Comparative Neuroanatomy of the Antennal Lobes of 2 Homopteran Species

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Abstract

We compared the morphology of the primary olfactory center, the antennal lobe (AL), in 2 homopteran insects, Hyalesthes obsoletus Signoret (Homoptera: Cixiidae) and Scaphoideus titanus Ball (Homoptera: Cicadomorpha). The comparison between the ALs of the 2 species is particularly interesting considering that, although both use volatile cues to locate their host plants, their feeding behavior differs considerably: specifically, H. obsoletus is a highly polyphagous species, whereas S. titanus is strictly monophagous (on grapevine). Our investigation of the AL structure using immunocytochemical staining and antennal backfills did not reveal any sexual dimorphism in either the size of the ALs or in the size of individual glomeruli for either species. Instead, the AL of H. obsoletus displayed numerous and well-delineated glomeruli (about 130 in both sexes) arranged in a multilayered structure, whereas the smaller AL of S. titanus contained fewer than 15 glomerular-like structures. This difference is likely to reflect the comparatively reduced olfactory abilities in S. titanus, probably as a consequence of the reduced number of volatiles coming from the single host plant. Instead, in H. obsoletus, the ability to distinguish among several host plants may require a more complex olfactory neuronal network.

Key words: antennal lobe, confocal microscopy, glomeruli, Hyalesthes obsoletus, olfaction, Scaphoideus titanus

Introduction

Insects are highly dependent on chemical information detected by peripheral sensory neurons (SNs). This information, which is processed in primary neuropils in the brain, determines specific behaviors involved in survival and reproduction. Structures involved in the detection of chemical stimuli are distributed mainly in sensory organs on mouthparts and antennae. The latter, which are considered to be the major olfactory organs, have most of the olfactory SNs (OSNs); OSNs detect stimuli from conspecifics, hosts, or food. Antennal OSNs extend their axons to the deutocerebrum where they transmit information to second-order neurons at the antennal lobe (AL) level. SN axonal terminals, local neuron arbors, and projection neuron (PN) dendrites together form spherical neuropils, known as glomeruli, which are typical of the ALs in insects (reviewed in Galizia and Rössler 2010). The number, size, and arrangement of glomeruli are species specific, but in general, the number of glomeruli appears to be remarkably stable within various clades (e.g., Schachtner et al. 2005). The highest number of glomeruli is reported in Caelifera (Orthoptera), with from 1000 up to 3000 isomorphic microglomeruli (Ernst et al. 1977; Laurent 1996; Ignell et al. 2001; Schachtner et al. 2005), whereas in dipteran larvae fewer than 25 glomeruli are present (Python and Stocker 2002; Ramaekers et al. 2005). Usually, however, the AL contains 40–450 glomeruli arranged in a single or double layer (Hansson and Anton 2000; Nishikawa et al. 2008; Galizia and Rössler 2010; Kelber et al. 2010). In some Palaeoptera orders, that is, Ephemenoptera, Odonata, and Plecoptera, considered totally or nearly anosmic, the ALs appear reduced (Panov 1961; Strausfeld et al. 1998). Nevertheless, recent data demonstrate that dragonflies (Odonata) do have olfactory sensilla, and glomerulus-like...
structures are present in the ALs (Rebora et al. 2012, 2013). Among the Hemiptera, the suborder Heteroptera displays clearly defined glomeruli, whereas in Homoptera, some species appear to lack such AL organization. Several reports regarding heteropteran families, such as Lygaeidae, Pentatomidae, and Reduviidae, show the presence of clearly defined glomeruli (Johansson 1957; Singh 1969; Villar et al. 1994; Kristoffersen et al. 2008; Barrozo et al. 2009), whereas in Homoptera, to which the species of our study belong, the situation is ambiguous, and examples of both glomerular (Pflugfelder 1937; Kollmann et al. 2011) and nonglomerular (Kristoffersen et al. 2008) organization of the AL have been found. Older morphological studies on several heteropteran species show differences in AL size for Geocorisae (land living) compared with Hydrocorisae (water living). Nevertheless, glomeruli were observed in both groups (Hansström 1928; Pflugfelder 1937; Guthrie 1961). For Homoptera, available information stems mainly from studies on the Sternorrhyncha (aphids, psyllids, white flies, and scales); very little is known from the Auchenorrhyncha (plant hoppers, cicadas, leafhoppers, treehoppers, and spittlebugs). In a recent comparative study, Kristoffersen et al. (2008) found nonglomerular ALs in 3 odor-guided Sternorrhyncha species (2 aphids and 1 psyllid). In contrast, antennal backfills performed in the pea aphid Acyrthosiphon pisum revealed OSN arborizations, indicating the presence of glomeruli (Kollmann et al. 2011). Strausfeld et al. (1998) consider cicadas (Homoptera: Cicadidae) as anosmic insects because they lack both ALs and mushroom body (MB) calyces. However, in a study of brain structure in Idiocerus atkinsoni Leth. (Homoptera: Jassidae), Singh et al. (1996) observed the presence of glomerular structures in the deutocerebrum.

