

Larval Antennal Sensilla in Water-Living Insects

ELDA GAINO* AND MANUELA REBORA

Dipartimento di Biologia Animale ed Ecologia, 06123 Perugia, Italy

KEY WORDS sensory systems; ultrastructure; hemimetabolous; holometabolous; freshwater larvae

ABSTRACT An overview of larval antennal sensilla in hemimetabolous and holometabolous water-living insects is given by updating current knowledge on the fine structure of these sensory systems. In the absence of successful electrophysiological studies, the possible function of sensilla is deduced from their architecture. Various kinds of sensilla are described in hemimetabolous insects, such as Ephemeroptera, Odonata, and Plecoptera, and holometabolous insects, such as Diptera Nematocera (Culicidae, Simuliidae, Psychodidae, Chaoboridae) and Trichoptera. Their possible function in responding to stimuli from the freshwater environment is illustrated and discussed. The importance of sensilla as taxonomic and phylogenetic traits is reported in Baetidae (Ephemeroptera) and in Diptera Nematocera. Some homologies outlined in Diptera Nematocera give evidence of a link between species, such as Chaoboridae and Psychodidae, whose antennae are highly modified, prehensile in the former and very reduced in the latter. Particular features, such as cuticle without pores in chemosensory sensilla and naked perikarya, are so far found exclusively in some water-living arthropods, thus reflecting a possible adaptation to the aquatic habitat. The structure of sensilla and chloride cells, which have a similar external morphology, is presented and discussed in various insect groups, considering the possible derivation of the chloride cells from sensilla. *Microsc. Res. Tech.* 47:440–457, 1999. © 1999 Wiley-Liss, Inc.

INTRODUCTION

Insect sensilla have been the object of intense scrutiny. Knowledge regarding their fine structure in the larval forms is, however, limited, even in species whose immature stages dominate the life cycle.

Most work, both morphological and physiological, has been done in adults that are commonly considered to need more information from the environment than larvae, which are generally restricted in habitat and mobility (Zacharuk and Shields, 1991).

Investigations of sensilla of water-living larvae have a special relevance in relation to the adaptation of these insects to the aquatic environment.

Current interest on larval sensilla of water-living insects has been focused on different body parts in which these structures may perform specific functions. Food selection and gustation are of primary importance to sustain growth and development of the voracious immature stages (Zacharuk and Shields, 1991). In this respect, some attention has been paid to mouthpart sensilla in Odonata (Bassemir and Hansen, 1980; Pritchard, 1965), Plecoptera (Kapoor, 1989), Diptera Simuliidae (Craig and Borkent, 1980), and Culicidae (McIver and Beech, 1986), involved in prey finding behaviour. Peculiar flower-shaped sensilla, present on the body surface of a stonefly nymph, gather information regarding flow rate and the chemical nature of water, thereby influencing movements of the nymphs (Kapoor and Zachariah, 1983, 1984).

Antennae are typically considered the main site for the reception of environmental stimuli (Zacharuk, 1985). Despite the relevant function they may perform, larval antennal sensilla have been studied only in some groups of aquatic insects: Ephemeroptera (Gaino and

Rebora, 1996, 1997, 1998, 1999), Plecoptera (Kapoor, 1985, 1987, 1991), Diptera Culicidae (Jez and McIver, 1980; Zacharuk and Blue, 1971a,b; Zacharuk et al., 1971), Diptera Simuliidae (Craig and Batz, 1982), Diptera Psychodidae (Seifert et al., 1990), Diptera Chaoboridae (Nicastro et al., 1995). A comparative study on some larval antennal sensilla carried out on numerous dipteran families, most of them aquatic, has been recently presented by Nicastro et al. (1998), who stressed that changes in the internal features of sensilla can be used as a tool for cladistic analyses.

Electrophysiological investigations are essential for assigning a definitive sensory function to any kind of sensillum, but no successful electrophysiological studies have been performed so far on larval antennal sensilla of water-living insects. As a consequence, functional interpretation of sensilla is mainly derived from their fine structure.

The commonly used procedures to study sensillar morphology are scanning electron microscopy (SEM) for investigating the external shape, and transmission electron microscopy (TEM) for the internal organisation allowing a putative function to be assigned to the sensillum. Permeable areas of the sensilla are analysed using both crystal violet (Slifer, 1960) and reduced silver (Schafer and Sanchez, 1976) methods.

The goal of this review is to provide an overview of larval antennal sensilla in hemimetabolous and holometabolous aquatic insects.

Contract grant sponsor: University of Perugia; Contract grant number: Progetto di Ateneo.

*Correspondence to: Prof. Elda Gaino, Dipartimento di Biologia Animale ed Ecologia, Via Elce di Sotto, 06123 Perugia, Italy. E-mail: gaino@unipg.it

Received February 1999; accepted in revised form June 1999.

**ULTRASTRUCTURAL ORGANISATION
OF LARVAL SENSILLA IN
HEMIMETABOLOUS INSECTS
Ephemeroptera**

Ephemeroptera are the most primitive winged insects and spend most of their life cycle as immature stages in water where they represent a fundamental level of the trophic chain.

A first approach on non-antennal sensilla in mayflies was reported by Eastham (1936) who described sensilla campaniformia on the gills of nymphs of the genus *Caenis*. In the tracheal gill epithelium of a few mayfly species, Wichard and Komnick (1971) visualised special cells, called chloride cells, which are involved in osmoregulation. Their morphology was very close to that of the devices present on the tracheal gills of *Palingenia longicauda*, called sensilla campaniformia by Csoknya and Halász (1972). Detailed investigation on these structures extended to several mayfly species (Filshie and Campbell, 1984; Komnick et al., 1972; Wichard and Komnick, 1973; Wichard et al., 1972, 1975) removed any doubt about their actual belonging to chloride cells and not to sensilla.

