NUZZACI G., DE LILLO E., PORCELLI F.*

Functional morphology of the mouthpart sensilla in females of *Varroa jacobsoni* Oudemans (Acari: Varroidae)**

ABSTRACT

The mouthparts of the *Varroa jacobsoni* Oudemans adult female (Acari: Varroidae) have been investigated by means of light microscopy, scanning and transmission electron microscopy revealing specialised sensory receptors on the chelicerae, corniculi and lateral lips. These receptors are described and a functional interpretation on the basis of their ultrastructure is given.

Each chelicera bears two uniporous sensilla on each tip of the digitus mobilis (one on the dorsal part and the other on the ventral one), a dorsal seta and an antiaxial lyrifissure both located on the distal part of each middle article. The uniporous sensilla could act as a contact chemoreceptor, the dorsal seta as a mechanoreceptor and the lyrifissure as a mechanosensitive proprio receptor.

Each corniculus tip bears a supposed uniporous sensillum that could be contact chemoreceptors.

Each lateral lip bears a short cuticular process set on the adoral wall at a slightly distal level with respect to the base of the labrum. Each process is provided with an apical pore. It could be a contact chemoreceptor.

Key words: anatomy, gnathosoma, cuticular sensilla, uniporous sensilla, lyrifissure, dorsal seta, subcapitular setae, ultrastructure.

INTRODUCTION


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The authors have contributed to the setting up of this research, to the analysis and to the interpretation of the observations jointly and in equal measure.

The anatomical observations, previously made on the sensory structures of the females, pointed out different types of sensilla: two uniporous sensilla on the tip of the digitus mobilis (Milani, 1987; Nuzzaci & De Lillo, 1992); a dorsal seta and an antiaxial lyrifissure on the distal part of the second cheliceral article (El-Banhawi, 1984; Akimov et al., 1988; Nuzzaci & De Lillo, 1992); a sensillum on the tip of the corniculus and another one on the adoral side of the lateral lips, both supposed chemoreceptors (Nuzzaci & De Lillo, 1992); chemoreceptive sensilla on the foretarsi (Ramm & Böckeler, 1989).

Up to now, the above mentioned sensilla found on the mouthparts have not been sufficiently described and understood from a functional point of view.

Our aim was to study in detail the morphology of the specialised sensilla arranged on the mouthparts of the adult females of *V. jacobsoni* in order to obtain fine evidences of their kind, on the basis of their ultrastructural resemblance to the previously described structures in Acari and Insects, and to make hypotheses about their functional and ecological-behavioural role.

**MATERIALS AND METHODS**

Adult females of *V. jacobsoni* were collected from sealed cells of an infested colony of *Apis mellifera ligustica* Spinola reared in Bari (Southern Italy).

For light microscopy observations, whole specimens of *V. jacobsoni* were fixed for three hours in Karnowsky's fixative prepared with cacodylate buffer (pH 7.2-7.4) containing 5% sucrose. A drop of Tween 80 (a wetting agent) was added per 100 ml of Karnowsky's fixative to improve the contact between the fixative solution and the mite surface. The specimens were rinsed overnight in several changes of cacodylate buffer and then were immersed in a solution of Crystal violet (5% in distilled water, Baker reagent lot n. E 3349) as suggested by Slifer (1960). After the first four hours, the specimens were periodically examined. Well stained ones were obtained after five or six hours of staining. These were dissected in distilled water and immediately observed and micrographed using a Zeiss III contrast phase microscope.

For transmission electron microscopy observations, some specimens of *V. jacobsoni* were deep-frozen in liquid air and dissected with a scalpel to remove the gnathosoma. This last part was fixed for 4 hours in a solution of 4%
glutaraldehyde in 0.1% M phosphate buffer (pH 7.2) containing 5% sucrose. Moreover, other specimens were fixed in Karnowsky’s fixative for 6 hours. The first specimens were rinsed overnight in several changes of the phosphate buffer, the second ones in the cacodylate buffer. Then they were post-fixed for 3 hours in 1% osmium tetroxide, and rinsed as above. The pieces were dehydrated through a graded series of ethanol, some of them were embedded in Araldite 502 and others in Araldite M and then polymerized at 70°C. The cross sectioning was made using an ultratome III LKB 8800 by a diamond knife starting from the gnathosomal apex. The sections were stained with 5% Uranyl acetate and Lead citrate, observed and micrographed using a Zeiss EM 109 transmission electron microscope.

