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SCANNING ELECTRON MICROSCOPIC EXAMINATION OF THE PUTATIVE OLFACTORY STRUCTURES POSSESSED BY THE PHORID FLY, *Megaselia halterata* (DIPTERA, PHORIDAE)

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Abstract

The antennae, palps, and mouth parts sheath of *Megaselia halterata* (Wood) (Diptera, Phoridae) were examined by scanning electron microscopy to locate putative olfactory sensilla (POS). Most POS were found on the third antennal segment (Johnston's organ). These POS included a lanceolate peg, a bulbous peg, and two types of pit sensilla. Female flies could be distinguished from males by the predominance of the lanceolate peg on the female Johnston's organ. One type of pit sensillum with a single exterior opening was located below the cuticular surface and housed several pegs. The other type of pit sensillum was domed with a single exterior opening, housed a single peg, and the lower half of this sensillum was embedded into the wall of the Johnston's organ. This type of pit sensillum was also found on the sixth antennal segment of both sexes. A bulbous peg was found on the palps of both sexes. No POS were found on the mouth parts sheath. Specimens were prepared in the traditional manner for scanning electron microscopy examination. Also specimens were embedded in Paraplast and sections of the Johnston's organ clarified the internal structure and distribution of the pit sensilla on this organ.

Key Words: *Megaselia halterata*, Phoridae, Diptera, antennae, palps, Paraplast, sectioning, scanning electron microscopy.

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Introduction

The majority of olfactory chemoreceptors are located on the antennae of insects (Schneider and Steinbrecht, 1968; Keil and Steinbrecht, 1982). These receptors are involved in the insect's search for suitable sites for such functions as egg laying, feeding, and seeking the opposite sex for mating purposes. The attraction of the insect pest, *Megaselia halterata* (Wood) (Diptera, Phoridae), to the volatiles from the commercial mushroom, *Agaricus bisporus* (Lange) Imbach (Fungi, Agaricaceae), has been investigated (Pfeil and Mumma, 1991, 1993). Female *M. halterata* were strongly attracted to mushroom compost, whereas, mated male flies were not as strongly attracted and unmated males were not attracted. Female flies were attracted to mushroom compost for the purpose of egg laying (Hussey, 1961). With regards to mating, female flies release a male-alluring sex pheromone at four days after emergence from the pupal case stage (Richardson and Chanter, 1979). A component of the pheromone has been identified as 3, 6-dimethylheptan-2, 4-dione (Baker *et al.*, 1982). The adult flies were small (1-2 mm in length) and moved in a stop-and-go behavior. This behavior is characteristic of the Phoridae and is hypothesized as a mechanism for the phorid fly to process information collected from the fly's receptors about the external environment (Miller, 1979).

To better understand the behavior of *M. halterata*, the antennae, palps, and mouthparts sheath of *M. halterata* were examined by scanning electron microscopy (SEM) to locate putative olfactory sensilla (POS). Also the differences between the sexes regarding POS were investigated.

Materials and Methods

A culture of *Megaselia halterata* was established in the laboratory from a collection of flies taken from a commercial mushroom house in Kennett Square, PA. The fly culture was maintained using mushroom compost colonized by the commercial mushroom.

Using the traditional method, whole flies were anesthetized and killed with CO_2 and separated according to

sex. Flies were transferred to specimen-processing carriers and fixed in 3% glutaraldehyde in 0.15 M sodium cacodylate buffer, pH 7.1 [Electron Microscopy Sciences (EMS), Fort Washington, PA]. After discarding the fixative, the flies were washed three times with double distilled water and dehydrated through a gradient series of ethanol which included three baths (five minutes each) in absolute ethanol. The samples were critical point (CP) dried from liquid CO_2 (CP: 31.5°C and 7.5 x 10^{-6} Pa) using a Polaron E3000 critical point drier. Dried samples were mounted onto aluminum stubs with double stick tape and silver paint and sputter coated with 56 nm of gold under vacuum in an International Scientific Instrument (ISI) PS2 Sputter-Coater (Topcon, Pleasanton, CA).

