (*Quercus* spp.), and specimens of *Naiadacarus arboricola* (Family Acaridae) and *Algophagous pennsylvanicus* (Algophagidae) from water-filled treeholes, all in eastern Virginia, U.S.A. All three species were used for studying setiform sensilla, whereas only *H. janehenleyi* was used for studying cupules.

For observation using phase contrast and interference DIC microscopy, specimens were cleared in Nesbitt's solution and mounted in Hoyer's medium on microscope slides. Since mite cuticle will autoflouresce, slide mounted specimens were also used for confocal microscopy. Confocal imaging was performed on a Bio-Rad Radiance 2100MP equipped with a Nikon TE2000-E inverted microscope and an HeNe laser with an excitation wavelength of 543 *nm*.

For transmission electron microscopy (TEM), the integument of the idiosoma was first ruptured with a minutin nadle to facilitate fixation and specimens then placed in a fixative of 3.5% gluteraldehyde, 2.5% paraformaldehyde, and 2% acrolein in cacodylate buffer (pH 7.4) for 12 h at 4$^{o}$C. After several brief cacodylate buffer rinses, they were post-fixed for 1.5 h at 4$^{o}$C and an additional 1.5 h at room temperature in 1% $OsO_4$ in cacodylate buffer. Specimens were then briefly rinsed in 50% acetone and soaked overnight in a 2% uranyl acetate 70% acetone solution at 4$^{o}$C. Dehydration was completed in acetone, and Spurr's medium used for infiltration and embedding. Thin sections were stained in lead citrate, and TEM was performed on a Zeiss EM 109.

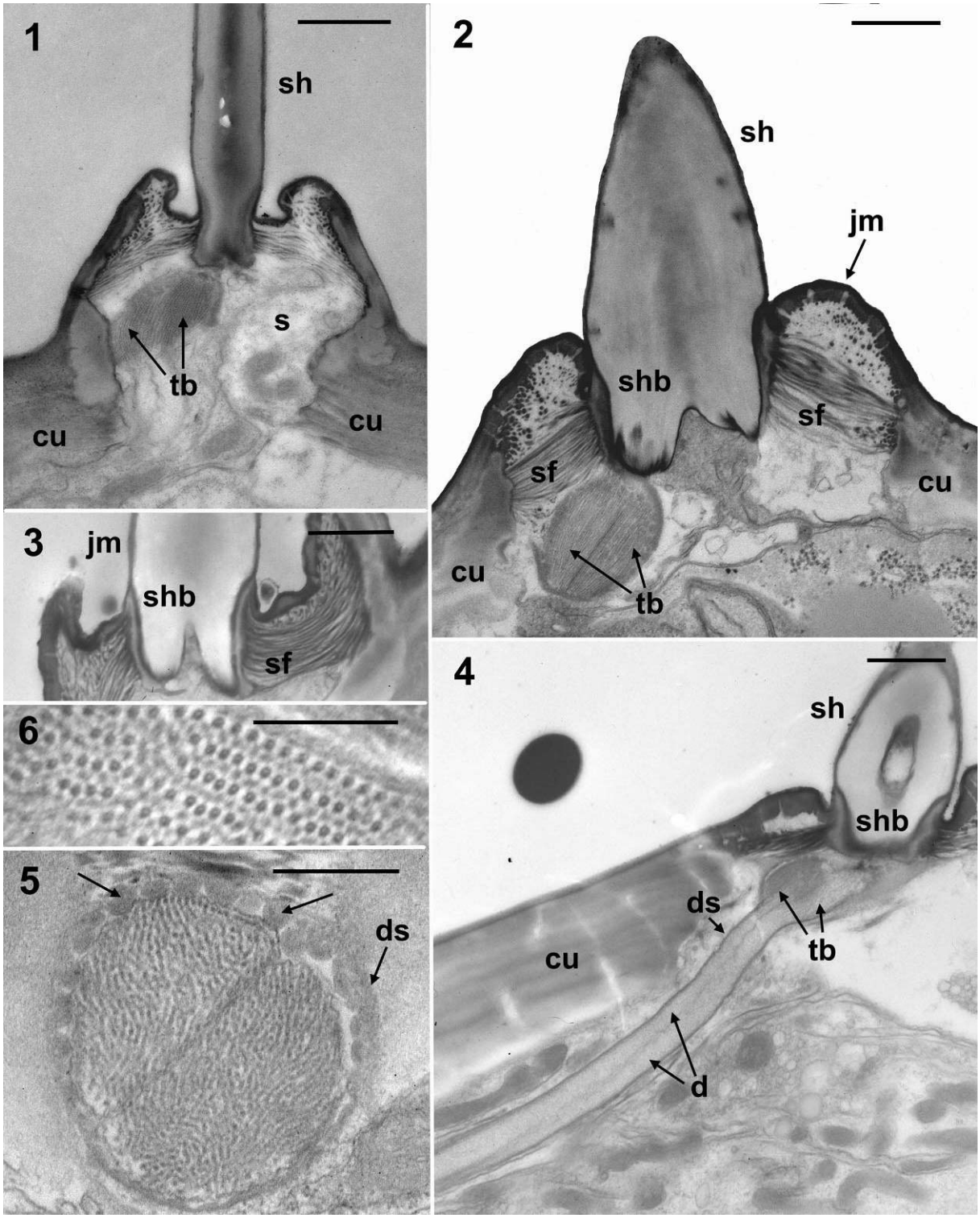
For scanning electron microscope (SEM), living mites were first placed in a bath of distilled water and sonicated at a low frequency. This was repeated several times in an attempt to cleanse them of debris. They were then briefly submerged in distilled water near boiling point in order to force protraction of appendages, dehydrated in ethyl alcohol, dried using the critical point procedure, individually affixed to stubs using double-sided sticky tape, and coated with gold-palladium in a sputter coater. Microscopy was performed on an AMR 1810.