The first ultrastructural study on the morphological organisation of antennal sensilla in mayfly nymphs was carried out by Schmidt (1974) on *Ephemerella* sp. The author described the structure of an inner ring of mononematic scolopidia located on the pedicel. Each mononematic scolopidium is commonly innervated by two sensory cells and three accessory cells (scolopale cell, trichogen cell, tormogen cell). The scolopidia have here been proposed as homologous of the Johnston's organ of other insects, thereby performing a mechano-sensory function allowing insects to sense the movement of the flagellum over the pedicel (for review see McIver, 1985).

A survey at the ultrastructural level carried out mainly under SEM of antennal sensilla of a few species highlighted that nymphs have a variety of sensilla, mainly located on the pedicel and flagellum (Fig. 1a-c), while adult antennae are strongly reduced and their morphology changes even from subimago to imago, in which sensilla are very few and less diversified (Gaino and Reborá, 1997).

Sensilla Trichodea. The most common sensilla in the nymph are sensilla trichodea single or in groups, located mostly along the distal border of each flagellar segment (Fig. 1c,d). In *Choroterpes picteti*, longitudinal sections observed under TEM revealed the presence of a tubular body ending at the base of the hair and two dendrites extending along the shaft (Fig. 1e and inset). This organisation is consistent with a chemo-mechano-sensory function even if, at present, we have no data about the occurrence of pores in the cuticle. Nevertheless, the lack of pores does not preclude a sensory function, since aquatic insects could sense the environment by simple diffusion of chemical compounds through the cuticle (Zacharuk et al., 1971).

Flat-Tipped Sensilla. A similar chemo-mechano-sensory function has been attributed to a peculiar kind of sensillum called flat-tipped sensillum, which is characterised by an apical pore (Fig. 1b and inset). This sensillum is present along the distal border of the antennal articles in the nymphs of some Heptageniidae

and of Baetidae (Gaino and Reborá, 1996, 1997, 1998, 1999).

Among Baetidae, the general shape and fine organisation of the flat-tipped sensillum have been studied in *Baetis rhodani*. In this species, the flat-tipped sensillum emerges from a well-defined socket located among the distal lobes of each antennal article and bends toward the antennal axis, contacting the next adjacent article to form a bridge connecting the two (Fig. 2a,b). The apical part of the sensillum greatly enlarges to give rise to a thin flange, closely apposed to the cuticular surface (Fig. 2b), where the pore is located (Fig. 2b, inset). On the whole, the sensillum assumes the shape of a spatulate bristle. This type of sensillum is also present on scape and pedicel, cerci and urotergites. Wherever located, it keeps the typical bent configuration, being differently oriented on the antennal segments and on the rest of the nymph body. In the antennal segments, the flat-tipped sensilla extend from the basal region to the tip whereas on the rest of the body they are oriented backwards.

In the heptageniids *Rhithrogena loyolae* and *Epeorus sylvicola*, this sensillum type differs slightly from that of *B. rhodani*. Indeed, the flange has an arrowhead shape and consists of two lateral expansions and an apical pore where they join together (Fig. 1b and inset). Sections of the flat-tipped sensillum of *B. rhodani* showed the presence of a tubular body (Fig. 2c) and dendrites extending along the shaft (Fig. 2d). These features corroborate a possible double chemo-mechano-sensory function of this sensillum (Gaino and Reborá, 1998).

Among a large variety of mechanisms evolved for adapting organisms to live in lotic waters, chemo-mechanosensory sensilla are relevant sensors owing to their dual modality. Indeed, as chemosensitive devices scattered on different body parts, they should be regarded as specialised structures providing gustatory input for the reception of environmental food resources; as mechanosensitive devices, their function could be linked to what is termed "wall effect" (Vogel, 1981), since they act as flow receptors through which the insects can perceive any nearby object (Wolfe and Zimmerman, 1984).

In addition to the physiological function performed by this kind of sensillum, a survey on its presence/absence in almost all the European genera belonging to the family Baetidae, has revealed that it is a useful trait for taxonomic and phylogenetic studies (Gaino and Reborá, 1999).

Other Sensilla in Ephemeroptera. In the nymph of *B. rhodani*, other kinds of sensilla have been described (Gaino and Reborá, 1998), such as sensilla chaetica, in the form of thick bristles set in a socket, which emerge from the ventral surface of scape and pedicel. They are delimited by an indented border (Fig. 2e). A tubular body and a joint membrane support a mechanosensory function. A ring of sensilla campaniformia is present along the distal border of the pedicel and permits the sensing of the movement of the flagellum in relation to the pedicel. Sensilla basiconica in groups of three or four elements are present on scape, pedicel and flagellum, and due to the dendrites along their shaft they are supposed to be chemosensors.