The second method adapted the technique of using paraffin-embedded sections to precisely locate internal structures (Gaudet and Kokko, 1984). Whole flies were separated according to sex, fixed in formalin-acetic acid-ethanol (1:1:18) for 4 hours at room temperature (27°C) and for 4 hours at 4°C. Samples were dehydrated through a gradient series of tertiary butyl alcohol (TBA) including three changes in 100% TBA, one of which was overnight. Samples were then transferred to 50:50 paraffin oil:TBA for 2 hours. One-half of the mixture was decanted and replaced with melted Paraplast Plus (EMS, Fort Washington, PA) and placed in an oven at 58°C to start the infiltration series. Four additional decantings were made at 30 minute intervals; after which time most of the liquid paraplast was replaced with fresh paraplast and stored at 58°C overnight and embedded the following day. Blocks were stored at 4°C and sectioned on an American Optical/Spencer 820 rotary microtome (A/O Co., Buffalo, NY) at a thickness of 20 μm. Slips with sample attached were deparaffinized in xylene for 24 hours, rinsed in two changes of 100% ethanol, and air dried. Samples were mounted on stubs and sputter-coated with 28 nm of gold as described above. Samples were examined in an ISI-60 SEM operated at an accelerating voltage of 10 kV, 8 mm working distance, and at tilts varying from 10° to 45°. Photomicrographs were taken with a Polaroid Land 545 camera using Polaroid 52 film (ISO-400).

Results

The antennae, palps, and the mouth parts sheath of both sexes of *M. halterata* were examined by SEM (Figure 1). The POS were referred to by common nomenclature as suggested by Zacharuk (personal communication, 1994).

Johnston's Organ (Third antennal segment)

Several different sensilla were observed on the Johnston's organ. The male fly possessed a bulbous peg (Figure 2) throughout the entire Johnston's organ interspersed with numerous microtrichia. The bulbous peg appeared to collapse readily (Figure 2); the bulb was thin-walled, and its lumen was connected to the interior of the Johnston's organ (Figure 3). The collapse of this sensillum probably occurred during the preparation process. The female fly possessed bulbous pegs only in the basal portion of the Johnston's organ where this sensillum was interspersed with microtrichia and a few lanceolate pegs (Figure 4). Lanceolate pegs were predominant in the middle to distal portions of the female Johnston's organ (Figure 5) but absent in the male.

Both sexes of flies had domed pit sensilla with a single external pore occurring in pairs, on the lateral, outer sides of the Johnston's organ (Figure 6). The base of this sensillum was embedded into and below the wall of the Johnston's organ (Figure 7). The peg housed in the dome had a pitted surface along its distal portion. The basal portion of each pit sensillum may have been capped-off as seen in Figure 8. Additionally subcuticular pits with single external openings were observed on the surface of the Johnston's organ (Figure 9). Each pit housed numerous pegs which easily collapsed and possessed thin walls and a spacious lumen (Figure 10). Numerous subcuticular pits were located around the base of the Johnston's organ of both sexes (Figure 11).

Sixth antennal segment, palps, and mouth parts sheath

The sixth antennal segment in both sexes possessed a pair of domed pits (Figure 12). This sensillum had a single external opening and housed one short peg (Figure 13).

Bulbous pegs seated in circular sockets (Figure 14)

Figures 1-6 on facing page 689.

Figure 1. Frontal view of male *Megaselia halterata* head. J: Johnston's organ, P: palp, M: mouth parts sheath, CE: compound eye; bar = 100 μm.

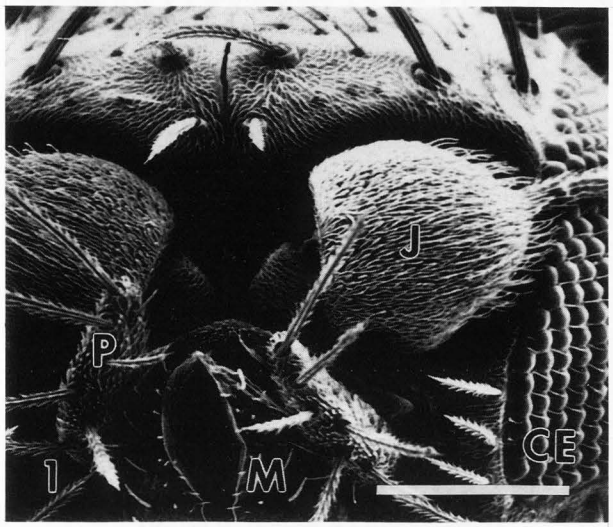
Figure 2. Basal portion of male Johnston's organ. A: bulbous peg, CP: trichoid cuticular projection; bar = 10 μm.