The description and classification of the observed sensilla were made mainly according to Zacharak (1958), McIver & Siemicki (1975), and Altner & Prillinger (1980).

Explanations of symbols:

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<thead>
<tr>
<th>Abbreviation</th>
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<td>ap</td>
<td>apical pore</td>
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<td>BB</td>
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<td>CU</td>
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<td>DD</td>
<td>distal dorsal sensillum of the digitus mobilis</td>
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<td>dm</td>
<td>digitus mobilis</td>
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<td>DoS</td>
<td>dorsal seta</td>
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<td>DS</td>
<td>dendritic sheath</td>
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<td>DV</td>
<td>distal ventral sensillum of the digitus mobilis</td>
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<td>iD</td>
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<td>outer dendritic segment</td>
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<td>salivary stylus</td>
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<td>tubular body</td>
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RESULTS

SENSILLA ON THE TIP OF THE DIGITUS MOBILIS

On the digitus mobilis, light microscope observations have shown two traces of crystal violet (fig. 1), and scanning electron microscope observations have shown two cuticular processes on the tip of the digit (fig. 2). One process is
on the dorsal side (distal dorsal) and the other is on the ventral one (distal ventral). This latter appears a bit more proximal than the first one (fig. 2).

Both processes appear very reduced, look like two blunt tips and shortly protrude beyond the cuticular surface. Centrally, they show a pit in which there is a pore appearing as a simple round shaped depression (fig. 2).

In a distal cross section, the digitus mobilis shows two separate lumina which are connected to the pores. They appear distally filled with a strongly electron-dense substance (figs 3-4).

In a more proximal level, two groups of several outer dendritic segments are encased in two well distinct dendritic sheaths. The distal part of these outer dendritic segments appear granular, and the microtubules are not visible. In a subdistal level (fig. 5), the dorsal dendritic sheath contains an electron-lucent matrix and is enveloped by a well defined sheath cell.

At the same level and in ventral position (fig. 5) there is a second dendritic sheath which is structurally similar to the dorsal one, except for a coarse electron-dense matrix, and is enveloped by a well defined sheath cell.

In a more proximal level, the dorsal dendritic sheath disappears and the ciliary regions of four sensory cells are contained in the ciliary sinus which appears filled with a moderately electron-dense matrix (fig. 6). The complex of the ciliary regions and of the ciliary sinus seems to be enveloped, at least, by one sheath cell.
Fig. 2 - Scanning electron micrographs of the mouthparts: a) from a dorsal view. Scale bar = 10 μm; b and c) details of the digitus mobilis tip. Scale bar = 1 μm. (by courtesy of dr N. Milani).
Figs 3-4 - Cross sections of the digitus mobilis: 3, at a more distal level than 4. Scale bar = 1 μm.
Figs 5-8 - Cross sections of the chelicera showing the cellular parts of the digitus mobilis sensilla: 5, through the outer dendritic segments; 6, at the ciliary sinus level of the dorsal sensillum; 7, at level of the inner dendritic segments of the dorsal sensillum; 8, at level of the ciliary sinus of the ventral sensillum. Scale bar = 1 μm.
Even more proximally, the presence of four inner dendritic segments has been observed (figs 7-8) in continuation with the dorsal dendritic parts.

At the same level and in a ventral position, one can find an evident ciliary sinus that seems to be produced by the confluence of three sheath cells (fig. 8). This sinus contains many microlamellae and the ciliary regions of five sensory cells (figs 8-10).

A similar structural arrangement has also been observed for the dorsal sensilla in a slightly more distal level.

No additional mechanosensitive dendrite, containing a tubular body, has been observed for these sensilla as reported for some insect gustatory receptors (Altner & Prillinger, 1980; Zacharuk, 1980; Steenbrecht, 1984).

**Dorsal seta**

The seta is paraxially located on the dorsal surface of the distal part of the second cheliceral article. It is about 20 µm long, bent forward and directed towards the tip of the digitus mobilis. The shaft of the dorsal seta is very close to the cheliceral cuticle surface, it has quite thick aperous walls with outer smooth surface and its lumen is devoid of any innervation.

The shaft arises from a flexible socket delimited by a rim (fig. 11). The articulation of this seta within the cuticle consists of a joint membrane.

Radial fibers ("suspension fibers" according to McIver, 1985) completely surround the distal part of the outer dendritic segments, as a cap (figs 12-13).

