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Porous or non-porous? The challenge of studying unusual placoid sensilla of *Megaphragma* wasps (Hymenoptera, Trichogrammatidae) with electron microscopy

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Abstract

Extreme miniaturization implies a high degree of optimization, rendering the retention of non-functional organs almost impossible. Two unique non-porous placoid sensilla on the antennae of females of *Megaphragma* were described in the literature. Placoid sensilla in Hymenoptera have an olfactory function and always bear pores; the apparent absence of pores therefore raises the questions whether such sensilla are functional in *Megaphragma* and whether their surface sculpture had been sufficiently well examined. We examined in detail the external microsculpture and internal ultrastructure of the placoid sensilla using Focused Ion Beam Scanning Electron Microscopy and Scanning Electron Microscopy with various types of sputtering and show that these sensilla actually have a porous cuticle and are innervated by 11 or 12 neurons with branched cilia, which is typical of olfactory sensilla. Comparison of various methods of electron microscopy allows us to conclude that for an accurate determination of the morphofunctional types of sensilla, especially in miniature insects, it is necessary to study both the internal ultrastructure of the sensilla and their external morphology using carefully selected scanning electron microscopy methods.

Keywords

antenna, SEM, sensilla, sputtering, TEM, Trichogrammatidae

Introduction

Placoid sensilla are common in Hymenoptera and are always multiporous (Chiappini et al. 2001; Romani et al. 2010). It has been shown that in *Apis mellifera* Linnaeus they have an olfactory function (Lacher 1964). They are involved in complex processes such as detecting airborn chemicals related to communication (deliberate or unintended) within a species or among species, specifically, in host location in chalcidoid females (Baaren et al. 2007).

The third flagellomere of the female antenna of the parasitic wasp *Megaphragma* (Hymenoptera: Trichogrammatidae) bears two peculiar placoid sensilla (PS), described as having no pores (Diakova et al. 2018), which would fundamentally distinguish them from other hymenopteran placoid sensilla and raise doubts about their possible sensory function. Since *Megaphragma* wasps are extremely miniaturized insects, the presence of non-functional sensory organs in them is unlikely.

The purpose of this study was to perform a comprehensive analysis of the external and internal ultrastructure of the PS in *Megaphragma amalphitanum* using FIB-SEM and various SEM methods to determine if the placoid sensilla really do not have pores.

Materials and methods

Adult females of *Megaphragma amalphitanum* Viggiani, 1997 were reared from eggs of *Heliothrips haemorrhoidalis* (Bouché, 1833) (Thysanoptera: Thripidae).

The study of the external morphology and microsculpture of the placoid sensilla (PS) was performed by Focused Ion Beam Scanning Electron Microscopy (FIB-SEM) and Scanning Electron Microscopy (SEM). The SEM used was a Jeol JSM-6380 (accelerating voltage (AV) 20–30 kV, working distance (WD) 10–20 mm) and FEI Inspect F50 (AV 10 kV, WD 5–10 mm), following fixation, dehydration, critical-point drying, and gold sputtering of the specimens (Giko IB-3, sputtering thickness 20–25 nm; for more details see Diakova et al. (2018)). Additionally, we studied samples prepared according to the same protocol but with chromium sputtering (Cressington 208HR, sputtering thickness 15 nm, orbital rotation with an inclination of up to 45°) using an FEI Quattro S microscope in the environmental SEM mode (ESEM, AV 5–10 kV, WD 10 mm, Pressure 200 Pa, water vapor) or gold sputtered under the same conditions and using the same microscope, but in the high vacuum mode (HV, AV 8–15 kV, WD 5–10 mm).

The analysis of internal ultrastructure was based on the three-dimensional electron microscopy data obtained from a custom FIB-SEM (Zeiss Merlin scanning electron microscope with a Zeiss Capella focused ion beam), following fixation of the samples, en block staining, and embedding in EPON (for more details, see Polilov et al. (2021)).

The three-dimensional reconstruction was performed using the Bitplane Imaris software. All structures were manually segmented using the "Surfaces" function. The resulting reconstructions were processed in the Blender program (reduction of the number of polygons, smoothing of artifacts, rendering).

Results and discussion

The earlier examination (Diakova et al. 2018) and our re-examination of the external microsculpture of the PS after sputtering with gold 20–25 nm thick using a Giko IB-3 system did not reveal any pores, and irregularities of sputtering were found on the cuticle surface (Fig. 1B, C).

Study of the internal structure of the PS showed the presence of the poorly discernible pores with a slight relief (less than 0.01 μm), 0.12 ± 0.02 μm deep 0.10 ± 0.02 μm in diameter (mean ± SD) (Fig. 1H, I, J). Structurally similar bell-shaped pores with a smooth relief were described in females of the parasitic wasp *Anagrus atomus* (Hymenoptera: Mymaridae), in which their presence was detected using TEM (Chiappini et al. 2001). The ventral and dorsal PS are innervated by 11 and 12 bipolar neurons, respectively, the dendrites of each of them branching many times (Fig. 1H, I, J). Dendritic branches are located on the sides of the PS under the cuticular ledges; pores are also located on the sides; in the central portion of the sensillum pores are absent, distinguishing these PS from most of the previously described multiporous PS, in which dendrites occupy the entire cavity of the sensillum and pores are present over the entire surface of the sensillum (Chiappini et al. 2001; Romani et al. 2010). The PS protrude apically from the cuticle of the flagellomere (Fig. 1A) and, as seen in cross section (Fig. 1J), form outgrowths, in which the arrangement of dendritic branches and pores is preserved. For PS, no auxiliary cells (tecogenic, tormogenic, or trichogenic) were found, which is most probably a consequence of the extreme miniaturization of the wasp. Since auxiliary cells perform important functions, including support, nutrition and secretion of the cuticle of the sensillum, it can be assumed that their disappearance takes place after the formation of the PS. Such a reduction in sensillum cells was not previously known and requires further, detailed study.

