Odonatologica 39(3): 235-241 *September 1, 2010*

A SCANNING ELECTRON MICROSCOPE STUDY OF THE ANTENNAL SENSILLA IN ADULT ZYGOPTERA

S. PIERSANTI, M. REBORA* and E. GAINO
Dipartimento di Biologia Cellulare e Ambientale, Università di Perugia,
Via Elce di Sotto, I-06123 Perugia, Italy

Received October 18, 2009 / Revised and Accepted March 26, 2010

Scanning electron microscope studies of the antennal flagella of *Coenagrion puella* and *Ischnura elegans* (Coenagrionidae), *Platycnemis pennipes* (Platycnemididae), *Lestes barbarus*, *L. viridis* (Lestidae), *Calopteryx virgo* and *C. haemorrhoidalis* (Calopterygidae) reveal the presence of pits containing sensilla on the latero-ventral side of the antenna. All these pits are the opening of deep cavities bearing the same sensilla previously described on Anisoptera antennae. These sensilla are represented by: (i) coeloconic porous sensilla, visible on the antennal surface, whose structure is in agreement with that reported for single walled olfactory receptors, and by (ii) two types of sensilla styloconica (type-1 and type-2), located at the bottom of the cavities and sharing common features typical of thermo-hygroreceptors. The present data allow us to extend previous considerations on the sensory role of the dragonfly antennae to the whole order Odonata, suggesting that olfaction, together with the ability to perceive temperature and humidity, are the main sensory functions of the antennae of these insects.

INTRODUCTION

An early overview of the sensory structures located on the antennal flagellum of Odonata reported the presence of coeloconic sensilla located in pits in both the suborders, Anisoptera and Zygoptera (SLIFER & SEKHON, 1972). Recently, more detailed fine structure (SEM, TEM) investigations on some Anisoptera species (REBORA et al., 2008; 2009a) revealed that these sensilla belong to three different types. One consists of single-walled, olfactory coeloconic sensilla (equipped with pores and pore tubules) visible on the antennal surface; the other two are sensilla styloconica (type-1 and type-2), sharing common features typi-

* **Corresponding author**: rebora@unipg.it, – tel. ++390755855729, – fax ++390755855733

cal of thermo-hygroreceptors and located at the bottom of cavities, which are evident as simple openings on the antennal surface.

On the basis of these data, the present research aims to investigate whether the sensilla described in Anisoptera are also present in Zygoptera. The results presented here are part of a research programme on the fine structure of the antennal sensilla in adult Odonata (REBORA et al., 2008; 2009a).

MATERIAL AND METHODS

Adults of both sexes of *Coenagrion puella* and *Ischnura elegans* (Coenagrionidae), *Platycnemis pennipes* (Platycnemidae), *Lestes barbarus* and *L. viridis* (Lestidae) and *Calopteryx virgo* and *C. haemorrhoidalis* (Calopterygidae) were collected in Umbria (central Italy) during summer 2008.

In the laboratory, antennal flagella were dissected from anaesthetised specimens and fixed for 12 hours in 2.5% glutaraldehyde in cacodylate buffer at pH 7.2. The fixed material, repeatedly rinsed in the same buffer, was then dehydrated by using a graded series of ethanols, followed by critical-point drying in a critical-point dryer CPD 030 Bal-Tec (Bal-Tec Union Ltd., Balzers, Liechtenstein). Specimens were mounted on stubs with silver conducting paint, sputter-coated with gold-palladium in a sputterer Emitech K550X (Emitech, Ashford, England), and observed with a Philips XL30 scanning electron microscope (Philips, Eindhoven, the Netherlands), at an accelerating voltage of 18kV.

For observations of the inner cuticular wall of the antenna, the dissected flagella were longitudinally sectioned with a razor blade and cleaned with KOH-solution. The flagellar cuticle was then dehydrated in a graded ethanol series, dried in an oven and mounted as above.

RESULTS

Zygoptera antennae consist of a scape, a pedicel and a monoarticulated flagellum. In all the analyzed species the latero-ventral side of the flagellum shows pits that represent the opening of cavities. On the edge of the pits coeloconic sensilla, characterized by a porous cuticle, are visible on the antennal surface while at the bottom of the cavities both types of deeply sunken sensilla styloconica are located. Type-1 sensilla have a cone with cuticular fingers in the distal portion and type-2 sensilla have a cone with an irregular rugged cuticular surface. No pore and no socket are present in either types. The pits are mostly concentrated on the proximal portion of the flagellum and differ in shape and abundance in the different families. No obvious differences were observed between the examined species belonging to the same family. No intra-specific differences have been observed between males and females.