The outer dendritic segment displays a complex composed of two tubular bodies deepened into the chelicera, parallel to the longitudinal cheliceral axis, encased in a dendritic sheath and innervating the socket region of the seta (figs 12-13). Each tubular body complex consists of a thick and strong electron-dense dendritic sheath. It is elliptical shaped in cross section (fig. 13) and divided by a median septum in two semi-elliptical halves. The septum is interrupted in its middle part and it is not continuous between the two tubular bodies (figs 13-14).

A bundle of about 150 microtubules, forming the tubular body, has been observed inside each half. The microtubules appear immersed in a moderately electron-dense matrix, and the bundle appears surrounded by an electron-lucent area, probably an artifact (shrinkage?) (fig. 14).

In a more proximal level (fig. 15) two ciliary regions are in continuation with the tubular bodies. For one of them, it was possible to count 15 groups of microtubules set in a circle; in the centre of it was not observed any other microtubules.

Neither sheath cells nor microvilli or microlamellae have been recognised.
Figs 9-10 - Cross sections of the chelicera showing the cellular parts of the digitus mobilis ventral sensilla: 9, at level of the ciliary sinus; 10, detail of the inner dendritic segments at a level more proximal than the ciliary constrictions. Scale bar = 1 μm.
Figs 11-13 - Cross sections of the second cheliceral article at the level of the dorsal seta: 11, at base level; 12, 13, detail of the tubular bodies. Scale bar = 1 μm.
ANTIAXIAL LyrIffissure

One lyrifissure is present on the antiaxial surface of the second cheliceral article. It is slightly more distal than the dorsal seta and looks like a small transverse fold on the cuticle (fig. 16) with its long axis set obliquely with respect to that of the chelicera.

The cuticular parts consist of an outer layer, that is more electron-dense than the adjacent cuticle, a middle layer, and an inner apparently fibrous layer, just less electron-dense than the surrounding cuticle (figs 17-18).
Fig. 15 - Cross section of the dorsal seta ciliary regions. Scale bar = 0.5 μm.

On the lyrifissure no pore has been observed. Between the lyrifissure and its surrounding there is not any particular cuticular separation, such as a ring or a rim.

Only one outer dendritic segment connected to the lyrifissure has been observed. It contains a tubular body which is encased in a thick and strong electron-dense dendritic sheath surrounded by the three layers previously described. The tubular body appears surrounded by an electron-lucent area.
Figs 16-20 - Antiaxial lyrifissure: 16, light microscope micrograph from a lateral view; 17, cross section of the chelicera at level of the tubular body; 18, cross section at a level more proximal than 17; 19, cross section at level of the outer dendritic segment; 20, cross section at level of the ciliary region. Scale bar = 1 μm.
In a more proximal level, the dendritic sheath appears thin and irregularly folded. It contains the outer dendritic segment (fig. 19) and is surrounded by a sheath cell in which was observed a multilamellate body.

More proximally a ciliary constriction with microtubules is visible (fig. 20).

No microvilli or microlamellae have been observed.

**Sensillum of the corniculus**

The tip of the corniculus shows a cuticular structure very similar to the sensilla on the tip of the digitus mobilis.

A distal cross section of a lateral lip shows a lumen filled with an electron dense substance, like a plug (figs 21-22).

Proximally the lumen continues with an outer dendritic segment which appears granular (fig. 23), immersed in an electron-lucent matrix (fig. 24) and encased in a well defined dendritic sheath. This latter is enveloped by, at least, two sheath cells (fig. 24).

In a more proximal level, it is possible to distinguish a ciliary sinus containing microvilli, microlamellae and one ciliary region (figs 25-26).

No additional mechanoreceptor unit has been observed.

**Sensillum of the adoral wall of the lateral lips**

A very short and low cuticular ridge protrudes from the adoral wall of each lateral lip; it is located just ahead of the base of the labrum, and extends into the preoral groove.

It is not articulated and is provided with a terminal pore set inside a very reduced pit (fig. 27).

More proximal sections, cut at first longitudinally (figs 28-29) and then transversally (fig. 30), show a lumen which appears filled with an electron dense substance.

At a more proximal level, a group of four outer dendritic segments is recognizable. They are immersed in an electron-lucent matrix and encased in a well defined dendritic sheath whose border is irregularly folded (fig. 31).

In a more proximal level, a ciliary sinus, filled with a coarse matrix, contains the ciliary regions of four sensory cells (fig. 32).

Even more proximally, it is possible to observe the presence of four inner dendritic segments, which are in continuation with the above described dendritic parts. At this level the ciliary sinus appears to be produced by, at least, one sheath cell with many microlamellae and microvilli (fig. 33).