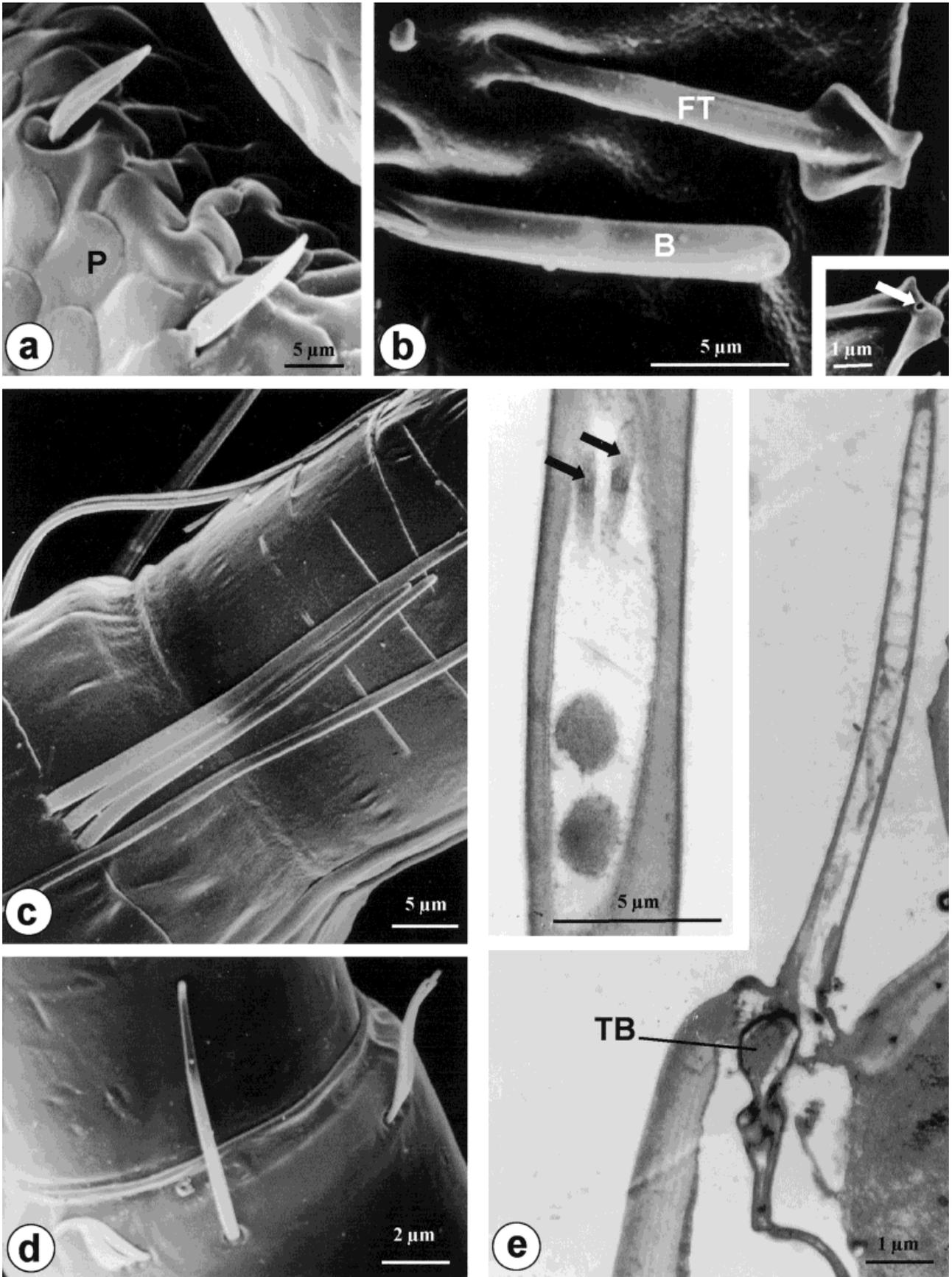


Fig. 1. SEM (a–d and inset in b) and TEM (e and inset) view of various kinds of antennal larval sensilla in Ephemeroptera. **a**: Sensilla chaetica on the distal portion of the pedicel (P) of *Siphonurus lacustris*; **b**: Flat-tipped sensillum (FT) and sensillum basiconicum (B) on the flagellum of *Rhithrogena loyolaea*. Note the pore (arrow) of the flat-tipped sensillum in the inset; **c**: Sensilla trichodea on the flagellum of *Habrophlebia eldae*; **d**: Sensilla trichodea on the flagellum

of *Choroterpes picteti*; **e**: Longitudinal section of a sensillum trichodeum in *C. picteti* showing the tubular body (TB) and the two dendrites (arrows) along the shaft (inset). Modified from Gaino E, Reborra M. 1997. Antennal cuticular sensilla in some mayflies (Ephemeroptera). In: Landolt P, Sartori M, editors. Ephemeroptera & Plecoptera: Biology-Ecology-Systematics. Fribourg: MTL. pp. 317–325.