In the present study, we compared the structure of the ALs of 2 homopteran Auchenorrhyncha species: Hyalesthes obsoletus Signoret (Cixiidae) and Scaphoideus titanus Ball (Cicadellidae). Both show a similar, vibration-based mate-finding strategy but differ significantly in feeding behavior (Mazzoni et al. 2010; Eriksson et al. 2011). The paleartctic H. obsoletus is highly polyphagous, feeding on different wild host plants, whereas the nearctic S. titanus is, at least in Europe, considered monophagous on grapevine, Vitis vinifera L. Both species are important grapevine pests, being vectors of phytoplasma diseases: H. obsoletus is responsible for the transmission of the Bois Noir (16SrXII-A group phytoplasmas); S. titanus is the vector of the Flavescence dorée agent (Candidatus Phytoplasma vitis, 16SrV group) (Lee et al. 1998; Martini et al. 2002; Firrao 2004; Lee et al. 2004).

The aim of our study was to increase our understanding of the structure of the AL of Hemiptera in groups for which information is so far unavailable. Furthermore, the comparison of the AL structure between 2 related species with such different foraging strategies is extremely interesting from an evolutionary and a functional perspective. Previous work on these 2 homopteran species have provided a complete overview on their antennal morphology (Romani et al. 2009; Rossi Stacconi and Romani 2012, 2013) and suggested that the number of OSNs in H. obsoletus is approximately 150 times higher than in S. titanus. We therefore hypothesized that the primary olfactory center in H. obsoletus is much more developed than that of several heteropteran species.

Materials and methods

Insects

Adults of both species were collected in vineyards; S. titanus were caught on grapevines in the Trento district (Italy), whereas H. obsoletus were caught on nettle plants (Urtica dioica L.) in the Ancona district (Italy). A modified leaf blower in which the intake port was fitted with a fine mesh fabric bag was used to catch the insects. Captured specimens were kept in a cages with fresh host plant leaves, until being brought to the laboratory. Insects were kept at 26 ± 1 °C and 60% ± 10% relative humidity, under a natural photoperiod. Insects were used for histology within a week of capture.

Histology

For general visualization of the brain anatomy, staining with Lucifer yellow solution (LY, 5% aqueous solution; Sigma-Aldrich) (Ai et al. 2009) or Karnovsky’s fixative (Karnovsky 1965) was carried out. The head capsules were opened in phosphate-buffered saline (PBS, pH 7.2) with sucrose (10%), and the exposed brains were fixed in 4% paraformaldehyde (PFA) for 2h at 4 °C. To facilitate antibody penetration and hence better staining, in H. obsoletus, the subesophageal ganglion (SOG) was cut away during all preparations. After washing 6 × 10 min in PBS, the preparations were dehydrated in an ascending ethanol series (100%, 70%, 50%) and then cleared in methyl salicylate (Sigma-Aldrich). For general visualization of the brain anatomy, staining with Lucifer yellow solution (LY, 5% aqueous solution; Sigma-Aldrich) (Ai et al. 2009) or Karnovsky’s fixative (Karnovsky 1965) was carried out. The head capsules were opened in phosphate-buffered saline (PBS, pH 7.2) with sucrose (10%), and the exposed brains were fixed in 4% paraformaldehyde (PFA) for 2h at 4 °C. To facilitate antibody penetration and hence better staining, in H. obsoletus, the subesophageal ganglion (SOG) was cut away during all preparations. After washing 6 × 10 min in PBS, the preparations were dehydrated in an ascending ethanol series (50%, 70%, 100%) and rehydrated in a descending ethanol series (100%, 70%, 50%). Specimens were subsequently rinsed in PBS and incubated in PBS-Tx (PBS with added 0.25% Triton-X; Sigma) for 1 h at room temperature. Parts of the brains were left overnight in a LY solution (1 μL LY/500 μL PBS-Tx), others were kept 5 days in Karnovsky (2% PFA, 2% glutaraldehyde in PBS) solution at 4 °C in a shaker. On the second day of incubation, LY-treated specimens were postfixed in PFA solution (4% in PBS) at 4 °C for 3h at room temperature. All specimens were subjected to a final treatment of 6 × 10 min washes in PBS, dehydrated in an ascending ethanol series, and then cleared in methyl salicylate (Sigma-Aldrich).