No additional mechanoreceptor unit has been individuated.
Figs 21-22 - Cross sections of the corniculus at a distal level: 21, slightly more distal than 22. Scale bar = 1 μm.
Figs 23-26 - Cross sections of the cellular parts of the corniculus sensillum: 23, at a distal level; 24, at level of the outer dendritic segment; 25, at level of the ciliary region; 26, slightly more proximal than 25. Scale bar = 1 μm.
Figs 27-28 - Sagittal sections of the distal part of the sensillum set on the adoral wall of the lateral lip: 27, detail at the level of the pore; 28, sensillum at a more proximal level. Scale bar = 1 μm.
Figs 29-30 - Sections of the distal part of the sensillum set on the adoral wall of the lateral lip: 29, sagittal at the tip of the sensillum; 30, slightly transverse at a more proximal level than 29. Scale bar = 0.5 μm (fig. 29) and 1 μm (fig. 30).
**SUBCAPITULAR SETAE**

They resemble structurally the dorsal seta with a socket innervated by two sensory cells which are provided with their tubular bodies.

**DISCUSSION AND CONCLUSION**

The structures observed on the tip of the digitus mobilis and of the corniculus could be considered an uniporous sensillum (TP-sensillum of STEINBRECHT, 1984; thick-walled sensillum of SLIFFER, 1970) (figs 34-35).

Whilst chemoreceptor activity has not yet been recorded from the uniporous sensilla of the digitus mobilis and from the sensillum of the corniculus by means of electrophysiological observations, their ultrastructural evidence, such as the presence of a terminal pore and of a viscous plug, the apical

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**Fig. 31** - Cross section of the sensillum set on the adoral wall of the lateral lip at the outer dendritic segment level. Scale bar = 1 μm.
Figs 32-33 - Cross sections of the cellular parts of the sensillum set on the adoral wall of the lateral lip: 32, at level of the ciliary sinus; 33, at level slightly proximal than 32. Scale bar = 1 μm.
settlement of the sensilla on the digitus mobilis, the absence of tubular bodies, for a gustative function is strong.

As far as one knows, a referable structure has not been described in other Acari; in the Tick *Boophilus microplus* (Canestrini) (Ixodida: Ixodidae) first the ultrastructural and then the electrophysiological results have shown the presence of a specialised tip pore sensilla on the inner digit which have partly a chemoreceptive function (*Waladde & Rice, 1977*).

Moreover, from the ultrastructural evidences, a chemoreceptor sensillum has been recently pointed out in the stylets of *Tetranychus urticae* Koch (Tetranychoidea: Tetranychidae) (*Nuzzaci & De Lillo, 1991*) and a suspected chemoreceptor structure has been observed in the stylets of *Cenopalpus pulcher* (Canestrini & Fanzago) (Tetranychoidea: Tenuipalpidae) (*Nuzzaci & De Lillo, 1989*).

These structures probably could be involved mainly in the feeding mechanisms.

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*Fig. 34 - Schematic representation of the uniporous digitus mobilis sensilla.*

*Fig. 35 - Schematic representation of the uniporous sensillum set on the corniculus.*
The number of the sensory cells found inside the distal dorsal sensillum (four) corresponds to most of the insect's taste hairs (Altner & Prillinger, 1980; Steinbrecht, 1984); the other two chemoreceptor sensilla have a more unusual arrangement with five and one sensory cells.

This kind of sensilla looks very much like the taste hairs of multimodal sensillum fields on cockroach and cricket palps where, such as in this case, an additional mechanoreceptor unit is missing (Steinbrecht, 1984).

Thus it could be thought that during the feeding activity, the uniporous sensilla of the digitus mobilis and corniculus are introduced through the wound on the honey bee integument and immersed in the host haemolymph.

The position of these sensilla on the mouthparts and the particular shape of their very reduced cuticular parts could allow a chemical communication between the dendrites and the external environment, as already reported for similar sensilla in some insects (Steinbrecht, 1984). It seems that they are well suited for providing a sensory feedback to the Mite from the internal host fluids.

The ultrastructure of the dorsal seta is in agreement with the description of known cuticular mechanosensillum belonging to the hair shaped type, called sensillum chaeticum (Schneider, 1964), and it is a no-pore sensillum, according to Steinbrecht (1984) (fig. 36).