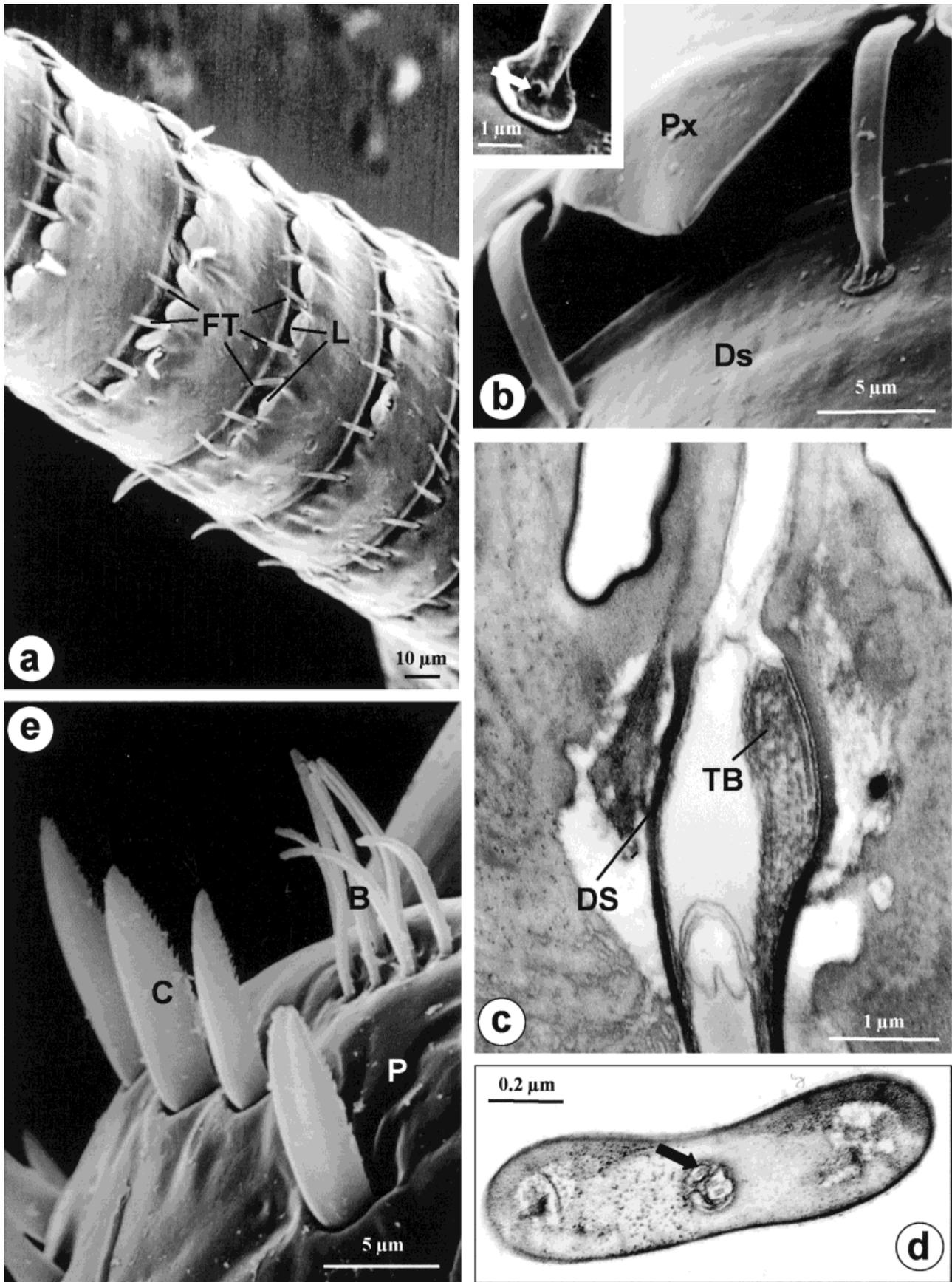


Fig. 2. SEM (a, b and inset, e) and TEM (c,d) view of various kinds of sensilla in the nymph of *Baetis rhodani*. **a**: Flagellum showing the regular arrangement of flat-tipped sensilla (FT) located among the cuticular lobes (L) on each flagellar segments; **b**: Flat-tipped sensilla forming a bridge between the proximal (Px) and the distal (Ds) flagellar segments. Note the apical pore (arrow) of the sensillum in the inset; **c**: Longitudinal section of the basal region of a flat-tipped

sensillum. Note the tubular body (TB) and the dendritic sheath (DS); **d**: Transversal section of the flat-tipped sensillum at the level of the shaft. Note the dendrites in the middle (arrow); **e**: Sensilla chaetica (C) and a group of sensilla basiconica (B) on the pedicel (P). Modified from Gaino E, Reborá M. 1998. Ultrastructure of the antennal sensilla of the mayfly *Baetis rhodani* (Pictet) (Ephemeroptera: Baetidae). *Int J Insect Morphol & Embryol* 27:143–149.

We have described three other kinds of sensilla on the flagellum (Gaino and Rebora, 1999). They include: (1) sensilla coeloconica, of pear-shaped appearance with a depression in the middle, located among the cuticular antennal lobes of the flagellum; (2) coeloconic-like sensilla found close to the cuticular lobes; (3) cuticular pits on the lateral surface. At present, we have no data on their internal organisation.

Odonata

Odonata, together with Ephemeroptera, represent the phylogenetically oldest pterygote insects. Like mayflies they have a long aquatic development but differ in having long-lived adults. Studies on the sensilla of the nymphs of this insect order are restricted to the mouthparts related to their specialisation for catching prey (Bassemir and Hansen, 1980; Chapman, 1982; Pritchard, 1965). No investigation has been devoted to the antennal sensilla. Chapman (1982) in a review on insect chemoreception mentions the occurrence of four or five sensilla on the tip of the antenna of *Aeshna* species nymphs, simply by reporting the old observations of Zawarzin (1912).

A preliminary study has been carried out by us on nymphs of *Libellula depressa**. The antenna consists of scape, pedicel, and a flagellum composed of five segments. Sensilla trichodea (long and short) are widely distributed on all the antennal segments. Among them sensilla basiconica are present. Sensilla coeloconica (4–5 per segment) are located on the ventral side of the first and second flagellar segments and at the tip of the flagellum.

Sensilla Coeloconica. The last flagellar segment of the nymphal antennae of *L. depressa* ends in a blunt tip (Fig. 3a) bearing a subterminal sensillum coeloconicum in the form of a globular peg sunken in a cuticular oblong pit (Fig. 3a). This sensillum is a compound sensillum innervated by two groups of three neurons filling the lumen of the peg (Fig. 3b); each group is wrapped by its dendritic sheath enveloping the outer dendritic segments and ending at the base of the peg (Fig. 3c). The peg shows an inner electron-dense coat (Fig. 3c). In longitudinal section, the cuticular surface of the peg is irregularly interrupted (Fig. 3c) in such a way that in tangential section it appears as a pierced cuticular surface (Fig. 3d and inset I). Longitudinal sections at higher magnification reveal that interruptions of the cuticular covering give rise to wide pore-like structures (inset II of Fig. 3d). These wide pore-like structures appear deep into the electron-dense coat so that the dendrites are separated from the outside only by a thin layer including electron-dense dotted material (inset II of Fig. 3d). The two groups of three dendrites within the peg are well separated from each other whereas the dendrites of each group are in close contact