Immunocytochemistry

A mouse monoclonal antibody nc82 (Hybridoma Bank) was used to visualize the compartments of the brain neuropils. Brains were fixed in 4% PFA, and dehydrated and rehydrated as described above. After being washed in PBS-Tx (3 × 10 min), specimens were preincubated for 60 min in a blocking solution with 5% normal goat serum (NGS).
(Sigma-Aldrich) in PBS-Tx. The primary antibody solution consisted of anti-mouse nc82 in PBSTx-NGS (5%) diluted 1:30; brains were incubated for 48 h at 4 °C on a shaker. After being washed in PBS (6 × 20 min), the preparations were incubated in the secondary antibodies solution consisting of 1:200 goat anti-mouse (Dianova) conjugated with a fluorophore, CY5 (633-nm excitation wavelength) or CY2 (488-nm excitation wavelength) in PBSTx-NGS.

Antennal backfills

To visualize the SN pathways, backfills were carried out on the antennae of both species. Living insects were immobilized and the ventral part of the head was exposed using double-sided adhesive tape. The antennae were cut at the pedicle level and inserted for 15 min in an appropriately sized glass microcapillary tip filled with distilled water. The microcapillary was then substituted with a second one filled with Microruby (Dextran, Tetramethylrhodamine and biotin, 3000 MW, Lysine Fixable; Molecular Probes). The preparation was placed in a dark box, containing moist paper, for 5 h at 18 °C to allow the fluorescent dye to diffuse through the antennal nerve into the brain.

Confocal microscopy and 3D analyses

Whole mounts of brains were viewed with a Zeiss LSM 510 confocal microscope equipped with ×20, ×40, and ×63 water immersion objectives. Scans were carried out using 488-nm excitation wavelength for Alexa 488, LY, and CY2 fluorophore, and 633-nm excitation wavelength for CY5 fluorophore. Image data were captured as serial stacks (speed 200–400 Hz, pinhole 1 AU, step size 0.4–1 μm). Three-dimensional reconstructions were obtained by segmenting the neuropil borders and then rendering these as polygonal surface models in AMIRA 4.1 or 5.1 (Visage Imaging); single images were cropped and arranged using Gimp 2.6 (http://www.gimp.org). The body axis was used to name the orientation of anatomical structures. Brain and AL volumes were calculated using AMIRA’s material statistics function. Student’s t-test for independent samples was used to compare left and right AL volume and to compare the AL volume as a percentage of the brain (proto-, deuto- and trito-cerebrum [TrC], excluding SOG) volume between males and females. Percentages were subjected to angular transformation in order to reduce data heteroscedasticity (Steel and Torrie 1980).

Results

General brain anatomy

In both species, the head capsule was opisthognathous with mouthparts directed backward. After the removal of the dorsal head cuticle and of the huge pharyngeal pump muscles, the brain and the SOG were visible. The brain was formed by the protocerebrum (optic lobes [OLs]; optic tubercles [OTs]; MBs; central complex [CC]; protocerebral lobes [PLs]; lateral accessory lobes [LALs]), the deutocerebrum (ALs; antennal mechanosensory and motor centers [AMMCs]); and the TrC. Antennae were positioned just below the large compound eyes (Figure 1a–d). The brain volume in H. obsoletus and S. titanus is reported in Table 1. The LY neuron background staining revealed the gross morphology of brain compartments. In both species, most of the major neuropils appeared delineated (lamina, medulla, lobula complex, CC, LALs, ALs, and AMMCs), whereas some areas of the brain mass (OTs of the protocerebral lobe) were undifferentiated or showed no clear boundaries. In H. obsoletus, the OLs were positioned anterodorsally with regard to the protocerebrum, protruding from it (Figure 1b), whereas in S. titanus the OLs had a more lateral position (Figure 1e). The lamina, the medulla, and the lobula complex were clearly distinguishable (Figure 1b,c,e). At the level of the CC, we identified the upper and lower divisions of the central body, the noduli, and the protocerebral bridge (Figure 2a,b). The latter was located dorsocaudally in relation to the central body and was divided into 2 bar-shaped hemispheres (Figure 1b,c,e). In H. obsoletus, the MBs were characterized by the absence of calyces (Figures 1b,c and 2b). Also, in S. titanus the MBs appeared devoid of calyces (Figure 2e), although their border was not well delineated in our preparations.