Figure 3. Longitudinal-section of bulbous peg (A). bar = 1 μm.

Figure 4. Basal portion of female Johnston's organ. A: bulbous peg, B: lanceolate peg; bar = 1 μm.

Figure 5. Middle portion of female Johnston's organ. B: lanceolate peg; bar = 1 μm.

Figure 6. Domed pit sensilla on lateral (outer) side of female Johnston's organ; bar = 1 μm.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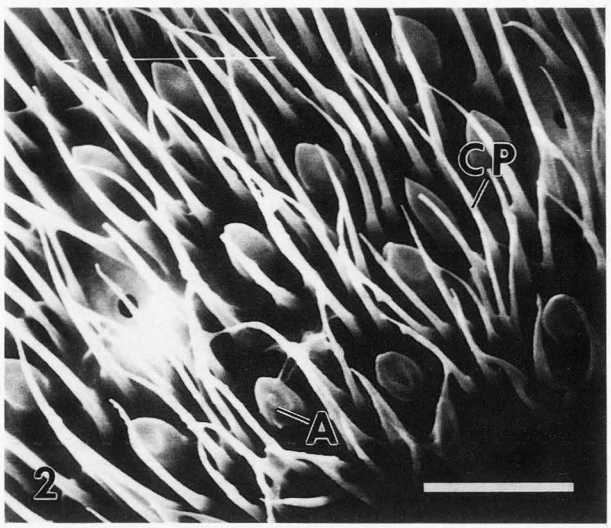
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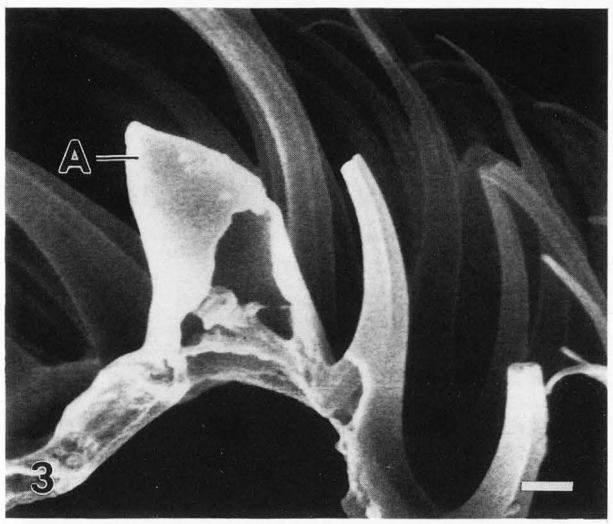
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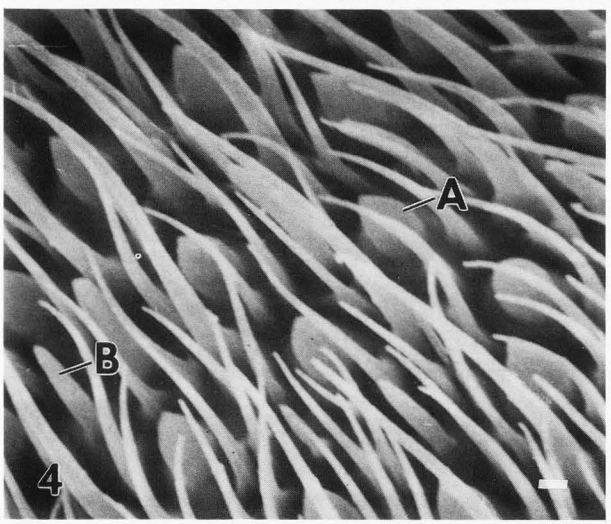
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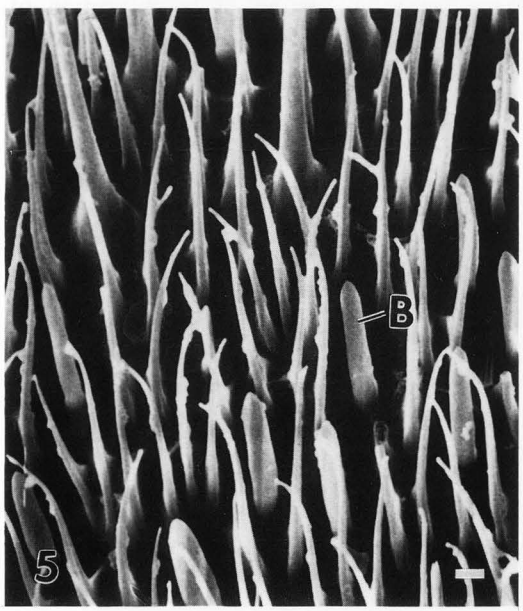
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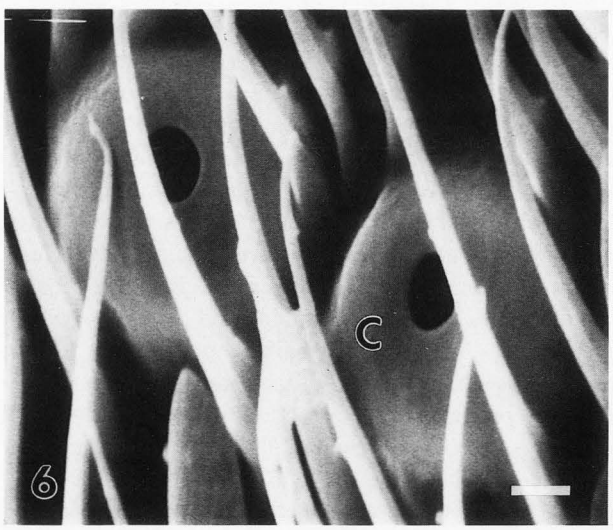
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Table 1. Description and distribution of putative olfactory structures in *Megaselia halterata*.