(Fig. 3b). The cuticle extends to envelop the apical portion of the outer dendritic segments (Fig. 3d). The ciliary constrictions with the ciliary rootlets are located far from the pit, thus giving rise to a very long outer dendritic segment (Fig. 3d). There is no articulation or specialised socket cuticle at the base of the peg. No pore channel system or any clear connection of the dendrites to the outside is evident. We have no data on the electrophysiology of the coeloconic sensilla of the nymph of *L. depressa*. However, on a morphological basis, we surmise a chemosensory function for this sensillum. Indeed, the interruptions of the cuticle described in this species are very similar to the pores of the wall of simple and compound coeloconic pit pegs located on the antennal flagella of adult damselflies and dragonflies, and interpreted as chemosensory devices by Slifer and Sekhon (1972). Likewise, we can hypothesise a possible diffusion of chemical compounds through the electron-dense coat delimiting the dendrites. On this account, adults and nymphs could have the same kind of sensillum, thus being active in different environments. Sensilla working in different environments have been described in the antenna of the diving beetle *Graphoderus occidentalis* by Jensen and Zacharuk (1992) and consist of multiporous olfactory sensilla sensitive to low concentrations of chemicals in both air and water. This assumption was mainly based on morphological studies and on previous electrophysiological investigations carried out by Behrend (1971) on the multiporous sensilla of *Dytiscus marginatus*. The liquor in the pore channel system of these sensilla was proposed to be both hydrophilic and lipophilic in transporting molecules of stimulant to the dendritic terminations.

Plecoptera

Important contributions to the structure of antennal larval sensilla have been given by Kapoor for the stonefly nymph *Paragnetina media* (Kapoor, 1985, 1987, 1991). The antennal segments are formed by scape, pedicel, and a long flagellum consisting of 76–81 segments.

Sensilla Trichodea. SEM images of the flagellar surface shows two kinds of sensilla trichodea (Fig. 4a). One is represented by long wavy hairs forming overlapping rows along the lateral surface of the entire flagellum, the other by straight hairs organised in a single row on the first 7–10 basal flagellar segments. The lack

*Immediately after dissection, selected material was fixed in Karnovsky's medium (1965) in cacodylate buffer, pH 7.2, for 1 hour, repeatedly rinsed in the same buffer and postfixed in 1% osmium tetroxide for 1 hour. For SEM observations, the specimens were dehydrated using an ethanol gradient, followed by critical-point drying in a CO₂ Pabisch CPD apparatus. Specimens were mounted on stubs with silver conducting paint, sputter-coated with gold-palladium in a Balzers Union Evaporator and observed with a Philips EM 515 scanning electron microscope. For TEM analysis, the tissue was dehydrated in the graded ethanol series and embedded in Epon-Araldite mixture resin. Thin sections, obtained with a Reichert ultramicrotome, were collected on formvar-coated copper grids, stained with uranyl acetate and lead citrate. The thin sections were examined with Philips EM 400 and EM 208 electron microscopes.

Fig. 3. SEM (a) and TEM (b–d and insets I and II) of a coeloconic sensillum in the nymph of *Libellula depressa*. **a:** Distal portion of the last flagellar segment bearing a subterminal coeloconic sensillum (arrow); **b:** Transversal section of the coeloconic sensillum at the level of the peg showing two subunits each formed by three dendrites (D) extending in the peg. Note the interruptions of the cuticle (arrows). A = algal filaments, C = cuticle; **c:** Longitudinal section showing the two dendrites (D) extending along the peg. Note the dendritic sheath (DS) ending at the base of the peg and the inner electron-dense coat (arrow). C = cuticle; **d:** Longitudinal section of the coeloconic sensillum showing the two long distal dendritic segments (D) enveloped in a common dendritic sheath (DS), ending with the ciliary constriction (CC). Note the pierced cuticular surface of the peg in **inset I** and the cuticle (C) deepening inwards and enveloping the apical portion of the two dendrites. **Inset II:** Magnification of the wide pore-like structures (arrows). Note that the dendrites (D) are separated from the outside only by a thin layer including electron-dense dotted material (asterisk).