The deutocerebrum, which is positioned just below the LAL (Figure 2c,f), consisted of the ALs and the AMMCs (Figure 2a,d). These neuropils showed a different shape and arrangement in the 2 species, and their morphology will be described below. The TrC was made up of 2 lobes positioned laterally to the esophagus (Figure 2c,f), receiving neural fibers both from the deutocerebrum and the SOG.

The deutocerebrum of H. obsoletus

In H. obsoletus, LY preparations revealed clearly glomerular ALs located ventrocaudally, near the esophageal connectives. Each AL was curved, elongated in the anteroposterior axis and partially surrounded the LAL (Figures 1b and 2a,c). The volume of the ALs for both sexes is reported in Table 1. In this species, the AMMC was located dorsally in regard to the AL (Figure 1c); it was round and its average volume was 1.789 ± 0.16 × 10³ μm³ in females and 1.554 ± 0.09 × 10³ μm³ in males (μm³ ± SD; n = 8). Immunocytochemical stainings with nc82 showed glomeruli with distinct borders. Three female and 4 male preparations had a staining intensity that made it easy to visualize the glomeruli borders for reconstructions. However, full antibody penetration at high resolution was achieved only in 2 specimens, a female (Figure 3) and a male (Figure 4), and we were thus unable to determine a sufficient number of glomeruli in a population.

On the basis of these preparations, the total number of glomeruli was 128 in the right AL of a male and 131 in the left
Figure 1  
(a–c) Hyalesthes obsoletus; (d–f) Scaphoideus titanus. (a and d) Scanning electron microscopy pictures of the head capsules of the 2 insects show the antennal nerve (AN) raising from the antenna: the flagellum (Fl), the pedicel (Pe), and the scape (Sc) are visible (b, c, e, and f). Three-dimensional reconstructions of the brains in detail: ventral views (b and e) and sagittal view (c and f). La: lamina; Me: medulla; LO: lobula complex; MB; PB: protocerebral bridge; UCB: upper central body; LCB: lower central body; NO: noduli; LAL; AMMC; AL; TrC; SOG. Scale bars: (a and d) = 500 μm; (b, c, e, and f) = 200 μm. Body axis: v: ventral; d: dorsal; p: posterior; a: anterior; l: lateral.
AL of a female. The glomeruli were arranged in a multilayered structure, the volumes of segmented glomeruli ranged from $0.19 \times 10^7$ to $4.2 \times 10^7 \mu m^3$, but 83.37% of all glomeruli ranged from $0.5 \times 10^7$ to $2.0 \times 10^7 \mu m^3$. The median glomerular size of the reconstructed specimens was $1.46 \times 10^7 \mu m^3$ in the male and $1.52 \times 10^7 \mu m^3$ in the female. The comparison of the AL scans between the 2 sexes did not reveal any sign of sexual dimorphism, and no macroglomerular complexes in either sex were found. The ALs volume, calculated excluding SOG, represents 0.57% and 0.59% of the total brain volume, in males and females, respectively, with no significant difference ($t = 0.92; df = 2; P = 0.45$). Within the sexes, no statistical difference was observed between the volume of right and left ALs (males: $t = 0.20; df = 3; P = 0.85$; females: $t = 0.48; df = 3; P = 0.66$).