## Results and Discussion

***No Pore Setiform Sensilla***

Detailed descriptions of arthropod setiform sensilla can be found in Altner (1977), McIver (1975) and Keil (1997, 1998), and specifically for mites in Alberti & Coons (1999), Coons & Alberti (1999) and de Lillo *et al*. (2004). From observations in this study, the np sensilla of astigmatic mites appear to be similar in most respects to those found in other arthropods including other mite suborders. They are made up of hair-like or bristle-like setae with a flexible or rigid shaft (fig 1) that is poreless and can vary in size and shape. The setae are distributed on the idiosoma and appendages in a predictable pattern (see Gandjean 1939; Griffiths *et al*. 1990), and are therefore important taxonomic characters. Each seta is located above procuticle that is much thinner than the surrounding cuticle, and is positioned in a flexible socket (= alveolus) (figs 1-4). The seta is anchored in the socket by a surrounding articulation membrane (= joint membrane) as well as by a layer of radiating suspension fibers that attach from the cuticle to a somewhat electron dense layer of procuticle that surrounds the base of the shaft (figs 2-4). Each seta is served by two dendrites and each dendrite terminates in an enlarged tubular body (figs 2, 4) that is tightly packed with microtubules (figs 5, 6). The tubular bodies are surrounded by a dendritic sheath of dense material that inwardly contains conspicuous semicircular bodies (fig 5) a condition observed in other Acari (Alberti & Coons 1999, Haupt & Coineau 1975, Mills 1973). In *Tetranychus urticae* (Tetranychidae) the tubular bodies connect to the articulating membrane (Mills 1973, Alberti & Crooker 1985), and in *Microcaeculus steineri* (Caeculidae) and *Phytoptus avellance* (Eriophyidae) they connect to the base of the seta (Haupt & Coineau 1975, Nuzzaci & Alberti 1996) In some thin sections in our study it appears that one of the tubular bodies connects to the base of the setal shaft and the other to the articulating membrane, however more work is needed to substantiate this. Distinct cell types such as tecagen, trichogen and tormogen could not be discriminated, nor were sheath cells or the cilliary region observed.

Like the np setiform sensilla of other arthropods, those of astigmatic mites are characterized by possessing tubular bodies and are therefore mechanoreceptors. In this regard, they can be stimulated by contact (touch) and movements caused by air and/or water.




