The sensory structures of the antennal flagellum in *Hyalesthes obsoletus* (Hemiptera: Fulgoromorpha: Cixiidae): A functional reduction?

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ABSTRACT

Despite their relevance as harmful pests on plants of economic importance, Hemiptera Fulgoromorpha have been poorly studied as regards their antennal sensory structures. In particular, the flagellum has been neglected and, therefore, to date there are no data on its structural organization and sensory equipment. In order to fill this gap, we carried out a study on the sensillum types and distribution on the flagellum of the planthopper *Hyalesthes obsoletus* Signoret, an efficient vector of the stolbur phytoplasma, the cause of various crop diseases. In this cixiid species the antenna is composed of three segments, the scape, an enlarged pedicel and a long flagellum. This latter is made of a single segment and presents a basal, bulb-like enlargement from which two processes arise, a short spur and a long arista. Combining scanning electron microscopy, transmission electron microscopy and focused ion beam investigations, we discovered the presence of a total number of 6 sensilla, belonging to 4 different types: a single scolopidium extending from the bulb to the arista, three sensilla styloconica within the cuticular spur and two different sensilla coeloconica inside the bulb. As far as structural data can suggest, these sensilla might be involved in the perception of mechanical stimuli (possibly air-borne vibrations), temperature and humidity variations and CO_2 concentration. The strong reduction in sensillum number in this species is discussed as possible functional specialization of the flagellum itself. The ultrastructure of the sensilla in the flagellum of a species of Fulgoromorpha is here presented for the first time.

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1. Introduction

Cixiidae, one of 21 families of Hemiptera Fulgoromorpha, are composed mainly of phytophagous species feeding on plant phloem (Bourgoin et al., 2004). Comprising about 160 genera and 2000 species, the family is distributed in all zoogeographical regions (Holzinger et al., 2002), species richness being higher in the tropics. Some cixiid species are considered to be pests of economically important crops, acting as vectors of plant pathogens such as viruses, bacterium-like organisms and phytoplasma.

Hyalesthes obsoletus Signoret (Homoptera, Fulgoromorpha, Cixiidae) is a palaearctic species of economic importance, since it is reported to transmit a phytoplasma known as Bois Noir (BN) or Vergilbungskrankheit (VK) to grapevine (*Vitis vinifera* L.) (Maixner, 1994; Maixner et al., 1995). *H. obsoletus* can acquire the phytoplasma during the post-embryonic development while feeding on the roots of several wild plants. Different preferred plant species

have been recorded in different countries or geographic areas [*Convolvulus arvensis* L. and *Urtica dioica* L. in Germany (Langer and Maixner, 2004); *Lavandula hybrida* Reverchon and *Lavandula angustifolia* Miller in France (Sforza et al., 1999); *Vitex agnus-castus* L. in Israel (Sharon et al., 2005)]. The winged adults then move to other host plants and, occasionally, to grapevine. Here, through feeding punctures, adults of *H. obsoletus* transmit the disease, which causes discoloration and necrosis of leaf veins and leaf blades, downward curling of leaves, lack or incomplete lignification of shoots, stunting and necrosis of shoots, abortion of inflorescences and shrivelling of berries. Although some aspects of the biology and behavior of this pest are known (Leclant, 1968; Alma et al., 1987; Sforza et al., 1998; Darimont and Maixner, 2001; Langer et al., 2003), there is still scant information on the mechanism that drive both host and mate selection. In particular, there are no data on the antennal sensory equipment of this insect, despite the crucial role that the antennae play in insect's host location and recognition (Anderson et al., 2000; Isidoro et al., 2001; Kristoffersen et al., 2006), as well as mating behavior (Bartlet et al., 1994; Romani et al., 2008). Antennal sensilla have been proved to be involved in the perception of different kind of stimuli in different insect orders (Altner and Loftus, 1985; Keil, 1999; Kristoffersen

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et al., 2008). As regards the Hemiptera Fulgoromorpha, descriptions of the external morphological features of antennal sensilla carried out using scanning electron microscopy techniques are reported in the literature (Marshall and Lewis, 1971; Bourgoin and Deiss, 1994; Liang, 2002). Besides the TEM investigation carried out by Lewis and Marshall (1970), the most comprehensive study was made on the delphacid *Nilaparvata lugens* Stal, a serious pest of rice in Asia. For this species, the ultrastructural features of the antennal sensory system and the electrophysiological response (EAG) to volatile organic compounds (VOCs) from the host plant were investigated (Aljunid and Anderson, 1983; Nam Youn, 2002). In the above mentioned studies, morphological investigation was focused on putative olfactory sensilla located on the second antennomere (i.e. the pedicel), while no data are available as regards the long, thread-like flagellum. As part of our ongoing study on the sensory bases of host plant location mechanisms in leafhopper vectors of phytoplasma, we need first to map and characterise antennal sensilla of *H. obsoletus*. Because of the lack of data in the literature regarding the antennal flagellum of leafhoppers, the goal of this study was to characterise the flagellum sensilla of *H. obsoletus*. We will present data showing how, in this species, the flagellum has undergone a strong reduction in sensillum number related to a possible functional specialization.

2. Materials and methods

2.1. Insects

Adults of *H. obsoletus* were caught on nettle in some vineyards of the Ancona district (Italy), using a modified leaf blower (Tanaka Togyo Co., THB-2510) in which the intake port was fitted with a fine mesh organza bag. Captured specimens were positioned in a cage (Bugdorm-I, Megaview) with wet paper and some fresh plant host parts (nettle), until arrival at the laboratory.

2.2. Scanning electron microscopy (SEM)

Ten individuals of each sex were used for the observations. Insects were anaesthetized using CO_2, then, individuals were dissected removing the antennae from the head capsule. In some cases the whole head with the antennae in their natural position was detached from the rest of the body. Specimens were dehydrated in a series of graded ethanol, from 50% to 99%. After dehydration, 99% alcohol was substituted with pure HMDS (Hexamethyldisilazane, Sigma®) and the specimens were allowed to dry under a hood, at room conditions. On each aluminum stub 5 specimens were mounted, taking care to place them with different orientations in order to obtain a clear view of the ventral, dorsal and both lateral sides. Mounted specimens were gold-sputtered using a "Balzers Union® SCD 040" unit. The observations have been carried out using a scanning electron microscope Philips® XL 30.

2.3. Focused ion beam technique (FIB)

Specimens prepared for the SEM were used for the FIB technique. We applied this technique to investigate internal structures within the enlarged base of the flagellum. For the experiments, we used an FEI® Helios NanoLab. This apparatus is capable of selectively ablating a previously marked region of the sample using a focused ion beam of gallium. The gold sputter-coated specimen was inserted into the chamber of the machine and the part of the antenna to be ablated was placed eucentrically, since the SEM electron beam is tilted 50° with respect to the ion beam. The FIB was operated at 1 μm depth and 0.96 pA current. A rectangular area (10 μm × 5 μm) was drawn over the specimen, and the milling

process was started and checked in real-time. Once the internal structures appeared, SEM pictures were taken approximately every 100 nm with an operating voltage of 5 KV and an applied current of 0.17 nA. An animated GIF representing the milling process, based on 8 SEM pictures, is added as supplementary file.