Letter	Description	Structure Location	Distribution Male	Distribution Female
A	bulbous	Johnston's organ	entire surface	basal portion only
B	lanceolate	Johnston's organ	not present	mid-distal portion
C	domed pit	Johnston's organ	present	present
D	subcuticular pit	Johnston's organ	present	present
E	domed pit	sixth antennal segment	present	present
F	bulbous	palp	present	present

were observed interspersed with numerous trichoid projections on the ventral surface of each palp of both sexes (Figure 15).

Microtrichia and potential mechanoreceptors were visible on the mouth part sheath.

Discussion

Several morphological features of these sensilla strongly suggest that these sensilla are olfactory in function. The occurrence of the sensilla only on the antennae and palps also indicates their possible involvement in olfaction, as has been demonstrated in other insects (Schneider and Steinbrecht, 1968). The lanceolate sensilla are similar to the types, A1-A3, found on the antennae of *Simulium rugglesi* (Mercer and McIver, 1973), the basiconica sensilla on the funicles of the onion fly, *Hylemya antiqua* (Honda *et al.*, 1983), and the multiporous sensilla described on the horn fly, *Haematobia irritans irritans* (White and Bay, 1980). The bulbous pegs are similar to the bulb organs, and pegs seated in circular sockets, on the palps of the mosquitoes, *Culex territans* (McIver and Charlton, 1970) and *Wyeomyia smithii* (McIver and Hudson, 1972), respectively. Kellogg (1970) performed electrophysiological tests on the sensilla basiconica, and the pegs on the palps, of *Aedes aegypti* and demonstrated that these sensilla were sensitive to changes in relative humidity and carbon dioxide, respectively. The lumen in bulbous peg appears to be continuous with the interior of the Johnston's organ suggesting a protoplasmic connection. Additionally, the bulbous pegs were collapsible indicating a thin, perhaps permeable, wall of the sensillum. The pegs in the subcuticular pit sensillum were thin-walled and possessed a large lumen as well. The pitted surface on the distal portion of the peg in domed sensilla suggests possible permeability to chemicals through these pits.