### Discussion

The 2 species studied here present divergent ecologies. *H. obsoletus* is a highly polyphagous herbivore, whose preferred host plants have been reported to depend on the geographical region (Sforza et al. 1999; Langer et al. 2003; Kessler et al. 2011). Host location and recognition involve the detection of numerous volatile organic compounds (VOCs) belonging to the specific bouquets from each of the host plants (Riolo et al. 2010). These complex ecological relationships are consistent with the presence of a well-developed antenna and AL, both of which are required to detect and process olfactory information deriving from a large variety of host sources. On the other hand, the European populations of *S. titanus* are specialized on *Vitis* sp. throughout their life. Olfactory cues have been shown to play an important role in identifying the host plant in this species, even if electroantennogram responses from olfactory sensilla were rather weak compared with those of adults of *H. obsoletus* (Sharon et al. 2005; Mazzoni et al. 2009a). Olfactory cues may act synergistically together with other kinds of sensory stimuli during host location and recognition phases (Mazzoni et al. 2009a).

Previous studies on the AL organization in Homoptera report the presence of small ALs that lack glomerular structure, although the presence of glomeruli has recently been reported in an aphid species (Kollmann et al. 2011). The 2 species investigated here present dramatically different structures both regarding the antennal sensory equipment (Romani et al. 2009; Riolo et al. 2012; Rossi Stacconi and Romani 2012, 2013) and the central nervous system organization. In *H. obsoletus*, we found well-developed ALs representing 0.60% of the brain.

### Table 1 Overview of main olfactory apparatus features

<table>
<thead>
<tr>
<th>Species</th>
<th>OSNs$^*$</th>
<th>Glomeruli</th>
<th>Brain$^b$ ($\mu m^3 \pm SD; n = 3$)</th>
<th>ALs ($\mu m^3 \pm SD; n = 8$)</th>
<th>AL/brain (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Scaphoideus titanus</em></td>
<td>16</td>
<td>—</td>
<td>$2.483 \times 10^7 \pm 0.031 \times 10^7$</td>
<td>$2.87 \times 10^7 \pm 0.2 \times 10^7$</td>
<td>0.11</td>
</tr>
<tr>
<td></td>
<td>$\sigma$</td>
<td>-13</td>
<td>$2.396 \times 10^7 \pm 0.037 \times 10^7$</td>
<td>$2.77 \times 10^7 \pm 0.12 \times 10^7$</td>
<td>0.11</td>
</tr>
<tr>
<td><em>Hyalesthes obsoletus</em></td>
<td>-2500</td>
<td>120–140</td>
<td>$3.956 \times 10^7 \pm 0.102 \times 10^7$</td>
<td>$23.53 \times 10^7 \pm 1.9 \times 10^7$</td>
<td>0.59</td>
</tr>
<tr>
<td></td>
<td>$\sigma$</td>
<td>120–140</td>
<td>$3.787 \times 10^7 \pm 0.036 \times 10^7$</td>
<td>$20.45 \times 10^7 \pm 2.2 \times 10^7$</td>
<td>0.57</td>
</tr>
</tbody>
</table>

SD, standard deviation.

$^*$Data from Rossi Stacconi and Romani (2012); Romani et al. (2009).

$^b$Excluding SOG.

### The deutocerebrum of *S. titanus*

After treatment with Karnovsky’s fixative, small ALs were identified. On the basis of 8 preparations, a visible texture resembling glomeruli borders was observed (Figure 5a) and a total of 13 glomeruli were identified. The ALs had a volume of $2.87 \times 10^7 \pm 0.2 \times 10^7 \mu m^3$ (SD; $n = 8$) in females and $2.77 \times 10^7 \pm 0.12 \times 10^7 \mu m^3 (SD; n = 8$) in males, representing 0.11% of the total brain mass in both sexes. Close to the AL, a columnar neuropil, the AMMC, was found. This neuropil region is located laterally with regard to the AL and continues posteriorly (Figure 5a–c). The posterior part of the AMMC was poorly delineated, and no clear borders to the TrC were found. The backfill stainings showed both the AMMC and the AL to be innervated by sensory axons from the antenna (Figure 5d,g,h). The antennal nerve split into 2 main tracts (Figure 5g,h). The first (T1) consisted of about 16 axons and innervated the AL region. In this area, several terminal areas of single-labeled neurons could be resolved (Figure 5e,g,h). These terminal areas belonged to at least 13 glomerular-like structures, a fact that supports the finite delineation observed in preparations using Karnovsky’s fixative (compare Figure 5a,b with Figure 5d–f). Moreover, some OSNs seemed to have 1–3 terminations ending in different areas of the AL (see Supplementary material). This means that single OSNs could innervate multiple glomerular structures (Figure 5e and Supplementary material); however, the staining quality did not allow a clear visualization of the terminal areas. The second tract (T2), containing at least 5 axons, innervated the AMMC where the axons branched moderately (Figure 5g,h). However, in this region, the Microrubry-Dextran staining was incomplete, so we could not determine whether T2 neurons always terminated in the AMMC or whether some neurons continued along the esophageal connective. Two sensory projections emanating from the inner dorsal side of the AL were filled (Figure 5f). These neurons projected ipsilaterally to the medial brain area in the PL, positioned anteroventrally to the CC.
mass (Table 1), with approximately 130 glomeruli clearly distinguishable in both sexes. On the other hand, in S. tianus, the ALs show few glomerular-like structures and are reduced in volume, representing 0.11% of the brain mass (Table 1), less than one-fifth of the ALs/brain volume ratio found in H. obsoletus, and approximately 13 glomeruli.