2.4. Transmission electron microscopy (TEM)

Ten individuals of each sex were anaesthetized with CO_2 and immediately immersed in a solution of glutaraldehyde and paraformaldehyde 2.5% in 0.1 M cacodylate buffer + 5% sucrose, pH 7.2–7.3. The flagellum was detached from its base to aid fixative penetration, and left at 4 °C for 2 h. The specimens were kept at 4 °C overnight in 0.1 M cacodylate buffer + 5% sucrose, pH 7.2–7.3, then the specimens were post fixed in 1% OsO_4 (osmium tetroxide) for 1 h at 4 °C and rinsed in the same buffer. Dehydration in a graded ethanol series was followed by embedding in Epon–Araldite with propylene oxide as bridging solvent. Thin sections were taken with a diamond knife on an LKB® "Nova" ultramicrotome, and mounted on formvar coated 50 mesh grids. Finally, the sections were investigated with a Philips® EM 208, after staining with uranyl acetate (20 min, room temperature) and lead citrate (5 min, room temperature). Digital pictures (1376 × 1032 pixels, 8b, uncompressed greyscale TIFF files) were obtained using a high resolution digital camera MegaViewIII (SIS®) connected to the TEM.

3. Results

3.1. General description of the antenna

In *H. obsoletus*, the antennae are located laterally on the head capsule, instead of being inserted frontally like in numerous insects. Each antenna is inserted just below the compound eye in a manner that the long, thread-like flagellum points toward the external side (Fig. 1A). In both male and female, the antenna is about 750 μm long, and is composed of three segments: a short scape connecting the antenna with the head capsule, a stout cylindrical pedicel and the flagellum (Fig. 1B). The pedicel is about 150μm long, and is covered by numerous sensilla trichodea and placodea (Fig. 1B). The flagellum consists of a single antennomere of about 500μm length. This segment can be divided into a proximal "bulb" and a distal arista (Fig. 1C). The bulb is proximally inserted on the pedicel at the level of a disk-like, flattened, smooth area. This area is encircled by rings of short cuticular spines, concentrically arranged (Fig. 1C). At the socket level, the bulb is quite narrow (Ø about 5.5 μm), then it becomes larger and assumes the shape of a flask (Figs. 1C and 3A). Two apodemes project inwards into the pedicel lumen (Fig. 3A). A single campaniform sensillum was found associated with the base of the flagellum (Fig. 1C). The distal part of the bulb gives rise to two non-socketed processes, a cuticular spur and a long, thread-like arista, about 440 μm long and ending with a sharp tip (Fig. 1B, C). The cuticular spur (about 15 μm long) appears like a short cone that distally divides into three blunt-tipped pegs (inset in Fig. 1C). Just at the base of the spur, a well evident aperture can be observed (Ø about 4 μm) (Figs. 1C and 2B).