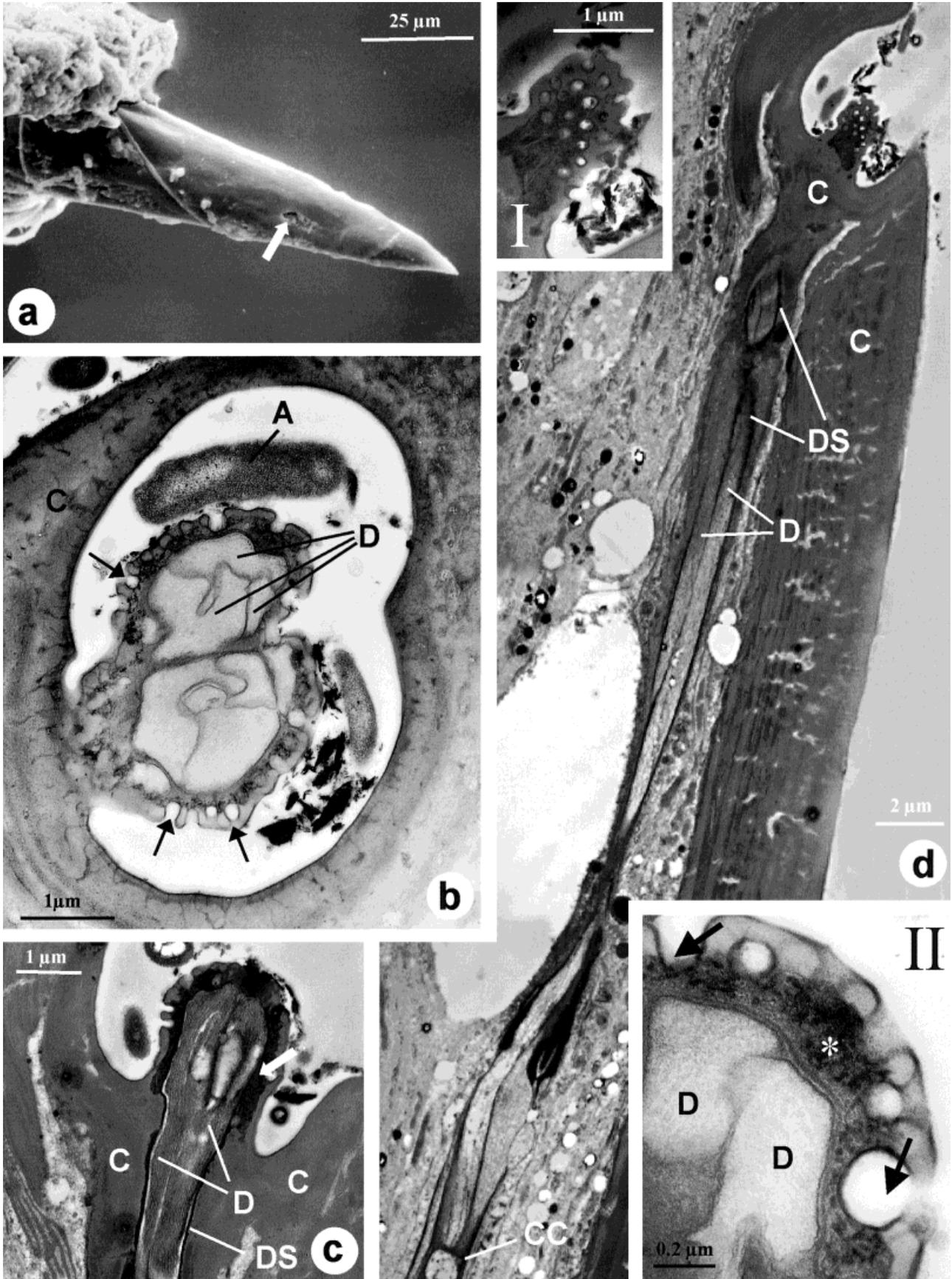


Fig. 3.

of pores in sensilla trichodea is deduced from non-negative staining using crystal violet solution applied to their outer surfaces.

Sensilla Basiconica. Two kinds of sensilla basiconica are visible under SEM: thick-walled sensilla basiconica and thin-walled sensilla basiconica. The first type bears a terminal pore (penetrated by crystal violet dye) and is forming both a crown of 60–70 club-shaped sensilla encircling the distal end of the pedicel (Fig. 4b), and groups of 2–4 sensilla, encircling all the flagellar segments (except segments one to seven) at their distal end (Fig. 4c). The second kind of thin-walled sensilla shows no discrete pores under SEM but is considered to be porous owing to its readily staining with crystal violet. Thin-walled sensilla encompass three kinds of sensilla basiconica on the pedicel and one kind on the flagellum. The first kind of sensilla basiconica is located on the distal part of the pedicel (Fig. 4b). These sensilla are long and hose-like, with a rounded tip and a longitudinally grooved surface. They are interspersed among the thick-walled sensilla. The second kind of sensilla basiconica (Fig. 4b) is interspersed between both the thick-walled sensilla and the thin-walled columnar sensilla, and is represented by short sensilla exhibiting light grooves on their surface. The third kind of sensilla basiconica includes slender sensilla arising in cluster from the entire surface of the pedicel. On the flagellum, thin-walled sensilla basiconica varies from a tapered to a blunt tip. They arise in groups of 2–4 from the distal end of each segment (absent from the first seven) (Fig. 4c).

Coniform Complexes. On the distal portion of the flagellar segments (from the fifth onwards), five to seven coniform sensillar complexes are evident (Fig. 4c,d). Each of them is made of 16–21 narrow cuticular spines of variable length. Kapoor (1987) devoted particular attention to this kind of sensilla. The spines are contained in a shallow pit from which they converge to form a conical tuft projecting out perpendicularly to the surface of the flagellar segments (Fig. 4e). The spines of each complex are smooth-walled and arise from an immovable socket and taper slightly to a blunt knob-like tip. The crystal violet solution stains the tip of the spines, thus indicating that these areas are porous. The spines are composed of exocuticle delimiting a central hollow channel that widens towards the knob-like tip. The thin wall of the tip is traversed by radiating channels that link the lumen of the spine with the exterior (Fig. 4f). Amorphous material extrudes from the channels to cover the tip of the spines. As in other insects, accessory cells are responsible for the secretion of this material that is assumed to be a part of the stimulus-conducting system. Each spine is innervated by two bipolar neurons (Fig. 4f, inset) whose distal dendritic segments are unbranched and always in contact with each other, enclosed by a single dendritic sheath (Fig. 5a). Each pair of dendrite extends into the lumen, right up to the tip of the spine. Three accessory cells are associated with the neurons in each spine (Fig. 5b). The inner accessory cell in the dendritic region is characterised by microvillate surfaces bordering a sensillar sinus (Fig. 4a), extensive infoldings of the plasma membrane, and a high concentration of mitochondria. These features suggest a metabolically active cell sur-

face that may be involved in transport of materials into the receptor sinus.