The re-examination of PS surface after sputtering with chromium made it possible to detect pores externally (Fig. 1D, E). Chromium sputtering is very smooth and even, but due to the complex relief and numerous articulations, the samples were heavily electrically charged, so we had to use ESEM mode to obtain high-quality images. Sputtering to produce a thin layer of gold with an orbital rotation and inclination help us to solve the problem of the sample charging, but the sputtering surface in this case is much less even (Fig. 1F, G).

Thus, the small size of the pores and their slight relief, together with the unevenness of the sputtering or its excessive thickness, can make the pores indistinguishable. Barlin and Vinson (1981) had already noted that the pores in the PS of chalcid wasps may not be evident in scanning electron micrographs of the cuticular surface. Our results confirm that for unambiguous conclusions about the absence of the pores, and, consequently, determination of the morphofunctional types of the sensilla, it is necessary to study the internal structure of the sensilla and/or very thoroughly study the surface using better electron microscopes.

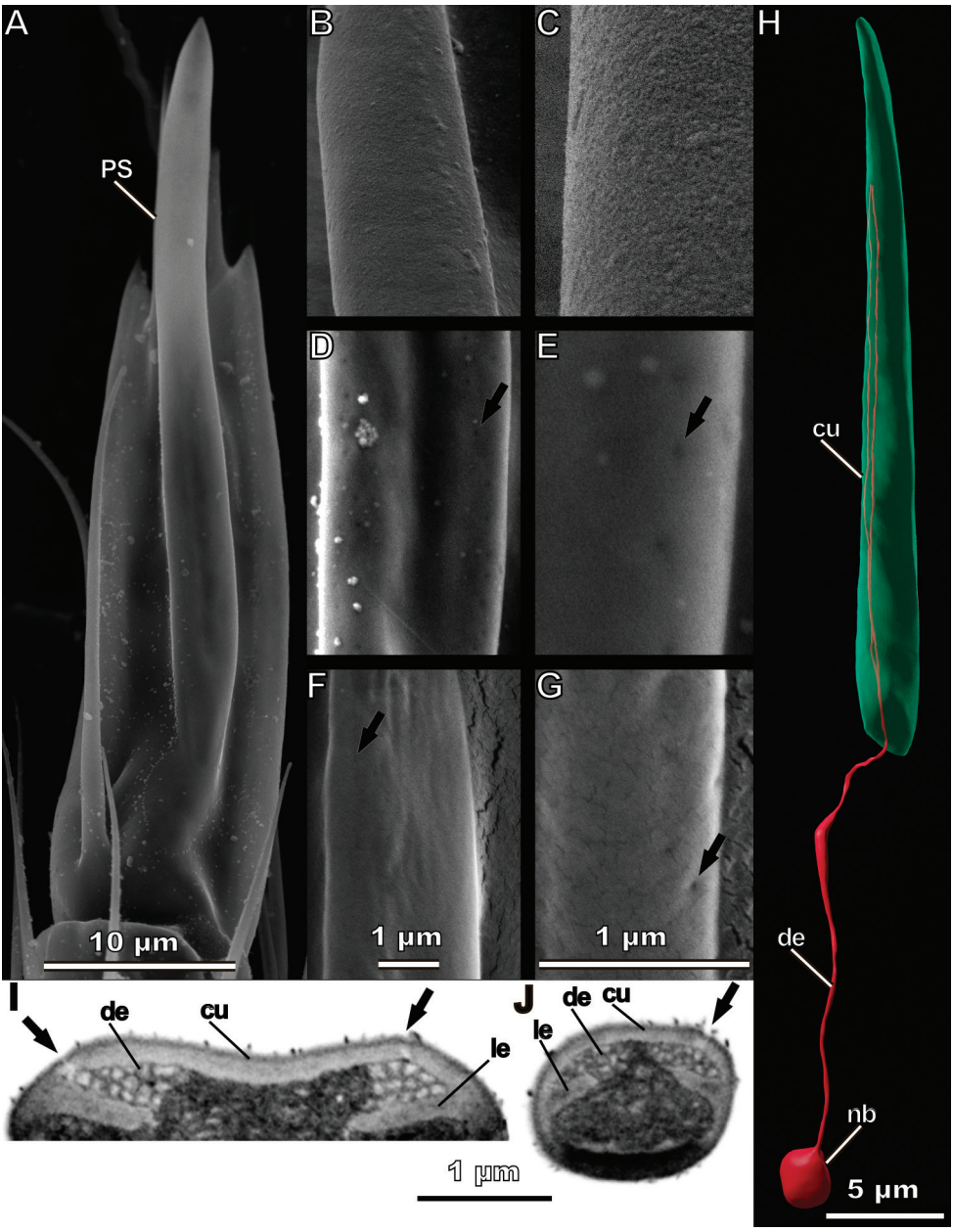

Figure 1. Structure and ultrastructure of unusual placoid sensella (PS) in female *Megaphragma amalphitanum* **A–G** SEM **H** 3D-reconstruction **I, J** FIB-SEM **A** third flagellomere bearing PS **B, C** PS wall sputtered with gold, sputtering thickness 20–25 nm **D, E** PS wall sputtered with chromium in ESEM mode **F, G** PS wall sputtered with gold, sputtering thickness 15 nm, orbital rotation with an inclination of up to 45° **I** cross section through the middle of PS **J** cross section through the outgrowth of PS **H** three-dimensional reconstruction of PS and one of the sensory neurons. Abbreviations: cu – cuticle, de – dendrite, le – ledge, nb – neuron body. Arrows designate the locations of the pores.

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