3.2. The sensilla of the flagellum

Ultrastructural investigations of the antennal flagellum revealed the presence of four types of sensilla: a single scolopidium, three sensilla styloconica and two sensilla coeloconica. The extensive wax covering of the external cuticular structures led in some cases to a sub-optimal fixation of the tissues, due to slow penetration of the water-soluble fixatives we used in our protocol.
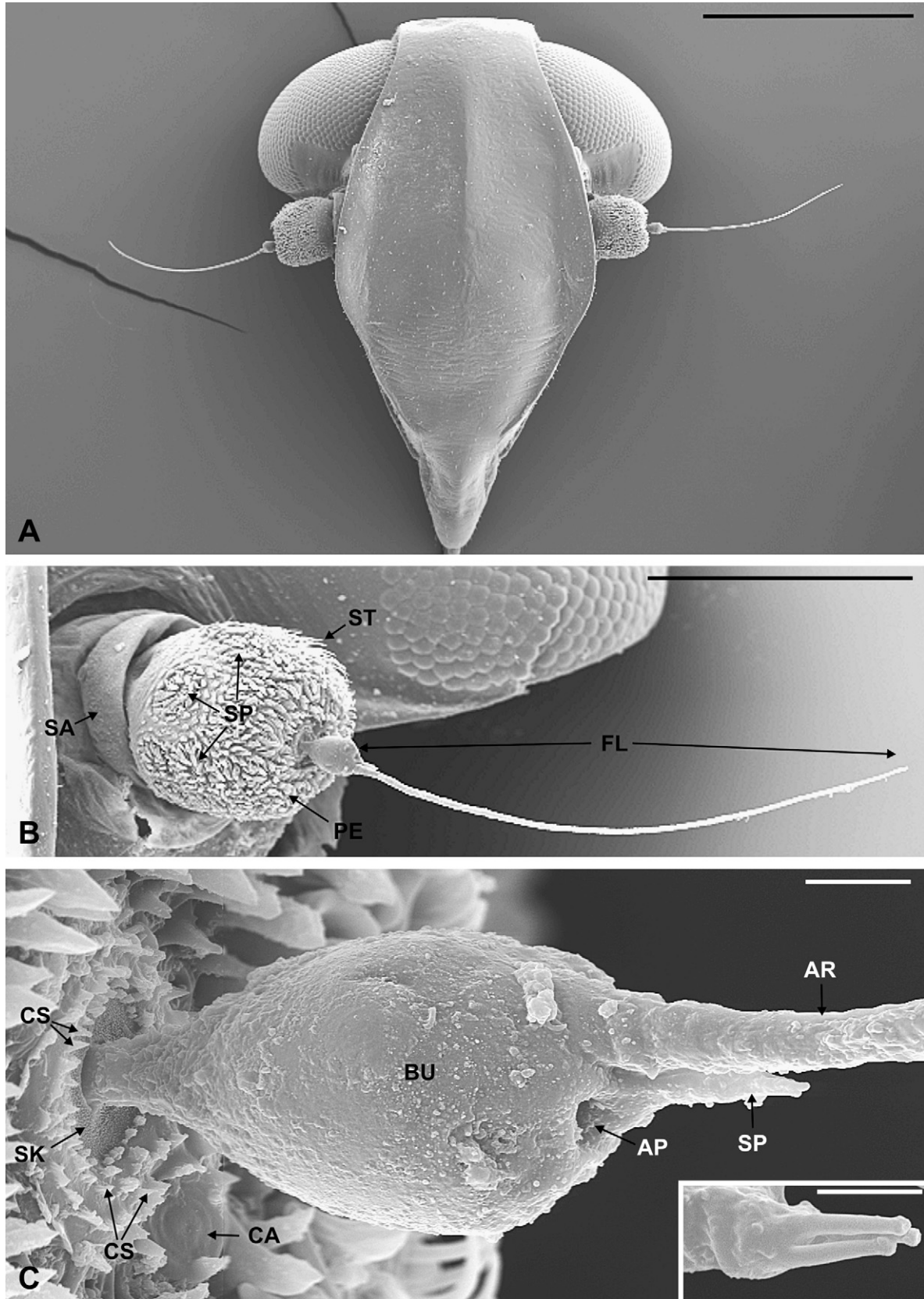
Fig. 1. SEM images of *Hyalesthes obsoletus*. In all the investigated specimens, abundant and almost insoluble waxes were found covering the external surface of the antennae. A) Head capsule of a female showing the position of the antennae. B) General view of the antenna showing the scape (SA), the pedicel (PE) with sensilla trichodea (ST) and sensilla placodea (SP), and the flagellum (FL). C) Proximal part of the flagellum; the bulb (BU) is inserted into a socket (SK) where a single campaniform sensillum (CA) is present. Rings of cuticular spines (CS) concentrically arranged are visible. On the distal part of the bulb, a spur (SP) with an aperture (AP) close to the base, and a thread-like arista (the prolongation of the flagellum itself) (AR) are visible. Inset: The spur is distally divided into three, blunt-tipped pegs. Scale bar: A: 500 μm; B: 200 μm; C: 10 μm; inset: 5 μm.
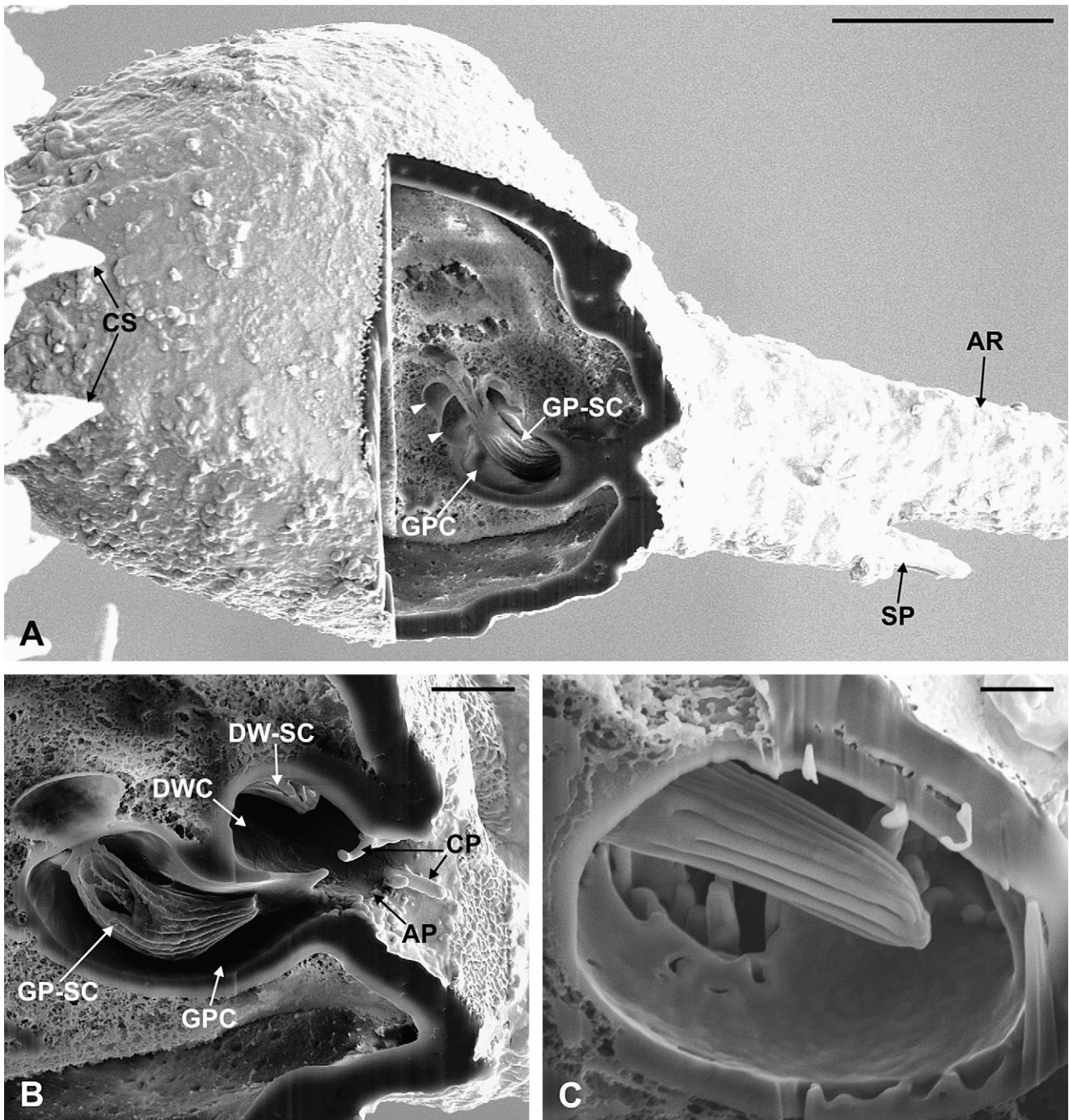
Fig. 2. FIB images of *Hyalesthes obsoletus*. A) The distal part of the bulb has been opened just below the arista (AR) and the spur (SP); the front cutting plane shows the grooved-peg sensillum coeloconicum (GP-SC) set in a chamber (GPC). Proximally, the GPC presents two small, spherical sub-chambers (arrowheads). B) Close-up view of the previous cutting plane, 2 μm deeper than the previous. The single aperture (AP) is clearly visible; the GPC and the double-walled peg chamber (DWC) have a common opening sheltered by a ring of cuticular projections (CP). C) Double-walled sensillum coeloconicum (DW-SC) in its cuticular chamber. The longitudinal grooves of the cuticular peg are visible. Scale bar: A: 10 μm; B: 2 μm; C: 1 μm.