COENAGRIONIDAE (*Coenagrion puella* and *Ischnura elegans*) – There are about five pits containing sensilla, located on small bulges arranged along the antennal surface (Fig 1a). Images of the inner cuticular wall reveal that the pits are the opening of wide and shallow cavities (about 30 μm in width and 10 μm in depth), which host sensilla coeloconica just inside the rim of the cavities (Figs 1b, d) and several deeply sunken type-1 and type-2 sensilla styloconica (Figs 1c, e, f).

PLATYCNEMIDIDAE (*Platycnemis pennipes*) – There are about five pits with sensilla. They are the opening of narrow and deep convoluted cavities (about 5-10 µm in width and 30-40 µm in depth) (Figs 2a-b), bearing coeloconic sensilla (Figs 2b,c) and deeply sunken type-1 and type-2 sensilla styloconica (Fig. 2b) as above. Each cavity is parallel to the longitudinal axis of the antenna (Fig. 2a).

LESTIDAE (*Lestes barbarus* and *L. viridis*) – The arrangement is the same as in *P. pennipes* with four or five pits opening into narrow and deep convoluted cavities (about 5 µm in width and 30-40 µm in depth), which host coeloconic sensilla (Figs 2d, e) and both types of deeply sunken sensilla styloconica (Fig. 2f). Each cavity is parallel to the longitudinal axis of the antenna.

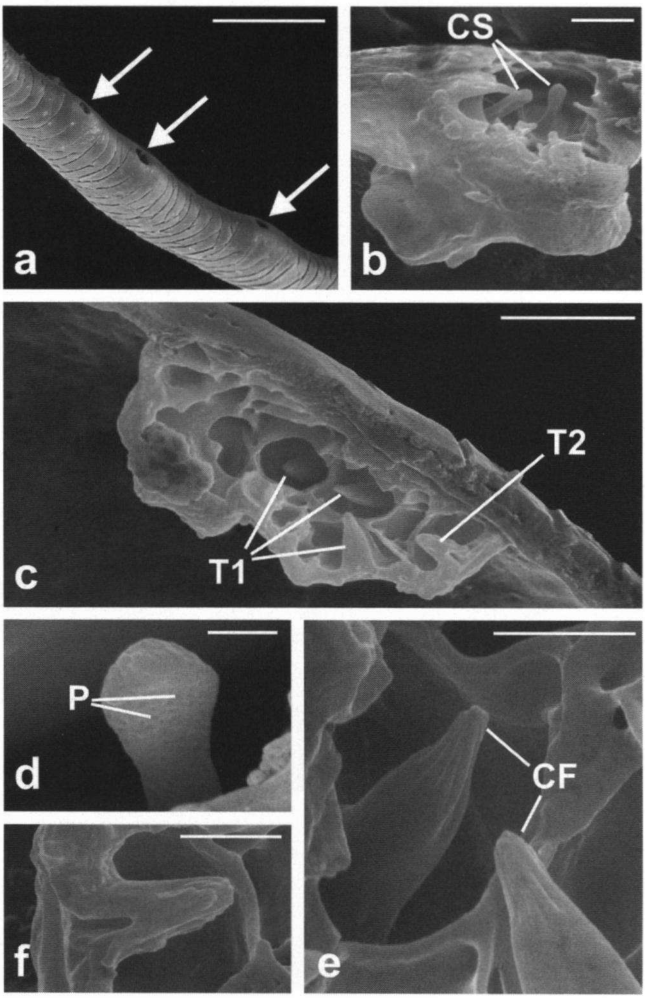

Fig. 1. Scanning electron micrographs of the antennal flagellum of *Coenagrion puella* (Coenagrionidae); (a) outer cuticular wall; arrows point to the pits located on small bulges. Scale bar = 50 µm; – (b) inner cuticular wall showing one of the cavity with coeloconic sensilla on its edge. Scale bar = 5 µm; – (c) view of a fractured cavity showing type-1 and type-2 deeply sunken sensilla styloconica. Scale bar = 10 µm; – (d) detail of a coeloconic sensillum showing pores on the peg surface. Scale bar = 1 µm; – (e) detail of type-1 deeply sunken sensilla styloconica showing the cuticular fingers at the apex of the cone. Scale bar = 2.5 µm; – (f) detail of type-2 deeply sunken sensillum showing the irregularly rugged surface of the cone. Scale bar = 2,5 µm. – [CF: cuticular fingers; – CS: coeloconic sensilla; – P: pores; – T1: type-1 deeply sunken sensilla styloconica; – T2: type-2 deeply sunken sensillum styloconicum]