The dorsal seta and the subcapitular setae correspond very much, both in cuticular and cellular components, to a mechanoreceptive sensillum and the basis of such setae is innervated by two receptor cells similar to other Arachnida (fig. 36) (McIver, 1975).

The position of the dorsal seta and the possible feeding mechanism make

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Fig. 36 - Schematic representation of the dorsal seta.
this seta well suited for monitoring the mechanical interactions between the chelicerae and the honey bee’s integument during the attachment procedure.

The presence of a tubular body, which is a specialised mechanosensitive dendritic ending (Thurm, 1964), the absence of a pore, and a structural similarity with the Insect campaniform sensillum (Thurm, 1964; McIver & Siemicki, 1975, McIver, 1985), with the distal tarsal slit sense organ of *Amblyomma variegatum* F. (Hess & Vilmant, 1984) and with the Araneid slit sense organ (Barth, 1972), suggest that the antiaxial lyrifissure, placed on the distal part of the second cheliceral article, may function such as a mechanosensory proprioceptor (fig. 37).

From a functional point of view, the lyrifissure could perceive the strains in the cuticle causing deformations of the cuticular fold such as occurs in some
Insect campaniform sensillum and where it is most likely related with the flexibility of the cuticle (Thurm, 1964; McIver, 1985). These deformations could be transmitted to the layers of the lyrifissure and so to the tubular body.

Because the tendons of the depressor and levator muscles of the digitus mobilis are at the same level of the lyrifissure, it could be supposed that this mechanoreceptor could also detect the muscular strain involved in the action of the digitus mobilis.

At last, morphologically speaking the sensillum placed on the adoral wall of lateral lips, located just ahead of the mouth, appears structurally similar to the chemoreceptive sensillum (fig. 38).

This sensillum could be involved in the gustatory activity (Steinbrecht, 1984) and could taste the host fluid a bit before this substance enters into the pharynx.

A similar structure has been observed also in Cenopalpus pulcher (Can. & Fanz.) (Nuzzaci & De Lillo, 1989) and in Tetranychus urticae Koch (Nuzzaci & De Lillo, 1991).

Our investigation has revealed the presence of some specialised sensilla, very probably related to the feeding mechanisms, on the mouthparts of the adult females of V. jacobsoni. They are:

- two uniporous sensilla on the tip of the digitus mobilis,
- a dorsal seta on the distal part of the second cheliceral article,
- an antiaxial lyrifissure on the distal part of the second cheliceral article,
- an uniporous sensillum on the tip of the corniculus,
- an uniporous sensillum on the adoral wall of the lateral lips,
- three pairs of subcapitular setae.

Their functions, supposed on the basis of their ultrastructure, need to be ascertained by means of electrophysiological studies.

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RIASSUNTO

MORFOLOGIA FUNZIONALE DEI SENSILLI DELLE PARTI BOCCALI IN FEMMINE ADULTE DI
VARROA JACOBSONI OUDEMANS (ACARI: VARROIDAe)

Lo studio delle parti boccali delle femmine adulte di Varroa jacobsoni Oudemans (Acari: Varroidae) mediante il microscopio luce, il microscopio elettronico a scansione e quello a trasmissione ha permesso di rilevare la presenza di strutture sensoriali specializzate situate sui cheliceri, sui corniculi e sulle labbra laterali. Di questi sensilli viene data una descrizione e vengono formulate ipotesi funzionali sulla base delle caratteristiche ultrastrutturali. Tali ipotesi si basano sulle analogie con i sensilli già descritti e di funzione nota in insetti e acari.

Ogni chelicero è provvisto di due sensilli unipori in corrispondenza dell’apice del digitus mobilis, di una setola dorsale e una lirifissura situati sulla parte distale del secondo articolato chelicerale. Si ritiene che i due sensilli del digitus mobilis possano agire da chemiorecettori di contatto, la setola dorsale da meccanorecettore e la lirifissura da propriorecettore.

In base alle caratteristiche ultrastrutturali, il sensillo situato all’apice dei corniculi può essere ritenuto di tipo uniporo e come tale potrebbe essere un chemiorecettore di contatto.

Ogni labbro laterale è provvisto di un corto processo cuticulare situato sulle pareti interne in posizione poco distale rispetto alla base del labrum. Questo processo è dotato di un poro apicale e potrebbe agire da chemiorecettore di contatto.

Parole chiave: anatomia, gnatosoma, recettori, sensillo con poro terminale, lirifissura, setola dorsale, setole subcapitulari, ultrastruttura.

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