3.3. The scolopidium

Serial longitudinal and cross sections of the bulb and the arista revealed the presence of a single scolopidium (Fig. 3A–G), made up of one sensory neuron and three accessory cells (Fig. 3B). The soma of the sensory neuron (about 10 μm long and 6 μm wide) is located in the medial region of the bulb, about 5 μm below the antennal wall. The inner dendritic segment (IDS) (Fig. 3D) extends for about 30 μm, showing a typical ciliary root (about 25 μm length) with a cross-banded pattern (transition of about 75 nm). Two basal bodies, a large proximal and a small distal, are found at the transition between the inner and the outer dendritic segment (ODS). The ODS (Fig. 3E, F) extends into the arista lumen for about 25 μm. The distal end of the ODS is surrounded by an electrondense dendrite sheath (Fig. 3F). The cilium is encircled by the scolopale cell that extends from the distal region of the soma up to the end of
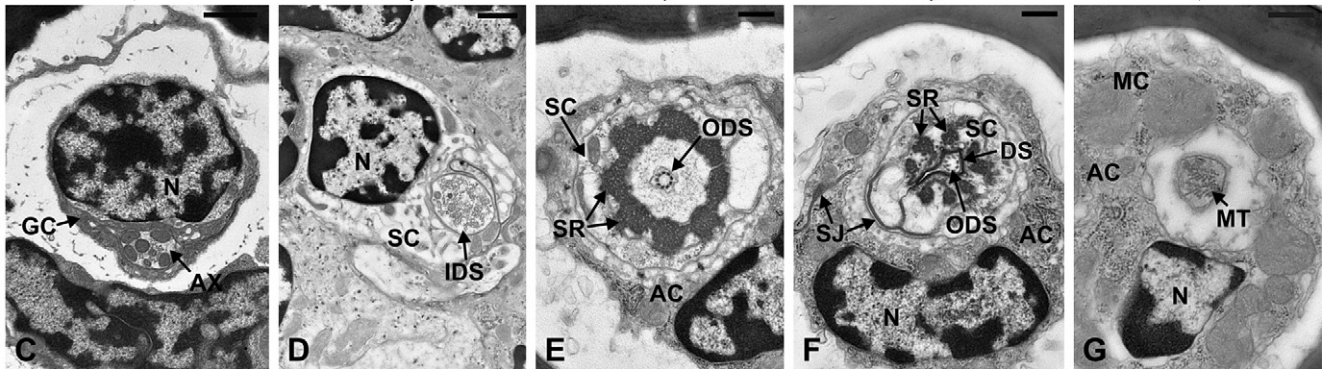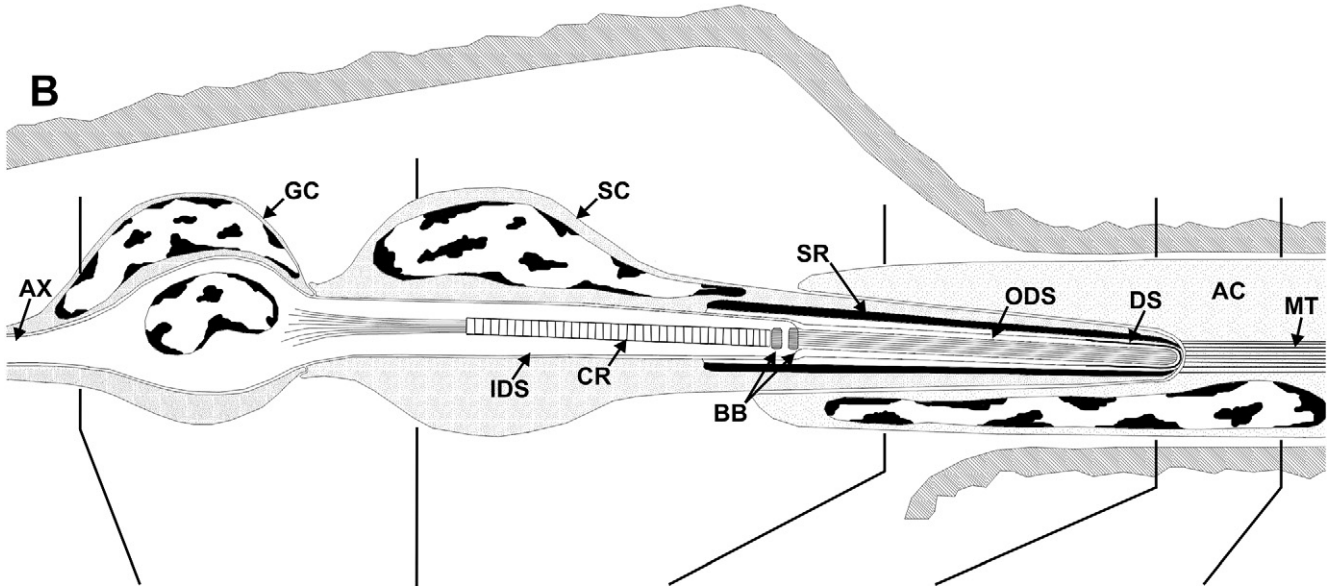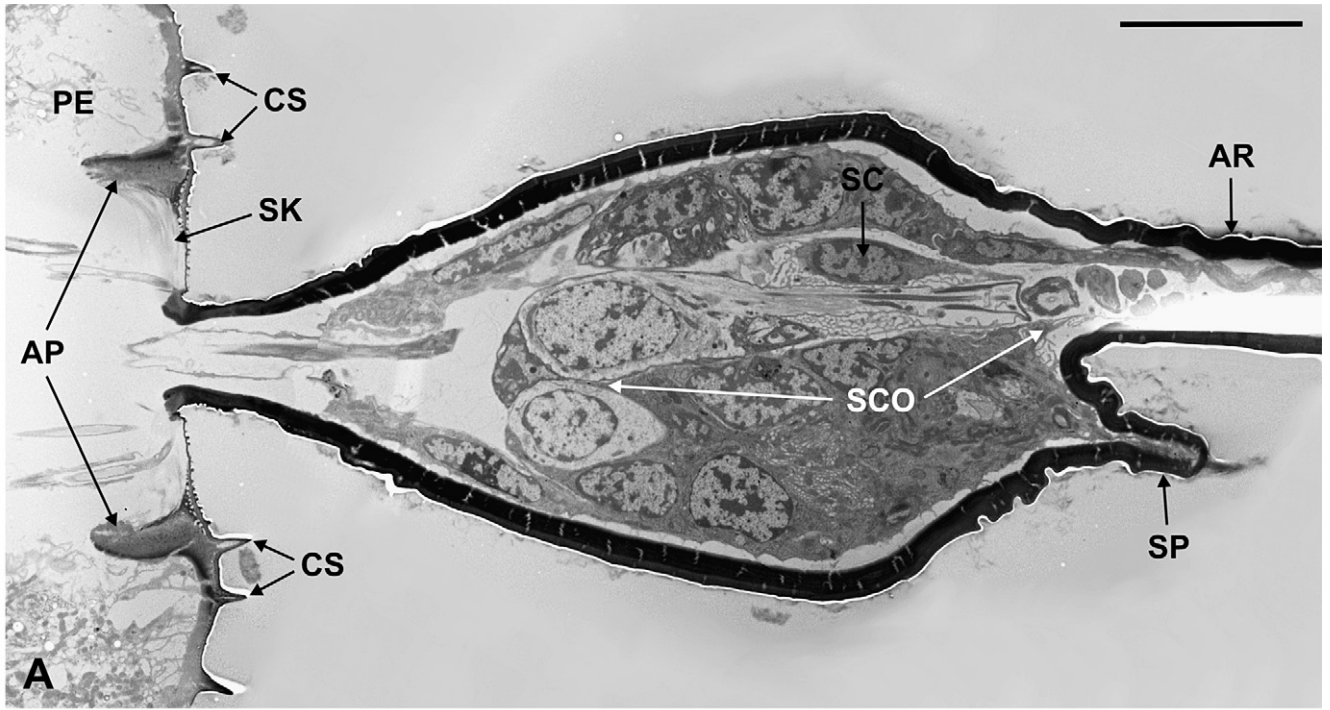
Fig. 3. TEM images of *Hyalesthes obsoletus*. A) Longitudinal section of the bulb showing its insertion into the pedicel (PE) with a socket (SK). At this level the cuticle is considerably thinner than the pedicel wall. Two cuticular apodemes (AP) projecting inside the pedicel lumen are visible, as well as the cuticular spines (CS) around the socket. A scolopidium (SCO) is visible almost in the centre of the bulb lumen; distally, it enters the arista (AR) (not shown on this section). B) Semischematic drawing of the scolopidium illustrating its cellular components on the basis of serial TEM sections. C–G) Selected TEM cross sections of the scolopidium. C) The glia cell (GC) at the axon (AX) level. D) The inner dendritic segment (IDS) of the scolopidium embedded by the scolopale cell (SC). E) The outer dendritic segment (ODS) surrounded by eight scolopale rods (SR) partially fused together. F) The scolopale cell (SC) close to the tip of the ODS. Eight scolopale rods (SR) are visible around the dendrite sheath (DS) that surrounds the outer dendritic segment; septate junctions (SJ) between the scolopale and the attachment cell (AC) are visible. G) Cross section taken distally on the arista, showing the attachment cell (AC) with an elongated nucleus (N), large mitochondria (MC) and numerous, bundled microtubules (MT). AR: arista, DS: dendrite sheath, N: nucleus, SP: spur. Scale bar: A: 10 μm; C–D: 1 μm; E–G: 0.5 μm.

the dendritic process (Fig. 3E). Eight scolopale rods surround the distal end of the ODS (Fig. 3F), while more proximally (about 15 μm below this level) the rods merge together forming the scolopale (Fig. 3E). At the ODS level, the scolopale cell is surrounded by the attachment cell, that presents several microtubules (Fig. 3F). Septate junctions between the two cells are often found (Fig. 3F). The attachment cell presents an elongated nucleus, large mitochondria and numerous elongated microtubules running in parallel according to the longitudinal axis of the scolopidium (Fig. 3G). These microtubules are bundled in a restricted region of the cell. The attachment site of the attachment cell is located distally within the arista (data not shown). A small glia cell is found wrapping around the sensory neuron axon and soma (Fig. 3C).