**Figures 1-6.** EM micrographs of np setiform sensilla: 1) *A. pennsylvanicus*, longitudinal section of seta and socket; 2) *N. arboricola,* longitudinal section of setal base and socket; 3) *H. janehenleyi*, longitudinal section through base of socket; 4) *H. janehenleyi*, longitudinal section illustrating dendrites with enlarged tubular bodies; 5) *N. arboricola*, cross section of tubular bodies; 6) *H. janehenleyi* enlarged view of microtubules in tubular body. Abbrev.: cu, cuticle; d, dendrite; ds, dendritic sheath; jm, joint membrane; s, setal socket; sf, suspension fibers; sh, setal shaft; shb, shaft base. Unlabeled arrows in figure 5 point to semicircular bodies of dense material adjacent to dendritic sheath. Scale bar = 1 $\mu$m (Figs 1-4), 0.5 $\mu$m (Fig 5), 0.25 $\mu$m (Fig 6).

With the exception of *Phytoseiulus persimilis* (Phytoseiidae) in which a palpal seta was found to have two pairs of tubular bodies, the Acari have been found to have only two tubular bodies (mechanoreceptor cells) (Alberti & Coons 1999), and the astigmatic mites in our study are no exception. Spiders and scorpions, however, have three and seven tubular bodies respectively (Folex 1985). It is interesting that species in the ricinulid genus *Pseudocellus* were found to have only two tubular bodies (Talarico *et al*. 2006), thus lending support to the theory that the Ricinulei are the sister-group of the Acari (Shear 1999).

***Cupules***

Astigmatic mites are characterized by four pairs of small, somewhat circular idiosomal organs designated from front to rear as *ia*, *im*, *ip* and *ih* (Griffiths *et al.* 1990). Their cup-like appearance under light microscopy has led to their designation as cupules by many authors, however, in other taxa they are often referred to as slit sense organs (see Alberti & Coons 1999) and sometimes lyrifissures (e.g., Penman & Cone 1974). Although they may sometimes falsely appear to be openings in the cuticle, their lucent appearance under the light microscope is due to a reduction in the thickness of the cuticle; the epicuticle remains intact and covers the cup. Cupules can be classified as "non-setal sensilla" since there is no seta-like shaft extending above the cuticle surface (Alberti & Coons 1999 ), however they also fit under the "intracuticular receptor" classification of Hess & Vliman (1986). They are considered to be homologous to the lyrifissures (= slit sensilla) of spiders (Alberti 1998), mechanoreceptive organs that have been extensively studied and are therefore well understood both morphologically and functionally (Barth 2002, Barth & Blickhan 1984). Although they are prominent features on the integument, the fine structure of the various intracuticular sense organs has not received much study in the Acari. In this regard, they have been examined in some detail only on the tarsi of the tick *Amblyomma variegatum* (Hess & Vlimant 1984), and to a lesser extent on the chelicerae of a few gamasid mites (Nuzzaci *et al*. 1992, de Lillo *et al*. 1996, 2002), and the opisthosoma of the spider mite *T urticae* (Penman & Cone 1974; Alberti & Crooker 1985) and the oribatid mite *Scutovertex minutus* (Alberti 1998). The following description is based on an examination of the cupules of the algophagid mite *H. janehenleyi*. Cross sections of three individual mites resulted in longitudinal sections of cupules *ih* (3 cupules) and *ip* (4 cupules). Horizontal sections of one individual resulted in cross-sections of all cupules on both sides of the idiosoma (therefore 8 cupules). All cupules were found to have similar morphology.