The antennae of *M. halterata* possessed the most diverse group of POS among the three cephalic appendages examined by SEM (Table 1; Figure 15). The Johnston's organ (third antennal segment) had bulbous and lanceolate pegs on the surface, domed pegs with the basal portions submerged below the cuticular surface, and pits which were subcuticular (Figure 15). Domed sensilla also occurred on the 6th antennal segment. No other antennal segment besides the Johnston's organ and the 6th segment possessed POS.

Figures 7-12 on facing page 691.

Figure 7. Tangential section of domed pit sensillum demonstrating pit sensillum (S), and a single peg with a pitted distal portion housed in the pit (El). O: circular opening to pit; bar = 1 μm.

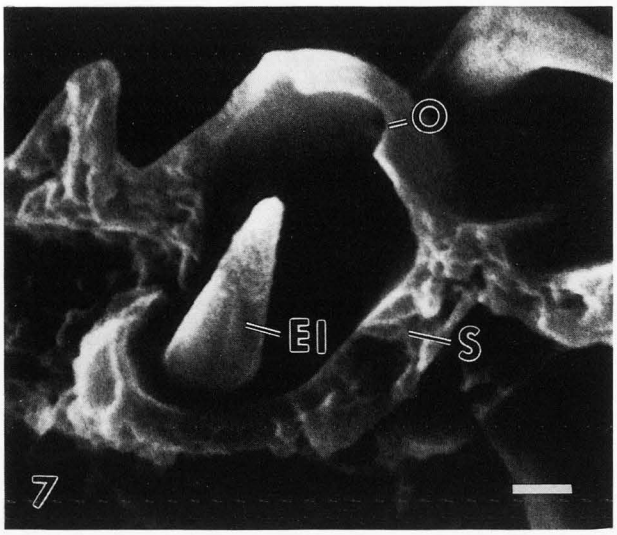
Figure 8. Interior view of cuticular wall of the Johnston's organ illustrating bases of the domed pits (Ba); bar = 10 μm.

Figure 9. Longitudinal section of subcuticular pit containing several pegs (El). O: opening in cuticle to pit; bar = 1 μm.

Figure 10. Cross-section of subcuticular pit containing a cluster of pegs (El). Pegs were thin-walled and collapsible; bar = 1 μm.

Figure 11. Tangential section of basal portion of Johnston's organ indicating the positions of several multipeg subcuticular pits (D); bar = 10 μm.