3.4. The sensilla styloconica

Cross sections of the cuticular spur show the presence of three sensory units (Fig. 4A–D). Each unit is distally separated from the others, and is made of a continuous, aporous cuticular shaft (thickness about 200 nm) (Fig. 4A). Each sensillum is innervated by three sensory neurons (Fig. 4F). More proximally (at about 5 μm from the tip of the sensillum), the three sensilla merge in a single cuticular shaft (Fig. 4B) that extends for 15 μm. The outer dendritic segments are embedded in a thick dendrite sheath forming a large, electron-dense body (Fig. 4C). The dendrite sheath envelops the sensory cells up to the level where each sensillum is separated from the others. Septate junctions between adjacent thecogen cells are frequently observed (Fig. 4E). Cross sections at the outer dendritic segment level just below the electrondense body show a thick electrondense dendrite sheath with three sensory neurons, one of them is clearly branched into 4–5 sub-spherical dendritic processes (Fig. 4E).

3.5. The sensilla coeloconica

Following the serial SEM images taken during the FIB investigation, the bulb of the flagellum revealed two cuticular cavities opening at the level of the external aperture located at the base of the cuticular spur (Fig. 2A–C). The external opening presents a ring of short, not innervated cuticular projections pointing towards the aperture lumen (Fig. 2B). The internal cavity then divides into two separate chambers housing two different types of sensilla coeloconica.

3.5.1. Double-walled sensillum coeloconicum (DW-SC)

This sensillum is found within the larger, rounded cavity (Ø about 6 μm) (Fig. 5A). The cuticular shaft is grooved in its distal part, and ends in a blunt tip (Fig. 2C). Cross and longitudinal sections revealed the typical double-walled organization. Apically, 13–14 cuticular ridges representing the external cuticular fingers are found arranged in a way that gives a stellate appearance to the structure (Fig. 5C). The outer dendritic segments (ODS) cannot clearly be distinguished due to artifacts in the preparation. The proximal half of the cuticular shaft is smooth externally. Longitudinal and cross sections at this level revealed the presence of an innermost chamber (Ø about 580 nm) occupied by 4 ODS (Fig. 5D), and an outermost chamber that is filled with electrondense material and small electronlucid vesicles (Fig. 5B). The sensory neurons enter the peg lumen without branching. The base of the sensillum has no socket, being inflexibly inserted at the bottom of the spherical cavity (Fig. 5B). At the ODS level, and before entering the peg, the four dendrites innervating this sensillum are enclosed by an electrondense dendrite sheath (Fig. 5E). Four ciliary constrictions are clearly visible in the inner sensillum lymph cavity, where several scolopale-like structures made of bundles of microtubules are also observed within the cytoplasm of the thecogen cell (Fig. 5F).

3.5.2. Grooved-peg sensillum coeloconicum (GP-SC)

This sensillum occupies the second, narrower but longer cuticular cavity (Fig. 5A). The cavity is more complicated compared to the one occupied by the DW-SC, particularly in its inner part where the cuticular peg arises. Here the cuticle of the cavity becomes thinner and is double-folded, therefore defining two small spherical sub-chambers (Figs. 2B and 6A). The peg (length about 15.5 μm) starts as a continuum of the inner part of the cavity, where it is inserted without flexible socket (Fig. 6A). The peg is sickle-shaped in a way that it runs within the cavity very close to the walls (Fig. 2A, B). Externally, the peg presents a constant number of about 22 cuticular ridges, the number of which decreases gradually towards the tip (where we counted about 14) (Fig. 6B). The cuticular ridges are separated by furrows, and they tend to disappear close to the base of the peg. Tiny pores can be observed opening at the base of the cuticular projections at various levels of the peg (Fig. 6B, D). The cuticle of the peg is thinner at the tip (70 nm) and becomes ticker close to the base (190 nm). The sensillum is innervated by a single sensory neuron (Fig. 6E, F), the outer dendritic segment of which enters the base of the peg and starts branching (Fig. 6A): we counted about 65–70 dendritic branches within the peg lumen, containing 1–2 microtubules each (Fig. 6C, D). The dendritic projections fill the sensillum lumen and are bathed by an electrondense lymph. The number of branches gradually decreases towards the tip of the peg (Fig. 6B).

4. Discussion

The antennal flagellum is reported as the most relevant part of the antenna in several insect orders, being composed of a varying number of antennomeres (ranging from a few up to 100 or even more) and housing the bulk of antennal sensilla (Zacharuk, 1985). In *H. obsoletus* the antennal flagellum revealed to be made of a single antennomere and, despite its exceptional length with respect to the rest of the antenna, we counted a total number of only six sensilla.