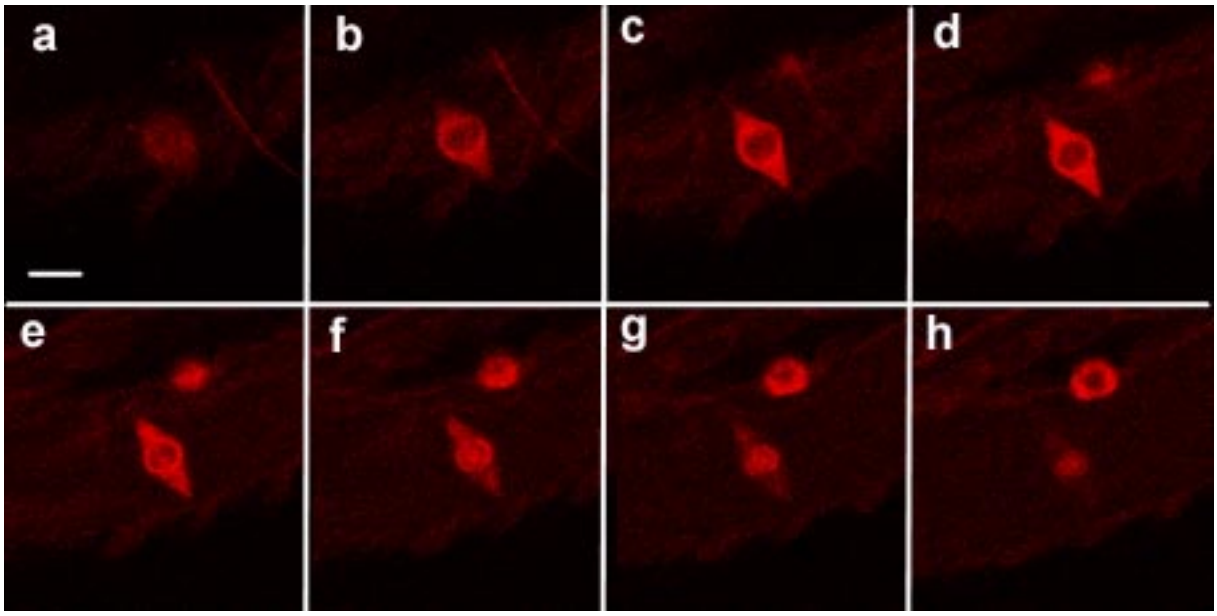
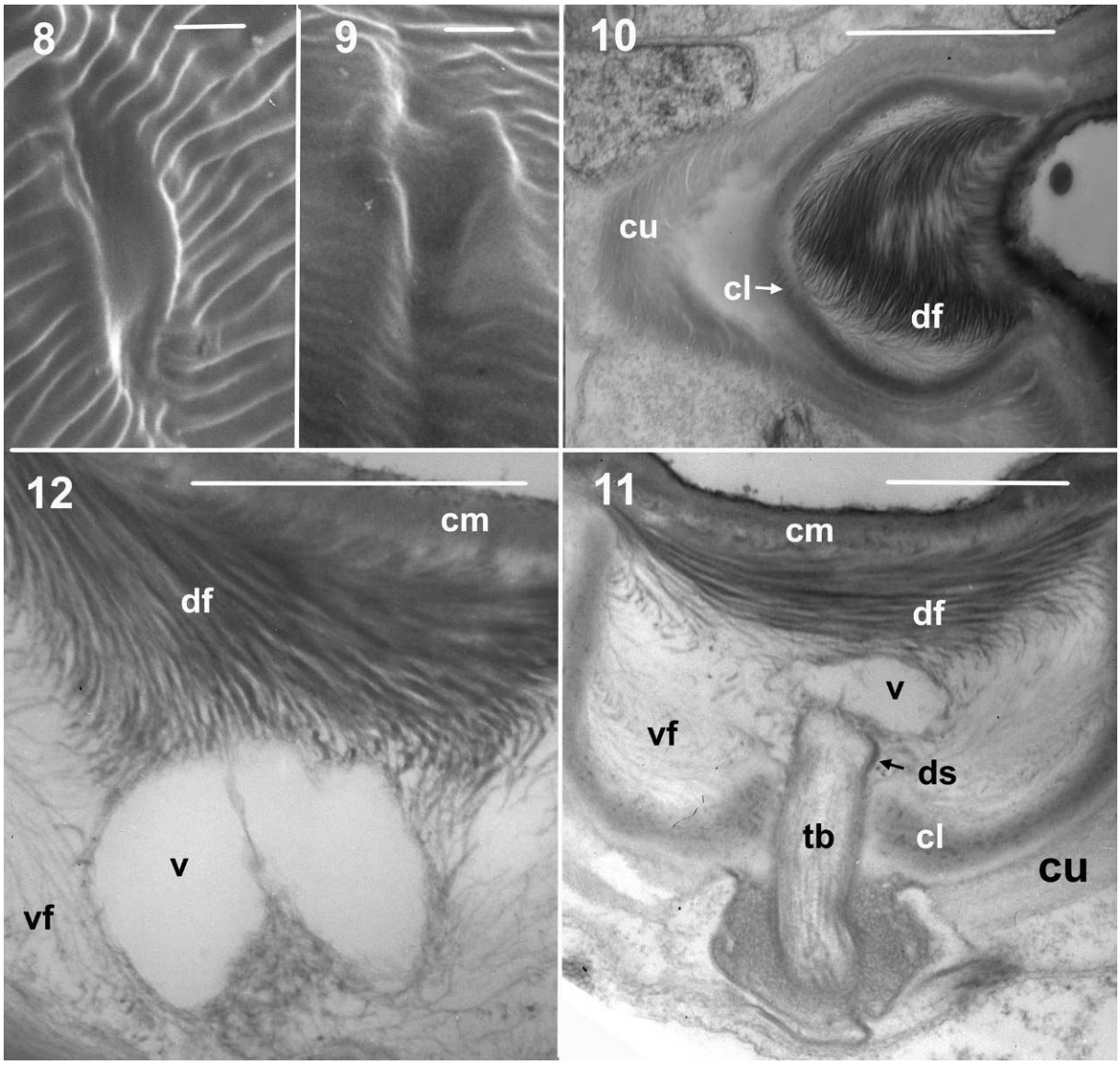