Compared with other investigated species, the AL/brain volume ratios we found in H. obsoletus and S. tianus are low. In Schistocerca gregaria (Orthoptera), both the ALs represent 5.6% of the total brain mass (Kurylas et al. 2008); in Apis mellifera (Hymenoptera), 5.67% in workers (Brandt et al. 2005); in Polistes dominulus (Hymenoptera), approximately 5% in foundresses (Ehmer et al. 2001), whereas in Drosophila melanogaster (Meigen) approximately 3% (Rybak 2013). H. obsoletus and S. tianus show differently sized and structured ALs, and this difference is consistent with

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**Figure 2** Comparative images between Hyalesthes obsoletus (a–c) and Scaphoideus tianus (d–f). (a and d) Ventral images of the central brain area; the upper (UCB) and lower central body (LCB) and the noduli (arrowheads) are visible at the level of the central complex. (b and e) Maximum intensity view of 7 sections taken ventrally with regard to the central complex reveals the MBs’ pedunculi. (c and f) Ventral images show the position of the TrC in both species. LAL; AMMC; and AL. Scale bars: (a–f) = 100 μm. Body axis: p: posterior; a: anterior; l: lateral.
data on their antennal sensory structures. Despite a similar gross morphology, the antennal sensory setup appears very different. In *S. titanus*, 12 sensilla are present on the flagellum and among these only 3 are olfactory sensilla (Mazzoni et al. 2009a; Rossi Stacconi and Romani 2012). A total of 17 OSNs innervate these sensilla (Rossi Stacconi and Romani 2012), making this insect comparable to other species with extremely reduced olfactory systems, like psyllids (Kristoffersen et al. 2006) and *D. melanogaster* larvae (Ramaekers et al. 2005).

As revealed in our backfills, at least 16 OSNs, forming the T1 branch of the antennal nerve, penetrated the AL in *S. titanus*.

Figure 3  (a) Three-dimensional reconstructions of the left AL of female *Hyalesthes obsoletus*, ventral view. (b–h) Confocal stack of the monoclonal nc82 staining of the same AL. Seven planes from the ventral to the dorsal side were selected every 10 μm from a total of 79 images. The left column displays the original confocal images, the right column displays demarcated glomeruli. Scale bars: (a–h) = 200 μm. Body axis: v: ventral; d: dorsal; p: posterior; a: anterior; l: lateral.
It may be that some of these OSNs arise from coeloconic sensilla located on the antenna. These sensilla show typical features of hygro-thermo and CO$_2$ receptors (Rossi Stacconi and Romani 2012). Therefore, the ALs could act as a first integration center between different types of sensory cues. Axons from CO$_2$ and thermo-sensitive sensilla projecting to specific regions of the AL have been observed in other insects. In Periplaneta americana, a small set of glomeruli receives axons from cold receptors, whereas other glomeruli are innervated by afferents from hygroreceptors (Nishikawa et al. 1995).
Figure 5  *Scaphoideus titanus*. (a–c) Frontal series of the deutocerebrum area. The AMMC starts laterally, close to the antennal nerve (AN) and continues posteriorly. The arrowheads indicate the clearly visible glomeruli in the AL. (d) Anterograde backfill from the antennae of both sides reveals primary afferents in the deutocerebrum and central brain. (e) Focused view of the same right AL where 2 glomerular-shaped terminal areas from the same olfactory receptor neuron are clearly visible (dashed lines). (f) Maximum intensity view of the right AL and part of the protocerebral lobe shows sensory projection fibers of the right antenna (SP). (g and h) Digital reconstructions of the receptor neurons penetrating the glomeruli at AL level and the AMMC. Two tracts, T1 and T2, can be recognized. T1 innervates the AL; T2 goes to the AMMC. The insets show the reconstructed AL and AMMC, LAL and TrC. Scale bars: (a–c, d, and f) = 100 μm; (e, g, and h) = 50 μm. Body axis: v: ventral; d: dorsal; p: posterior; a: anterior; l: lateral.
In some dipterans (Stocker 1994; Distler and Boeckh 1997) and in honeybees (A. mellifera L.) (Nishiino et al. 2009), glomeruli have been described to be the target of thermosensitive and CO₂-specific OSNs. In H. obsoletus, sensilla were observed on all parts of the antenna, except the scape, innervated by approximately 2500 OSNs (Romani et al. 2009; Riolo et al. 2012).