Figure 12. Domed pit sensilla (E) on the 6th antennal segment; bar = 1 μm.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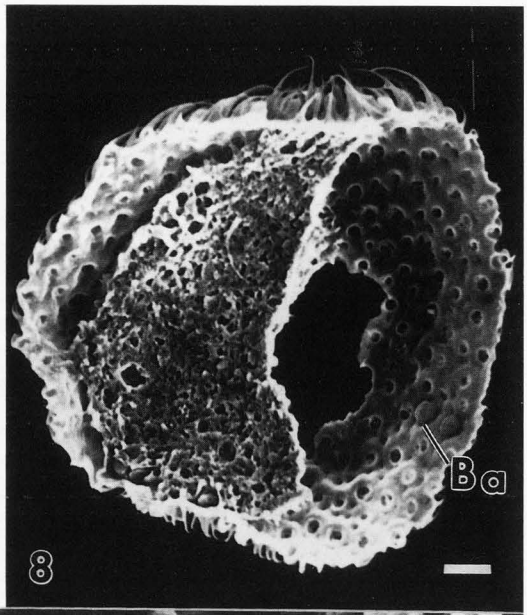
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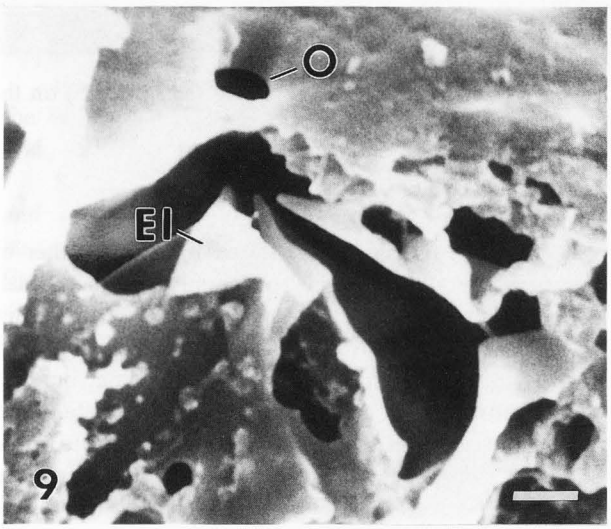
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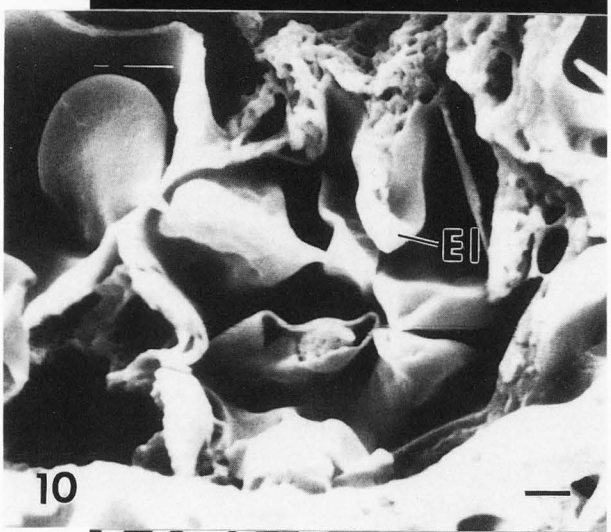
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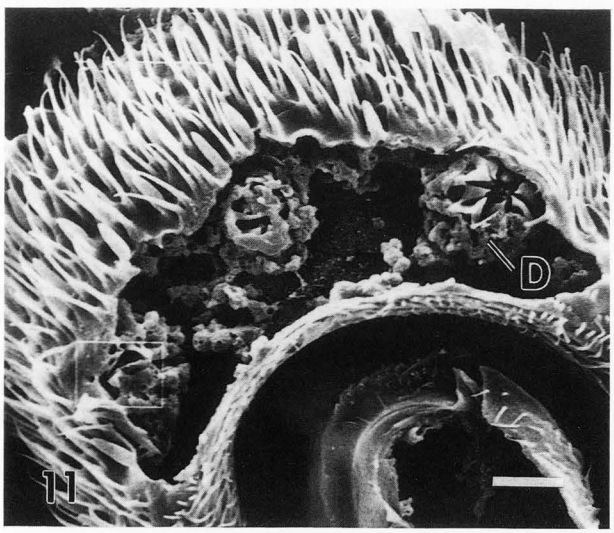
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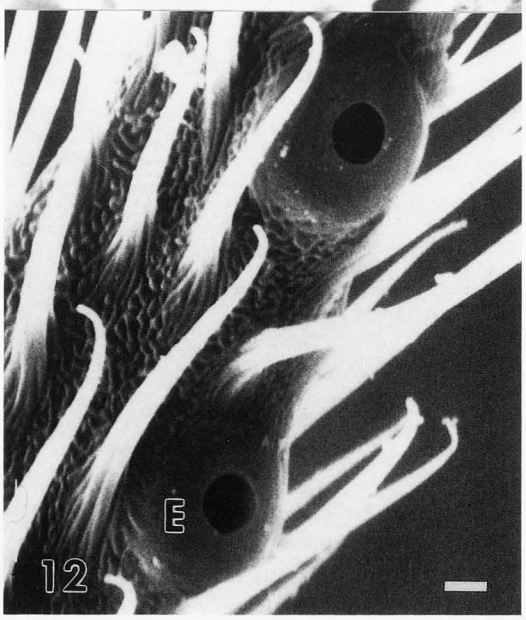
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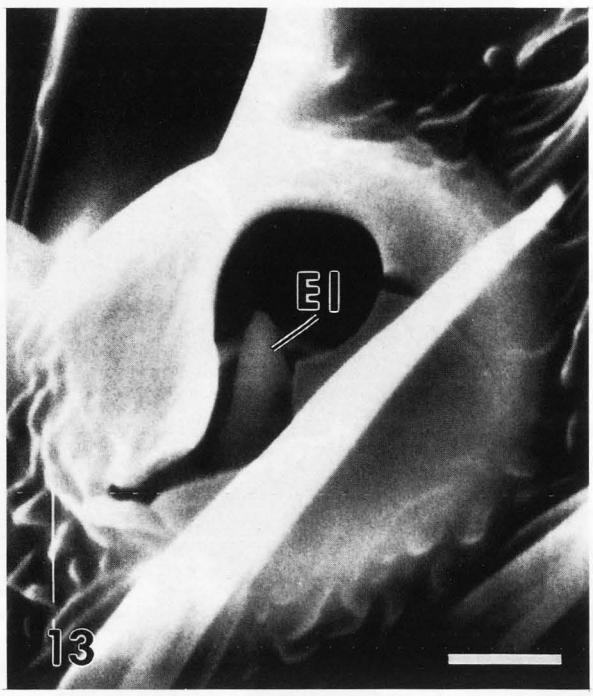