**Figure 7.** Confocal images of cupula *ip* sectioned from dorsum (a) through to ventrum (h). Note the round "cup" in the center and the extensions of electron dense material responsible for the "eye-shaped" appearance under light microscopy. Scale bar = 3 $\mu$m




**Figures 8-12.** EM micrographs of idiosomal cupules of *H. janehenleyi*: 8-9) SEM images of cupules *ia* and *ih* respectively; 10) TEM cross section of dorsal region of cupule *ia* just below the covering membrane; 11) TEM cross section through center of cupula *im*; 12) cross section through vacuole-like structure in center of cupule *im* presumable formed in part by fibers Abbrev.: cl, electron dense material lining cupula; cm, cover membrane; cu, cuticle; df, thick dorsal fibers; ds, dendritic sheath; tb, tubular body; vc, vacuole-like structure; vf, thin ventral fibers. Scale bar = 3 $\mu$m (Figs 8-9), 2 $\mu$m (Fig 10), 1 $\mu$m (Figs 11, 12).

The orientation of cupules is roughly at right angles with regard to the longitudinal axis of the idiosoma. Under phase contrast, interference DIC and confocal microscopy, a cupule appears as a circular central depression with longitudinal extensions of darkened cuticle in the upper layer that taper to a point, thereby giving it an "eye-like" appearance (fig 7). An examination with the SEM corroborates the eye-like appearance, and also reveals that the cupule is convex in its dorso-ventral axis and slightly concave in its lateral axis (figs 8, 9). In addition, the cupule is flanked by small elevations or ridges of the cuticle on its lateral margins (figs 8, 9). An examination with the TEM verifies the above and provides a detailed analysis of the internal ultrastructure (figs 10-15). The cuticle is separated to form a thin (~0.25 $\mu m$) electron-dense, dorsal external covering (= covering membrane) and a thicker, more lucent, internal layer that lines the walls and floor of the cup (figs 11, 13, 14). The circular dorsal portion of the cup measures approximately 3 $\mu m$ in diameter, and the cup depth in the center is approximately 2.5 $\mu m$. Internally, the cup is lined with a somewhat electron dense substance that is presumably more heavily sclerotized cuticle (figs 10, 11, 13, 14). The lining extends past the cup approximately 1 $\mu m$ as it tapers to a rounded point (fig 14), thereby giving the eye-like appearance seen with light microscopy. Dorsally, just under the

cuticular cover, thick, electron-dense, fiber-like strands transverse the cup at its cross-sectional axis (figs 10-14). In the center of the cupule, the fibers appear to form a vacuole-like, lucent structure or structures (fig 12). It could not be determined whether only one branching vacuole-like structure is formed or multiple vacuoles-like structures. A matrix of thinner and less densely packed fiber-like strands, presumably in a fluid, fill the remainder of the cupule. Only one dendrite serves a cupule and its outer segment containing the tubular body with its associated microtubules enters through the cupule center (figs 11, 13, 15).