Our investigations revealed the presence of a single scolopidium located within the flagellum. The scolopidium is of the "mono-nematic–monodynal" type, being innervated by a single sensory neuron ending in a terminal cap located in the subepidermal region of the antennal wall (Yack, 2004). Scolopidia are particular mechanosensory units acting as proprioceptors, or devoted to the perception of gravity forces, both air-borne or substrate-born vibrations (for reviews of this topic, see Moulins, 1976; Field and Matheson, 1998; Yack, 2004). Within insect antennae, scolopidia are typically found organized in the so-called Johnston's organ, i.e. a large chordotonal organ responsible for the detection of air-born vibrations, typically located at the level of the second antennal segment (pedicel). Johnston's organ has been described in particular in Dipterans (mosquitoes, chironomids and fruit flies), where it reaches a high degree of complexity related to the importance of sound communication in these insects (Boo and Richards, 1975; McIver, 1985). As regards Hemiptera, the Johnston's organ has been reported in aphids (Bromley et al., 1980), in the leafhopper *Oncopsis flavicollis* (Howse and Claridge, 1970) and in the stink bug *Nezara viridula* (Jeram and Pabst, 1996), while the occurrence of scolopidia within the flagellum is quite rare and, so far, has been reported only in some aphid species (Bromley et al., 1980). The single scolopidium found in the antennal flagellum of *H. obsoletus* is similar in structure to the one described in the 6th antennal segment (the apical one) in aphid (Bromley et al., 1980), except for the lack of an obvious cap. This sensillum could be used by the insect to detect air-borne vibrations, or to detect the respective flagellum position, thereby acting as a proprioceptor. It is also possible that this scolopidium could work together with a large chordotonal organ that we found within the pedicel (Romani et al., personal communication), which is
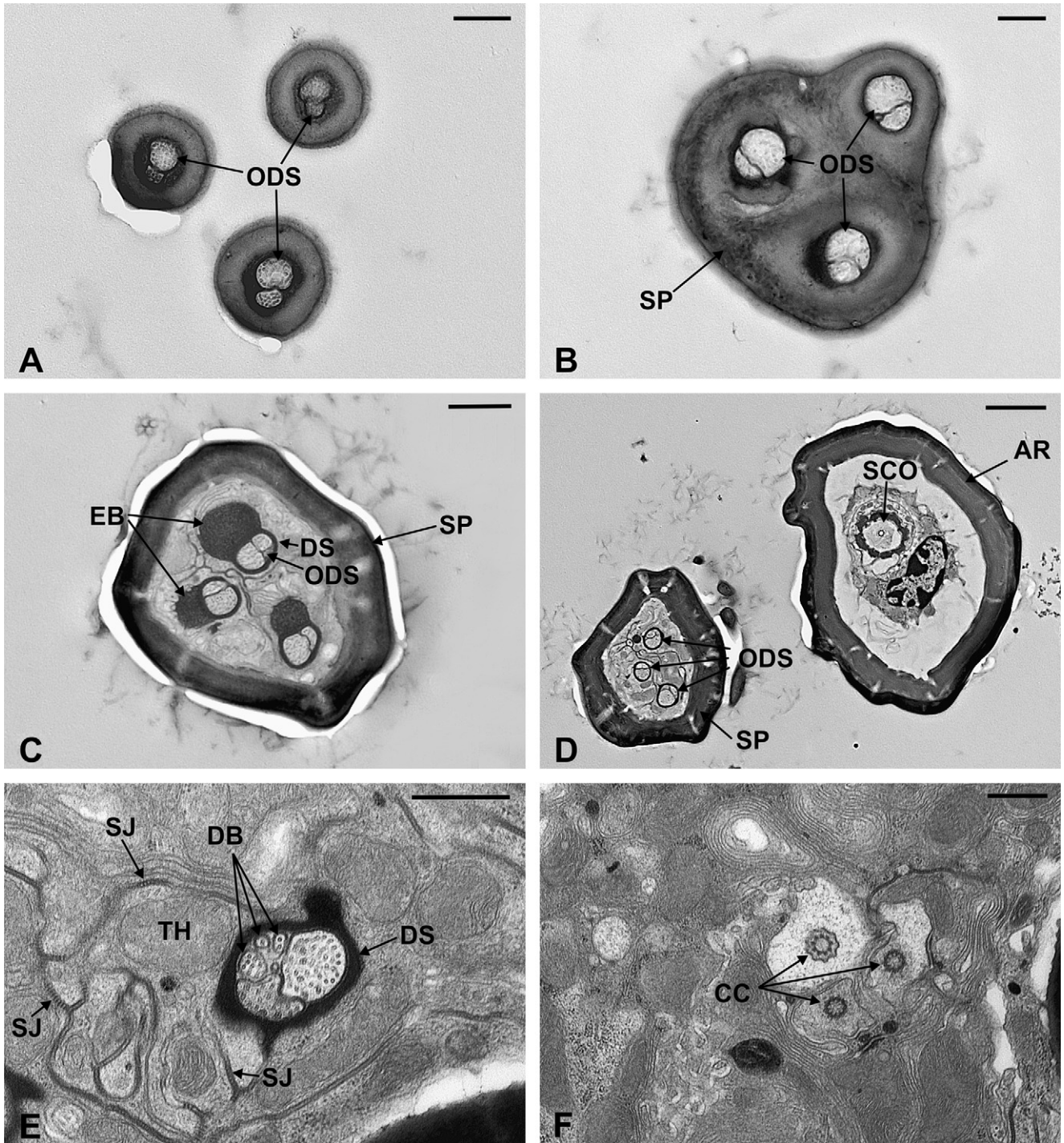
Fig. 4. TEM micrographs of cross sections of the sensilla styloconica in *Hyalesthes obsoletus*. A) The distal part of the spur where the three sensilla styloconica are separated. The outer dendritic segments (ODS) of the sensory neurons innervating each sensillum occupy the entire sensillar lumen. B) More proximally the three sensilla styloconica are fused together in the spur (SP). C) In the proximal region of the spur the three bundles of ODS are visible. At this level, the dendrite sheaths present a rounded, electrondense body (EB). D) Cross section of the arista (AR) and the spur (SP) showing the position of the scolopidium (SCO) and the outer dendritic segments (ODS) of the sensilla styloconica within the spur lumen. E) Proximal part of the spur; the dendrite sheath (DS) surrounds the outer dendritic segments. One of the ODS shows dendritic branches (DB). The thecogen cell (TH) shows several septate junctions (SJ). F) Ciliary constrictions of the three dendrites of a single sensillum styloconicum. Scale bar: A, B, E, F: 0,5 μm; C: 2 μm; D: 1 μm.

stimulated by mechanical stress onto the socketed base of the flagellum. In fact, the importance of acoustic signals for leafhoppers and planthoppers in the context of mating behavior was reported for several families (Claridge, 1985; Čokl and Virant-Doberlet, 2003; Virant-Doberlet and Žežlina, 2007). Moreover, the role of the antennae as sound-induced vibration detectors has been reported in mosquitoes and fruit flies (Göpfert et al., 1999; Göpfert and Robert,