CALOPTERYGIDAE (*Calopteryx virgo* and *C. haemorrhoidalis*) – There are about 10 pits containing sensilla and they may be isolated or gathered to form small groups of two/three (Fig. 3a). These pits are the openings of narrow and shallow cavities (about 10-15 μm in width and 15-20 μm in depth) (Fig. 3b), which host coeloconic sensilla just inside the rim of the cavities (Fig. 3c) and deeply sunken type-1 and type-2 sensilla styloconica (Figs 3b, d, e).

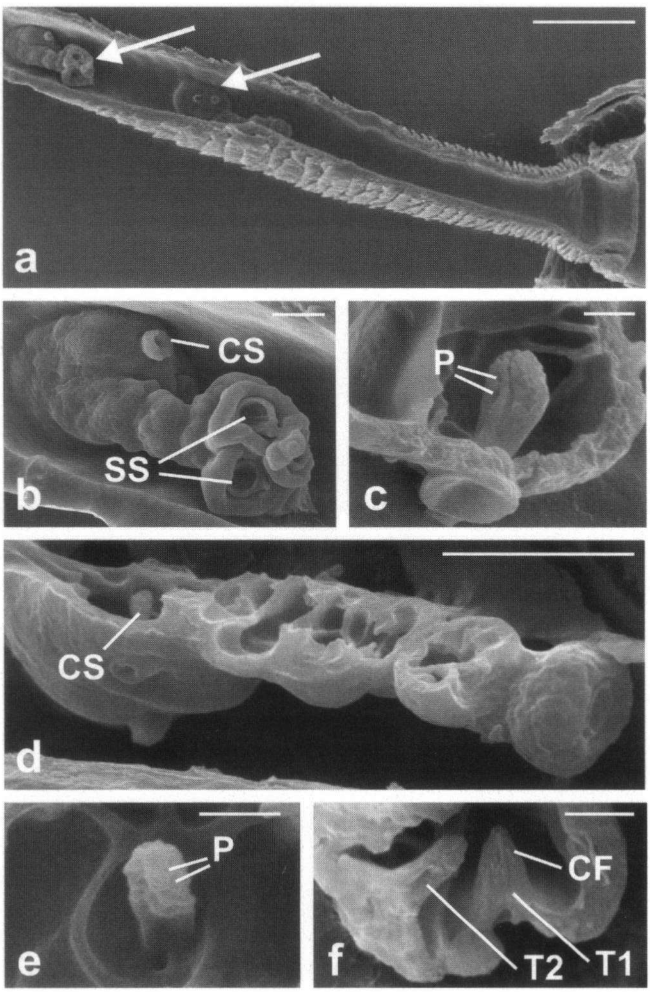

Fig. 2. Scanning electron micrographs of the antennal flagella of *Platycnemis pennipes* (Platycnemididae) (a-c) and *Lestes barbarus* (Lestidae) (d-f): (a) inner cuticular wall showing the cavities parallel to the longitudinal axis of the antenna (arrows). Scale bar = 30 μm; – (b) a cavity bearing a coeloconic sensillum on its edge and two sensilla styloconica at the bottom. Scale bar = 5 μm; – (c) detail of a coeloconic sensillum with pores on the cuticle. Scale bar = 2 μm; – (d) a longitudinally fractured cavity showing a coeloconic sensillum on its edge. Scale bar = 10 μm; – (e) detail of the coeloconic sensillum showing its porous cuticle. Scale bar = 2 μm; – (f) detail of type-1 and type-2 sensilla styloconica at the bottom of a cavity. Scale bar = 2 μm. – [CF: cuticular fingers; – CS: coeloconic sensillum; – P: pores; – SS: styloconic sensilla; – T1: type-1 deeply sunken sensillum styloconicum; – T2: type-2 deeply sunken sensillum styloconicum]