Figure 13. Fractured pit sensillum (structure E) revealing presence of a single peg (El); bar = 1 μm.

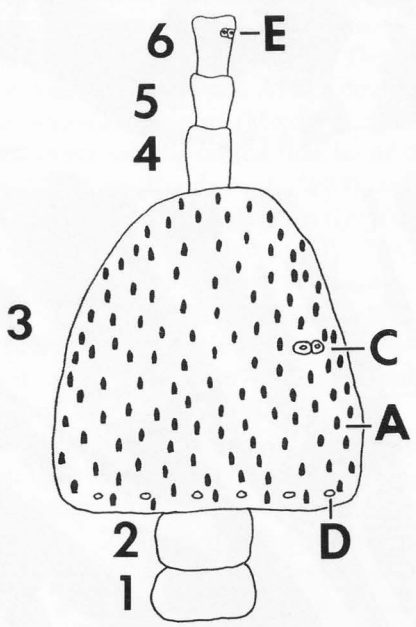

Figure 15. Relative positions of POS on male antenna. A: bulbous peg; C and E: domed pits, and D: subcuticular pits; antennal segments are numbered; figure not drawn to scale.

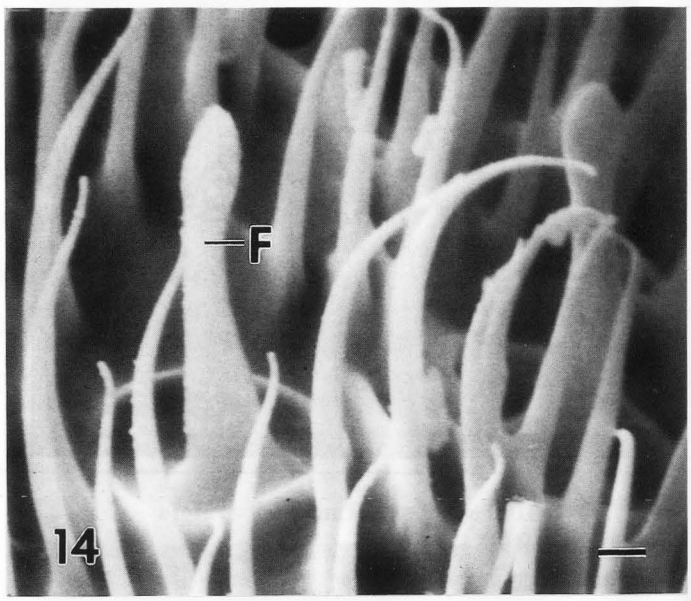

Figure 14. Individual bulbous peg (structure F) on the palp; bar = 1 μm.