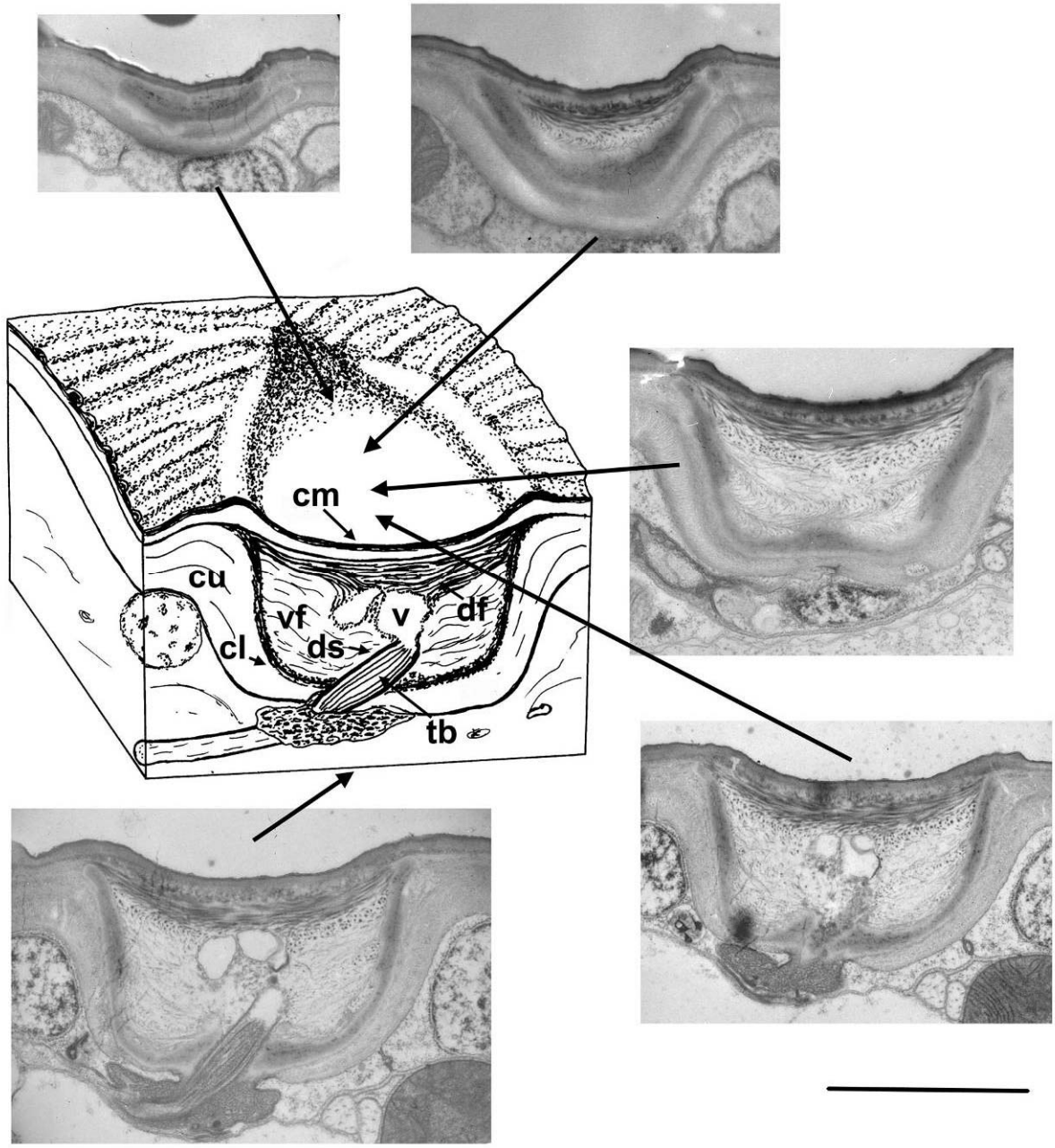



**Figure 13.** Diagram of *H. janehenleyi* cupula reconstructed from TEM cross sections, and TEM micrographs of cross sections. Arrows point to the approximate locations of TEM cross sections. See caption for Figs 8-12 for abbreviations. Scale bar = *2* $\mu$m.