2001, 2002). It is remarkable that the antennal gross morphology of *H. obsoletus* is quite similar to the one of *Drosophila* that is composed by only three antennal segments with a long terminal thread-like arista. The selective ablation only of the arista significantly affects the sound receptivity in *Drosophila*, revealing that this structure acts as sound receiver (Manning, 1967). In this view, the whole antennal flagellum of *H. obsoletus* could be considered as a sensillum itself
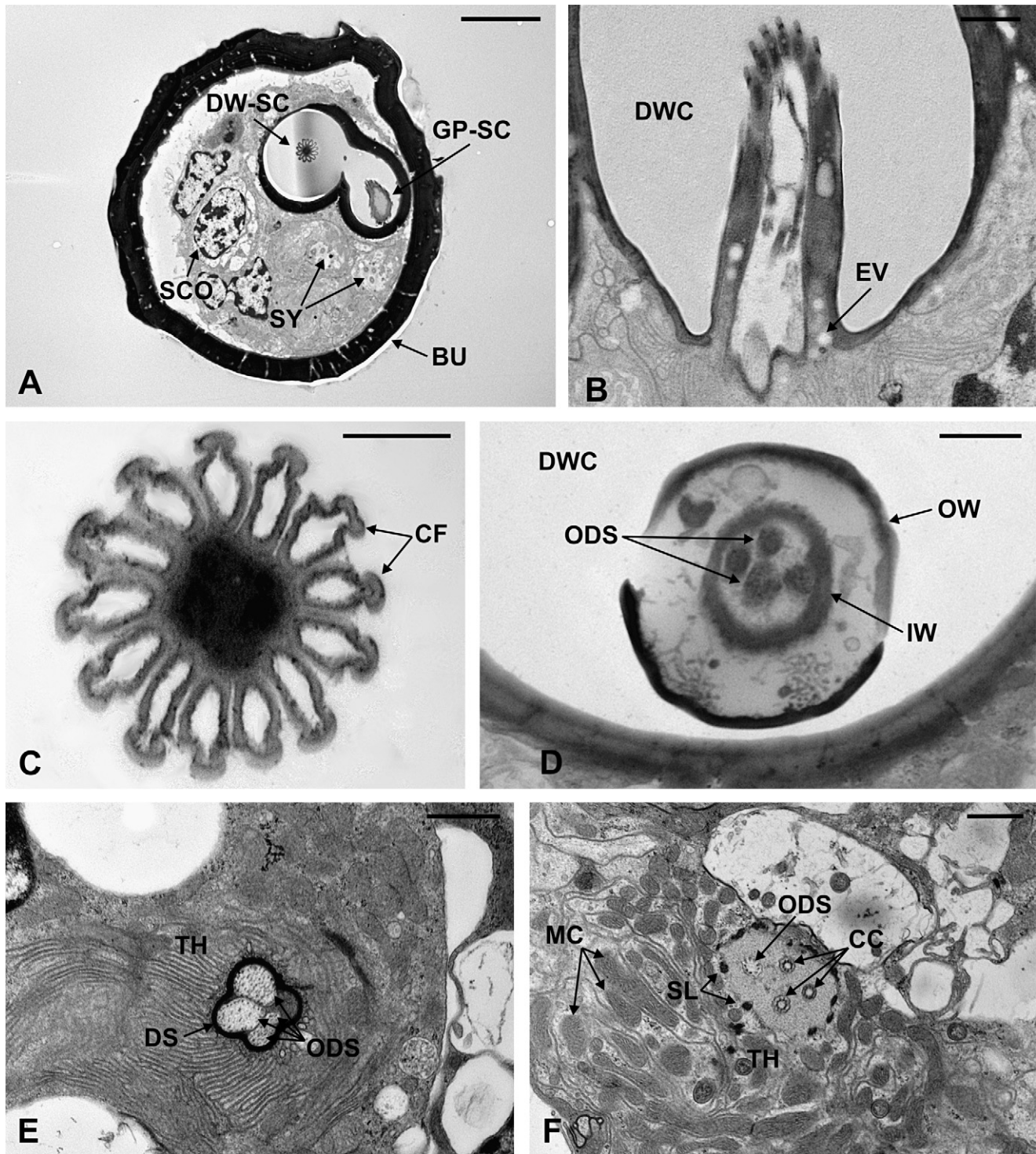
Fig. 5. TEM micrographs of the double-walled sensillum coeloconicum in *Hyalesthes obsoletus*. A) Cross section of the bulb (BU) showing the position of the scolopidium (SCO), two sensilla styloconica (SY) and the two sensilla coeloconica, the double-walled sensillum coeloconicum (DW-SC) and the grooved-peg sensillum coeloconicum (GP-SC). B) Oblique section of the double-walled sensillum coeloconicum within its cavity (DWC): in the outermost chamber of the peg electronlucid vesicles (EV) are visible. C) Cross section of the apical part of the DW-SC, showing 13 cuticular fingers (CF). D) Cross section of the proximal DW-SC; the outermost wall (OW) is externally smooth, the innermost wall (IW) surrounds 4 unbranched outer dendritic segments (ODS). E) ODS surrounded by the dendrite sheath (DS). F) Cross section through the ciliary constrictions (CC) of the sensory neurons. Scolopale-like structures (SL) are visible within the thecogen cell (TH). MC: mitochondria; Scale bar: A: 5 μm; B: 1 μm; C, D: 0.5 μm; E, F: 1 μm.

(functioning as a sound-receptive hair), but also as an antennal segment bearing at the same time other sensilla.

The cuticular spur located distally on the bulb revealed to be composed of three sensilla styloconica. The presence of a similar structure was reported as a general feature for the antennae of

Fulgoromorpha, with different names ("antennal second projection", Shih and Yang, 1996; "basal flagellar process", Liang, 2001), but to date there are no data available on the ultrastructural organization of these sensilla. From our observations, the sensilla styloconica present a thick, aporous cuticular wall, and are
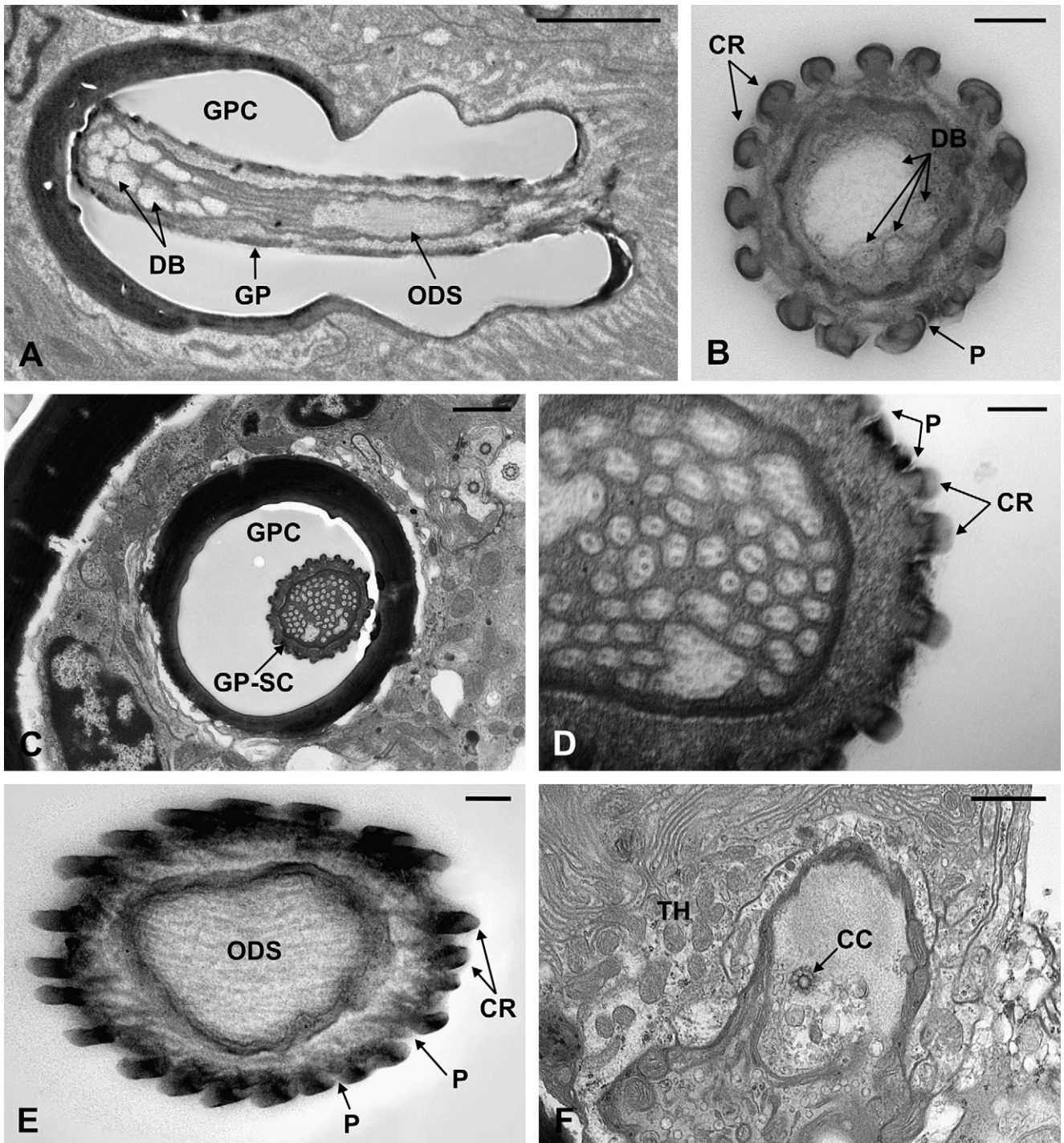
Fig. 6. TEM micrographs of the grooved-peg sensillum coeloconicum. A) Grooved-peg sensillum cavity (GPC), which is occupied by the grooved-peg sensillum coeloconicum (GP). In this longitudinal section of the peg, the outer dendritic segment (ODS) with its dendritic branches (DB) is visible. B) Cross section of the GP-SC close to the tip; pores (P) are present between the cuticular ridges (CR). C) Cross section of the grooved-peg sensillum cavity (GPC) with the GP-SC inside. D) High magnification of the GP-SC numerous cuticular ridges and small pores. The peg lumen is filled with dendritic branches. E) Proximal part of the grooved-peg: at this level the outer dendritic segment (ODS) shows no branches. F) Cross section of the GP-SC sensory neuron through the ciliary constriction (CC). TH: thecogen cell. Scale bar: A: 2.5 μm; B, D, E: 200 nm; C, F: 1 μm.