Male and female flies differed from each other by the presence of lanceolate pegs only on the female Johnston's organ (Table 1). Also the sexes could be distinguished by the distribution of the bulbous pegs over the length of the Johnston's organ in the male, but only over the basal portion of this organ in the female. Both sexes possessed the domed, single peg pit sensilla on the Johnston's organ and the sixth antennal segment and the multi-peg pit sensilla. The bulbous peg was found on the palps of both sexes also.

The traditional preparation of biological samples for SEM limits the study to surface sensilla only. Attempts to locate POS on antennal segments necessitated the removal and careful manipulation of the head and antennae. The second method of sample preparation produced serial sections through the entire fly. Sections of the Johnston's organ gave rise to a better understanding of the internal structure and distribution of the pit sensilla not possible by scanning of the cuticular surface.

Establishing the olfactory sensory function of the described POS would entail transmission electron microscopy (TEM) and single cell electrophysiology (EP). The number and type of neurons innervating the observed POS, the nature of their dendritic terminations, and the porosity of the sensory cuticles of these sensilla could be established by TEM. Inserting electrodes into the small pegs and the small size of the flies make EP studies difficult.

Acknowledgements

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Discussion with Reviewers

E. Kokko: Why do the authors not employ established nomenclature for sensilla description (e.g., sensilla basiconica, sensilla coeliconica, sensilla trichodea, sensilla chaetica)?
Authors: We do not know if the POS are innervated which would indicate that the POS were chemoreceptors. The identification of sensilla are based on several characters such as the number of neurons innervating the POS, thickness of the wall, etc. Because we did not have these types of data, we are not able to identify with any certainty the types of sensilla present in this phorid fly. We did suggest the type of receptors as done in the **Discussion** based on a comparison of the POS to published sensilla which have been identified.

E. Kokko: What evidence supports the conclusion that the "lanceolate" sensilla identified in Figures 4 and 5, viewed in profile, are indeed lanceolate? Are they not probably cylindrical?
Authors: Until now this point was not brought up. Either these POS are lanceolate in native state or a preferential collapse occurs in the cylindrical structure during sample preparation. If these POS are cylindrical in their natural state, then the POS described as "lanceolate" must have collapsed along the same plane to create the lanceolate appearance. Otherwise, we should see the 'blade' plane at different angles such as 45° but we do not see this occurring.

E. Kokko: The "peg (EL)" referred in Figure 9 looks like debris. What is the nature of the 'peg' and what is it supposed to be associated with?
Authors: Within each pit there are approximately 10-12 pegs. Each peg may be a POS. In Figure 9, we are looking down onto the cross-section of the pegs of this pit. The debris is probably the cellular components of a particular peg which was enclosed by the peg wall.

W.T. Wcislo: Are there qualitative and quantitative differences in sensilla among other *Megaselia* species?
Authors: We do not know of these differences in other *Megaselia* species, however, in mosquitoes, there are large differences (McIver, 1982, J. Med. Ent. **19**: 489-535).

J.J. Ruffolo: Are some of the various POS described here more likely than others to be olfactory receptors in a strict sense rather than contact chemoreceptors?
Authors: Probably not, because contact chemoreceptors are structurally distinct from the POS described here and usually occur on the tarsi and ovipositor.

J.J. Ruffolo: Is it feasible and potentially useful to examine POS by light microscopy of semi-thin sections?
Authors: Semi-thin sections were made and viewed with light microscopy but the depth of field was a problem. The sections examined confirmed the presence and location of Structure D around the base of the Johnston's organ. The method of Silfer and Brescia (1960. Entomological News, Vol. **LXXI**, pp. 221-225) was also attempted on the whole antennae of *M. halterata* to visualize pores in the surface of the POS. Pores were not observed, however, the use of this method did lead to the discovery of the POS on the sixth antennal segment.

J.J. Ruffolo: Can you speculate on how the sex differences in POS frequency and distribution might correlate with behavioral differences?
Authors: We do not know enough about the chemical communication system of this fly to be able to speculate.