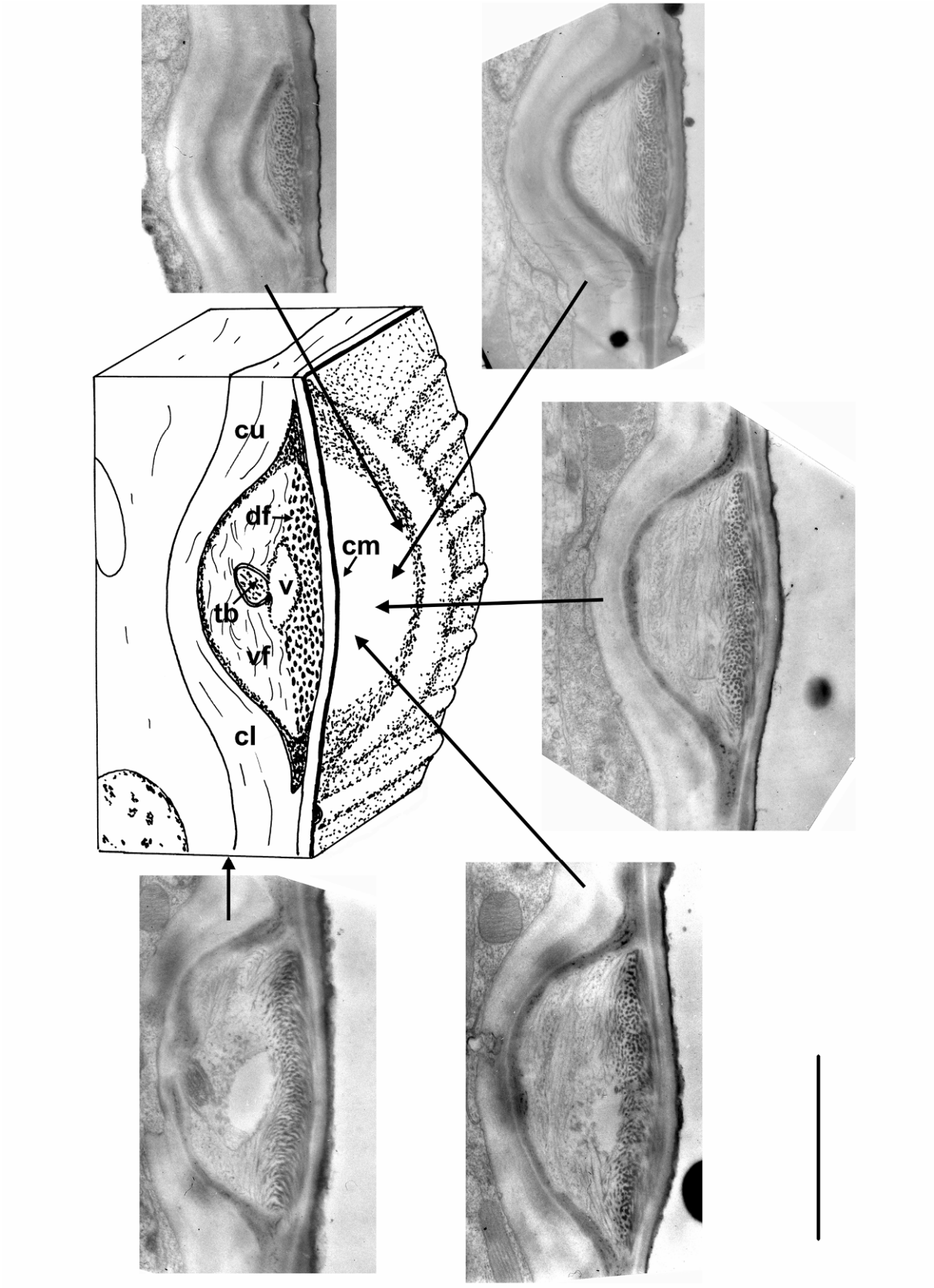



**Figure 14.** Diagram of *H. janehenleyi* cupula reconstructed from TEM longitudinal sections, and TEM micrographs of longitudinal sections. Arrows point to the approximate locations of TEM longitudinal sections. See caption for Figs 8-12 for abbreviations. Scale bar = *2 µ*m.

Just below the cupule at the point of entry, the dendrite passes through an electron dense material that possibly functions to seal the entrance hole (figs 11, 13, 15a). The dendrite does not extend to the cuticular cover, but ends approximately mid-way in the cup and in intimate contact with the "vacuole-like" structure (figs 11, 13, 15b-d). In a number of microtome sections the dendrite terminus appears to expand and actually form part of the vacuole wall (fig 15).