DISCUSSION

The present fine structural investigation on *Coenagrion puella* and *Ischnura elegans* (Coenagrionidae), *Platycnemis pennipes* (Platycnemididae), *Lestes barbarus* and *L. viridis* (Lestidae) and *Calopteryx virgo* and *C. haemorrhoidalis* (Calopterygidae), confirms the presence on the Zygoptera antennae of the same sensory structures already described in Anisoptera (REBORA et al., 2008; 2009a). Thus, damselfly flagella are equipped with porous sensilla coeloconica, already hypo-

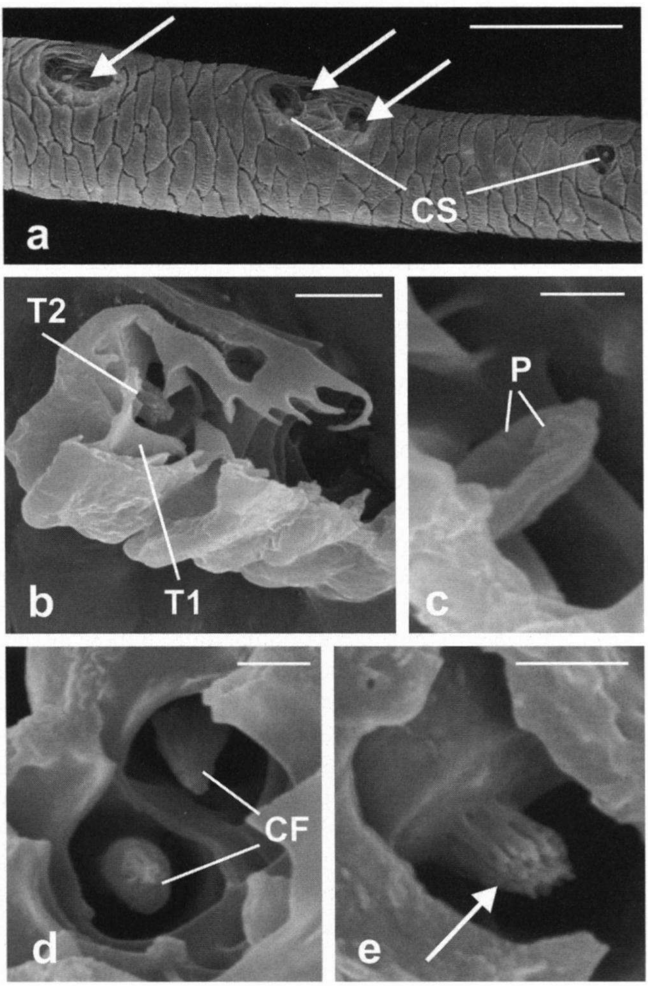

Fig. 3. Scanning electron micrographs of the antennal flagella of *Calopteryx haemorrhoidalis* (a) and *C. virgo* (b-e) (Calopterygidae): (a) outer cuticular wall; arrows point out the pits with coeloconic sensilla visible on their edge. Scale bar = 50 µm; – (b) detail of a fractured cavity: type-1 and type-2 deeply sunken sensilla styloconica are visible on the bottom. Scale bar = 5 µm; – (c) detail of a coeloconic sensillum showing the cuticular pores. Scale bar = 2 µm; – (d) detail of type-1 deeply sunken sensilla styloconica showing the cuticular fingers at the apex of the cone. Scale bar = 2 µm; – (e) detail of type-2 deeply sunken styloconic sensillum showing the irregularly rugged cuticular surface (arrow). Scale bar = 3 µm. – [CF: cuticular fingers; – CS: coeloconic sensilla; – P: pores; – T1: type-1 deeply sunken sensillum styloconicum; – T2: type-2 deeply sunken sensillum styloconicum]

thesized to be olfactory receptors in Anisoptera (REBORA et al., 2008; 2009a), and two deeply sunken sensilla styloconica, namely type-1, characterized by cuticular fingers, and type-2, characterized by a rugged cuticular surface, hypothesized to be thermo-hygroreceptors in Anisoptera (REBORA et al., 2008; 2009a).

The distribution of these sensilla is very similar in all the zygopteran species analyzed whereas in Anisoptera some differences emerged among the families examined (REBORA et al., 2008; 2009a). In the Zygoptera the sensilla are located in pits mostly concentrated in the proximal portion of the flagellum, along its latero-ventral side; all the pits open into deep cavities hosting the porous coeloconic sensilla on their edge and both kinds of sensilla styloconica (type-1 and type-2) at their bottom. This morphological uniformity could be relevant when considering the debate about the zygopteran monophyletic/paraphyletic origin: the first hypothesis supported by morphological data (REHN, 2003), and the second one sustained by molecular investigations (HASEGAWA & KASUYA, 2006).