The spines of the coniform sensillar complex show a typical organisation of chemosensilla (Kapoor, 1987) and could be involved in sensing osmotic conditions. However, histochemical and physiological studies are necessary to assign a definite sensory function to the coniform sensillar complex.

Sensilla Coeloconica. Flagellar segments bear sensilla coeloconica in the form of small pegs. They are absent from the basal 10–12 segments, and their number increases as the segment's length increases. Each sensillum is enclosed in a circular or oval pit. Two kinds of pegs are visible: uniporous (Fig. 5c) and nonporous. The uniporous pegs are located in a row of 4–6 on the distal margin of flagellar segments 20 onwards. Each peg shows ridges along most of its length; its apex narrows to a broad crest with a central hole. Crystal violet dye penetrated the terminal pore. Each peg is innervated by a single bipolar cell whose dendrite extends to the crest and ends below the pore. Three accessory cells are associated with the sensory cell. These sensilla are similar to those described in several insects and presumed to be chemoreceptors. The nonporous coeloconic sensilla are more numerous on the mid-flagellar segments. They have a single neuron and their organisation allows three different kinds to be identified: type 1 coeloconic pegs (broad base and short rounded apex), whose neuron enters the tip and completely fills the lumen ending in a tubular body; type 2 (narrow base and a pointed long apex), with a highly convoluted dendrite ending in a tubular body limited to the base of the peg; type 3 located in shallow pits. In the latter, the neuronal cell has a large body wrapped by an accessory cell; the neuron lacks a true tubular body but shows condensed microtubules in the terminal portion of the dendrite. Type 1 and 2 lack a dendritic sheath. Kapoor (1991) presumes a mechanosensory function for the nonporous sensilla (type 1 and 2) and a possible involvement in thermo- or hygroreception for the nonporous sensilla type 3. He stresses the need of electrophysiological studies to assign exact function to these pegs.

Sensilla Campaniformia. Sensilla campaniformia occur in groups of 40–50 around the distal portion of the pedicel (Fig. 4b). These sensilla are oval in shape, each of them lying in a depression, and sense the

Fig. 4. SEM (a–e) and TEM (f and inset) view of various kinds of sensilla on the nymphal antenna of *Paragnetina media*. **a:** Flagellum showing long wavy (T1) and straight (T2) sensilla trichodea; **b:** Pedicel showing thick-walled sensilla basiconica (TW) with an apical pore (arrow), long (B1) and short (B2) thin-walled sensilla basiconica and campaniform sensilla (Ca); **c:** Flagellum showing thick-walled sensilla basiconica (TW), thin-walled sensilla basiconica (B4), and coniform sensillar complexes (CS); **d:** Five coniform sensillar complexes (CS) on the distal end of a flagellar segment. M = intersegmental membrane; **e:** Detail of a coniform sensillar complex made of a tuft of cuticular spines; **f:** Longitudinal section of a spine showing the lumen (L), a dendrite (D) and channels (arrows) across the apical cuticle. **Inset:** Dendrites (arrow) in the shaft. a–c modified from Kapoor NN. 1985. External morphology and distribution of the antennal sensilla of the stonefly, *Paragnetina media* (Walker) (Plecoptera: Perlidae). *Int J Insect Morphol & Embryol* 14:273–280, with permission of the publisher; d–f and inset modified from Kapoor NN. 1987. Fine structure of the coniform sensillar complex on the antennal flagellum of the stonefly nymph *Paragnetina media* (Plecoptera: Perlidae). *Can J Zool* 65:1827–1832.