A peculiar feature in H. obsoletus is the organization of OSNs innervating placoid sensilla, arranged in neuronal units surrounded by a dendrite sheath (Riolo et al. 2012). An analogous situation has been observed in other hemipteran (Bromley et al. 1979; Romani and Rossi Stacconi 2009) and dipteran species (Jez and McIver 1980; Solinas et al. 1987). Despite this peculiar peripheral organization, we hypothesize that single glomeruli are innervated by OSNs expressing the same olfactory receptor protein in different sensilla. Unfortunately, we did not obtain any specific sensillum antennal backfills showing how afferents innervate the ALs, so this remains a future goal to investigate.

No obvious sexual dimorphism of the glomerular architecture was observed in H. obsoletus. This absence is consistent with the lack of volatile cues in the mating behavior of H. obsoletus and S. titanus. In fact, vibrational signaling has been demonstrated to mediate sexual communication in both species; such signaling is sensed and processed by sensory cells in the pedicel targeting the AMMC (Mazzoni et al. 2009b; Mazzoni et al. 2010). In S. titanus, the T2 branch of the antennal nerve projects mainly to the AMMC. A few neuronal fibers from the T2 bypass the AMMC and continue along the esophageal connective. These afferents could derive both from the mechanical proprioreceptors and exteroceptors located on the antennae. Nonolfactory afferents targeting the AMMC or the SOG have been extensively described in other Hemiptera (Barth 1976; Insausti and Lazzari 2000; Kristoffersen et al. 2008; Barrozo et al. 2009; Kollmann et al. 2011), Blattodea (Nishikawa et al. 1995), Lepidoptera (Jørgensen et al. 2006), Diptera (Ignell et al. 2005), and Hymenoptera (Maronde 1991; Ai et al. 2009). The ipsilaterally ascending primary sensory fibers lead to the protocerebral lobe and end ventrally to the central complex just where the pedunculi of the MBs are located. These fibers may consist in transsynaptically filled PNs transmitting the processed information to higher brain centers. Similar projections were observed by Dacks et al. (2006) in 2 hemipteran species, Oncopeltus fasciatus (Lygaeidae) and Largus cinctus (Largidae). However, since no somata have been stained near the AL, these ipsilateral neural projections could also stem directly from the antennae, as has been found in locusts (Ignell et al. 2001), in Hymenoptera (J. Rybak, unpublished results), and in dragonflies (Rebora et al. 2013).

We provide a comparative study of 2 species representative of Auchenorrhyncha, a neopteran group for which no detailed data regarding the structure of the primary olfactory centers are as yet available. The results show the presence of morphologically distinct glomeruli in Homoptera, contrary to what most previous studies in the literature claim. The differences in the structure of the ALs between the 2 examined insects are evident, confirming our initial hypothesis. In the polyphagous species, H. obsoletus, the olfactory-based discrimination process between several hosts requires a more developed olfactory sensory setup on the antennae and, consequently, a more complex olfactory integration center at the brain level. In contrast, the monophagous S. titanus spends its entire lifecycle on grapevines; therefore, the low numbers of olfactory sensilla on the antennae and of ALs are consistent with the perception that a single host plant has few VOCs. Our data form a base for future research that focuses on the specific characterization of the spatial organization of the ALs of these insect groups.

Supplementary material
Supplementary material can be found at [http://www.chemse.oxfordjournals.org](http://www.chemse.oxfordjournals.org)

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