The present data reinforce some functional and phylogenetic considerations already proposed for the sensory role of the dragonfly antennae (REBORA et al., 2008; 2009a), which can be extended to the whole order Odonata. In particular:

- The small and setaceous antennae of damselflies and dragonflies seem to play a relevant role in environment perception. Notably, thermo-hygroreception and olfaction could be important sensory abilities in Odonata. Thermo-hygroreception is intuitively relevant for aquatic insects for which thermoregulation is very important (MAY, 1976; McKEY & HERMAN, 2008) but the role of olfaction is more difficult to hypothesize about. In both cases the biological significance of these abilities needs to be better investigated with behavioural and electrophysiological researches.
- The widespread presence of putative olfactory sensilla in Anisoptera (REBORA et al., 2009a) and Zygoptera, together with the description of similar sensory structures in the mayfly *Rhithrogena semicolorata* (REBORA et al., 2009b), raises relevant questions on the ability of Paleoptera (Odonata and Ephemeroptera) to perceive odours. Indeed, these insects have been traditionally considered anosmic, because their brains lack glomerular antennal lobes and mushroom body calyces, typically involved in Neoptera odour perception (STRAUSFELD et al, 1998; FARRIS, 2005). Recent neuroanatomical studies report that Odonata possess a remnant calyx but it is claimed that it is probably related to the visual ability of this group (SVIDERSKY & PLOTNIKOVA, 2004; STRAUSFELD et al., 2009). Because these taxa are considered to be the oldest pterygote insects, studies on Paleoptera olfaction could contribute significantly to trace evolutionary trends in insect odour perception and also be relevant for phylogenetic considerations.

ACKNOWLEDGEMENTS

We are grateful to Dr FRANCESCA SCOCCIA for her helpful contribution in insect collecting. This research was supported partially by FISR MICENA funds.

REFERENCES

FARRIS, S.M., 2005. Evolution of insect mushroom bodies: old clues, new insights. *Arthropod. Struct. Dev.* 34: 211-234.

HASEGAWA, E. & E. KASUYA, 2006. Phylogenetic analysis of the insect order Odonata using 28S and 16S rDNA sequences: a comparison between data sets with different evolutionary rates. *Ent. Sci.* 9: 55-66.

MAY, M.L., 1991. Thermal adaptations of dragonflies, revisited. *Adv. Odonatol.* 5: 71-88.

McKAY, T. & T. HERMAN, 2008. Thermoregulation in three species of damselflies, with notes on temporal distribution and microhabitat use (Zygoptera: Lestidae). *Odonatologica* 37(1): 29-39.

REBORA, M., S. PIERSANTI & E. GAINO, 2008. The antennal sensilla of the adult of Libellula depressa (Odonata: Libellulidae). *Arthropod. Struct. Dev.* 37: 504-510.

REBORA, M., S. PIERSANTI & E. GAINO, 2009a. A comparative investigation on the antennal sensilla of adult Anisoptera. *Odonatologica* 38(4): 329-340.

REBORA, M., S. PIERSANTI & E. GAINO, 2009b. The antennal sensilla of adult mayflies: Rhithrogena semicolorata as a case study. *Micron* 40: 571-576.

REHN, A.C., 2003. Phylogenetic analysis of higher-level relationships of Odonata. *Ent. Sci.* 28: 181-239.

SLIFER, E.H, & S. SEKHON, 1972. Sense organs on the antennal flagella of damselflies and dragonflies (Odonata). *Int. J. Insect Morphol. Embryol.* 1: 289-300.

STRAUSFELD, N.J., L. HANSEN, Y. LI, R.S. GOMEZ & K. ITO, 1998. Evolution, discovery, and interpretations of arthropod mushroom bodies. *Learn. Memory* 5: 11-37.

STRAUSFELD, N.J., I. SINAKEVITCH, S. BROWN & S. FARRIS, 2009. Ground plan of the insect mushroom body: functional and evolutionary implications. *J. comp. Neurol.* 513: 265-291.

SVIDERSKY, V.L. & S.I. PLOTNIKOVA, 2004. On structural-functional organization of dragonfly mushroom bodies and some general considerations about purpose of these formations. *J. Evol. Biochem. Physiol.* 40: 608-624.