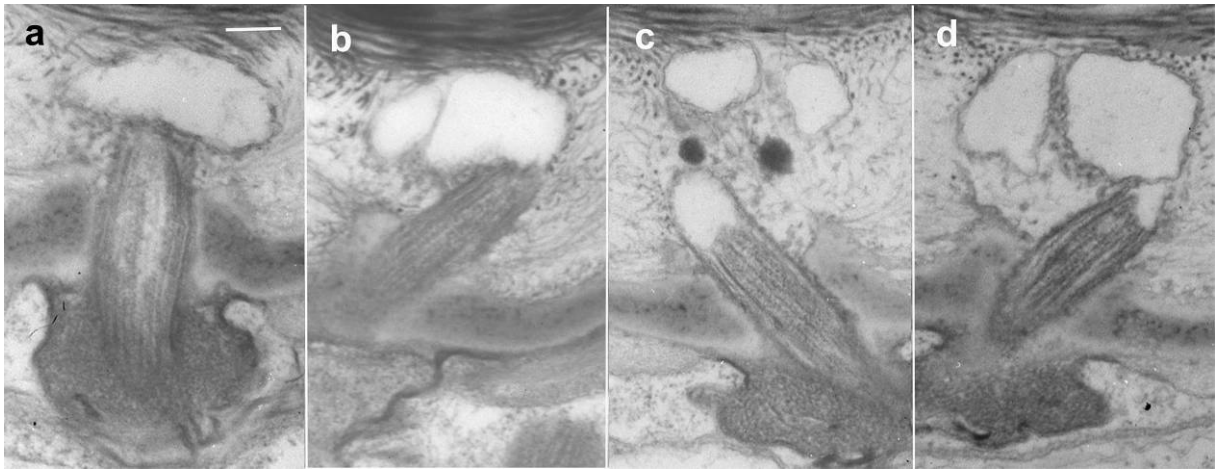



**Figure 15.** TEM micrographs of cross sections of *H. janehenleyi* cupula illustrating the association of the tubular body with the vacuole-like structure. a = cupule *im*, b = cupule *ip*, c, d = cupule *ih*. Scale bar = 0.5 $\mu$m.

Possessing a dendrite with a tubular body, there is no doubt that the cupule functions as a mechanoreceptor that responds to strains in the exoskeleton. It is possible that the longitudinal extensions of more heavily sclerotized cuticle aids in interpreting the directionality of the cuticular deformation. When the cuticle surrounding the cupule is mechanically stressed, the covering membrane is deformed which in turn somehow deforms the tubular body of the dendrite. Since the tubular body does not contact the covering membrane, it is postulated that the deformation of the covering membrane is relayed through the underlying thick fibers, thereby causing the vacuole-like structure to compress which in turn deforms the tubular body and thereby sets off the nervous response. Possible causes of cuticular stress are internal pressures or movements of the mite (proprioception), and/or external forces such as gravity and vibrations caused by substrate or air movement.

The fine structure of idiosomal cupules has been investigated in only two other species, the spider mite *T. urticae* and the oribatid mite *S. minutus*, and studies on both provide only a fragmentary description. The cross sectional images of *T. urticae* cupules published by Penman & Cone (1973) are similar in appearance to those of *H. janehenleyi* in that they have an outer cuticular cover and a cavity filled with loosely packed fibrous material. They did not, however, find a connecting dendrite. Alberti & Crooker (1985) later reported that cupules of *T. urticae* were innervated, but provided no details. In a report on the sensory structures of oribatid mites, Alberti (1998) provided a cursory description of cupules *ia* and *im* of *S. minutus*. He found the cupule to be a narrow portion of modified and flexible procuticle with a small attached cuticular process to which the tubular body of a dendrite purportedly connects. He did not determine the number of receptor cells.

The cupules of *H. janehenleyi* are unlike the cuticular stress detectors described for other arthropods. The tubular body does not connect to the cover membrane as found in the campaniform sensilla of insects and the slit sense organs of other arachnids. Whether this is true for all astigmatic mites and perhaps all actinotrichid mites awaits further investigation.

## Acknowledgements

We thank Jewel Thomas, College of William and Mary, for fixation of the specimens, and Drs. Eric Bradley and John Griffin, College of William and Mary, for discussions on sensory receptors. Research was supported by a SSR Grant awarded

to NJF by the College of William and Mary.

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