innervated by a set of three sensory neurons each, with one of them showing several dendritic branches. Sensilla styloconica have been described for several species of Coleopterans (Bland, 1981; Alm and Hall, 1986; Bartlet et al., 1999) and Lepidopterans (Haug, 1985; Steinbrecht, 1989). These sensilla are usually innervated by a triad of sensory cells, with two unbranched dendrites that occupy the cuticular shaft lumen, and a third dendrite ending at the base of the sensillum and giving rise to distally lamellated branches. The presence of this "sensory triad" has been demonstrated to be involved in the perception of humidity and temperature in numerous cases (Altner and Prillinger, 1980; Altner and Loftus, 1985; Yokohari, 1981; Zimmermann, 1992). Our TEM data are consistent with a possible role of these sensilla styloconica as thermo-hygroreceptors in *H. obsoletus*.

The implementation of FIB technique in the study of biological specimens revealed to be extremely useful when attempting to understand the ultrastructural organization of the internal structures of insects. This technique makes it possible to selectively ablate entire, pre-defined regions of insect cuticle, therefore allowing the examination of delicate internal structures. FIB investigations carried out on the distal part of the bulb in *H. obsoletus* flagellum revealed the presence of two distinct chambers housing two types of sensilla, a double-walled coeloconic sensillum and a grooved-peg sensillum. Both chambers open to the antennal surface at the level of a single aperture, partly hidden and protected by short cuticular projections. A similar structure was first described as "basal sensory organ" (Bourgoin, 1985), and later as "Bourgoin's organ" (Cobben, 1987), and seems to occur in several families of Fulgoromorpha (Liang, 2001). Although the presence of coeloconic sensilla within this cavity was already reported (Liang, 2001), previous studies were carried out using only external SEM observation, without providing data on the internal structure. We found that the DW-SC revealed ultrastructural features similar to those of the same sensilla described for other insects, i.e. the presence of spoke channels and unbranched outer dendritic segments, the absence of lamellated outer dendritic segments, and the presence of secretory vesicles at the base of the peg (Altner et al., 1981; Ruchty et al., 2009). For these sensilla, a bimodal function related to the detection of thermal stimuli, as well as olfactory cues, has been demonstrated (Altner et al., 1981; Ruchty et al., 2009), others had exclusively olfactory function (Pophof, 1997, Pophof et al., 2005). It is remarkable that, in *H. obsoletus*, a single double-walled sensillum coeloconicum was found, while sensilla coeloconica are often reported as grouped in small patches in specific antennal areas (Altner et al., 1981; Hunger and Steinbrecht, 1998; Ruchty et al., 2009). Whether this sensillum is a temperature/humidity or an olfactory receptor can only be decided after electrophysiological recording.

The structure of the GP-SC, which is located in the second subcuticular chamber, is very different from that of the DW-SC. First of all, it is set in a deeper and narrower cavity that opens externally through an aperture that is smaller than the one of the DW-SC. Deeply sunken sensilla (of the styloconica type) have been recently described for Odonata (Rebora et al., 2008), and on the basis of ultrastructural features, a possible role as thermo-hygroreceptors has been hypothesized. However, the GP sensillum we found in *H. obsoletus* seems to be more closely related to the sensilla ampullacea described in Hymenoptera, which represent one of the most extreme cases of sensilla hidden below the body surface. Sensilla ampullacea are characterized by the presence of a cuticular peg in a small chamber inside the antennal lumen, which is connected via a cuticular duct with the single external opening. Sensilla ampullacea have been described in ants (Dumpert, 1972; Kleineidam and Tautz, 1996; Kleineidam et al., 2000) as well as in other Hymenopterans (Ågren and Hallberg, 1996). They are reported to be innervated by a single sensory neuron (Kleineidam et al., 2000), they are of the thin-walled type with numerous pores opening on the sensillum wall (Lee et al., 1985; Bogner et al., 1986; Kleineidam et al., 2000), and the neuron is often highly branched and/or lamellated (Lee et al., 1985; Kleineidam et al., 2000). Sensilla ampullacea of ants have been proven to respond to CO_2 (Kleineidam et al., 2000). The single GP-SC of *H. obsoletus* seems to match almost completely with the above reported features, and a role in the perception of CO_2, therefore, is likely. For sensilla ampullacea of ants, the presence of a long and narrow duct was interpreted as an adaptation to dry environments by reducing water loss. The fact that the GP sensillum of *H. obsoletus* is not so deeply embedded inside the antennal lumen could be due to the habitat exploited, represented by the foliage of herbaceous and/or perennial plants, where the relative humidity never reaches very low levels.

This paper is the first comprehensive study carried out to reveal the ultrastructure of the flagellar sensilla on the antennae of a Fulgoromorpha species. Most remarkable is the low number of sensilla; the whole structure counts only 6 sensilla belonging to 4 different types of sensilla. It is of interest that the flagellum lacks any typical chemoreceptor, while it seems to be adapted to pick up air-born vibratory signals and environmental cues (temperature and humidity variations, concentration of CO_2). The absence of sexual dimorphism is supportive for this notion, since both males and females should have a similar number and type of sensilla responding to CO_2, temperature and humidity.

It is noteworthy that the complete absence or extreme reduction of olfactory sensilla on the flagellum is compensated by the presence of putative olfactory sensilla (placoid and trichoid) on the pedicel. This is of interest considering that olfactory sensilla are seldom found on the pedicel. A strong reduction in the number of the antennal sensilla on the flagellum was observed in other species of Auchenorrhyncha (Kristoffersen et al., 2006; Onagbola et al., 2008). Whether in *H. obsoletus* the reduction of the flagellar sensilla number is part of an evolutionary bauplan needs to be further investigated in other species of the infraorder Fulgoromorpha.

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Appendix. Supplementary information

Supplementary data associated with this article can be found in the version at doi:10.1016/j.asd.2009.08.002.

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