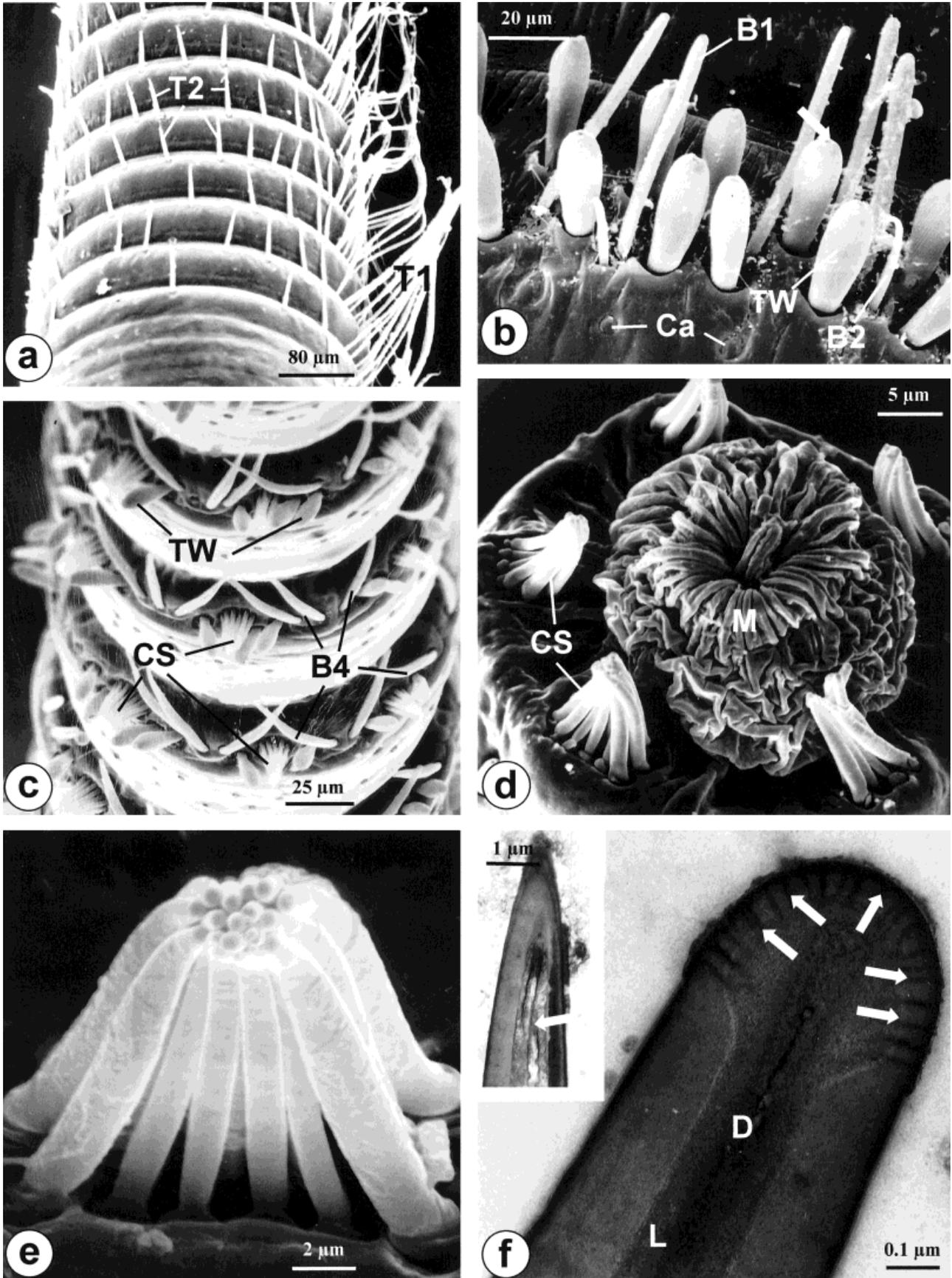


Fig. 4.

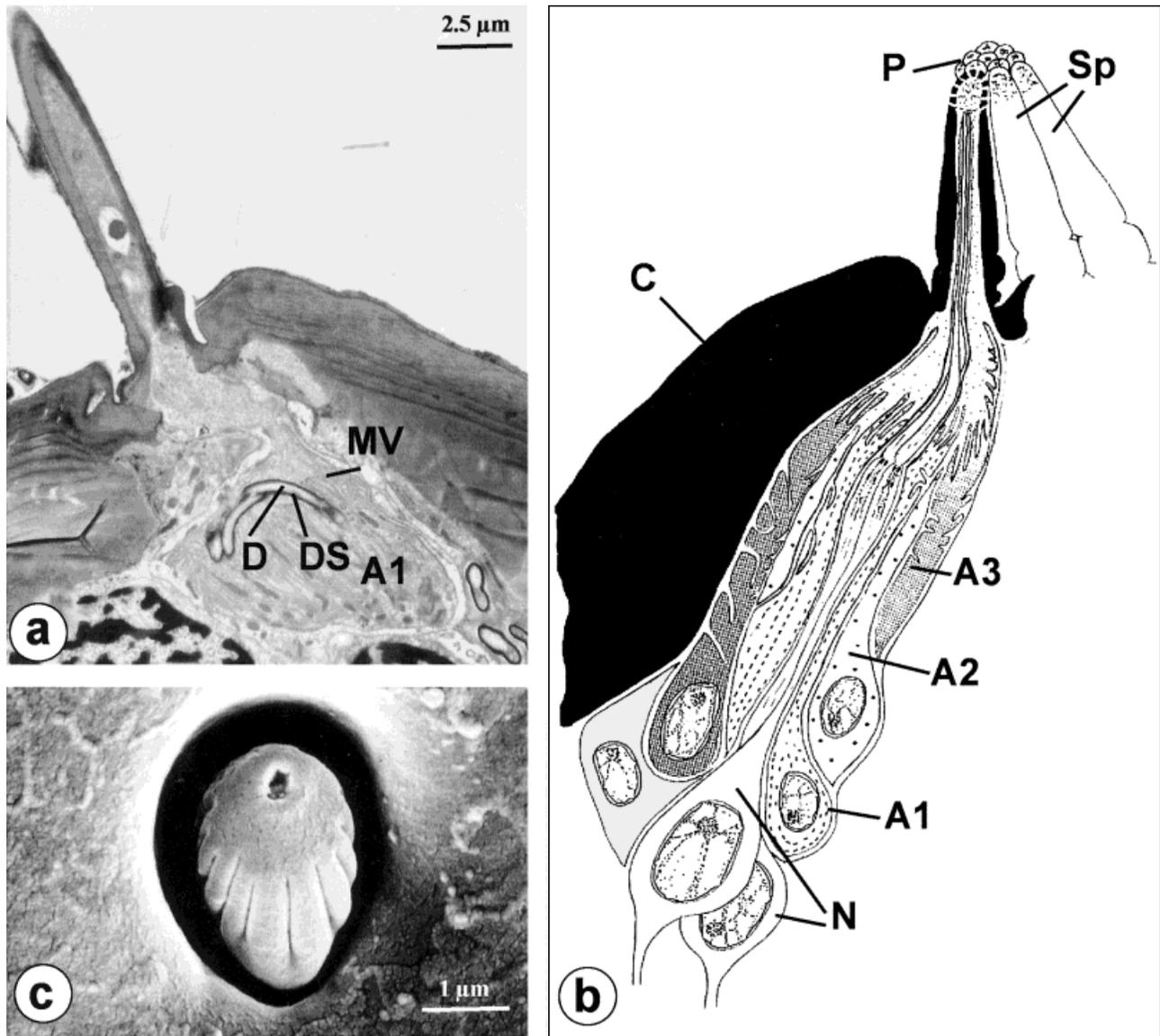


Fig. 5. Nymph of *Paragnetina media*. Organisational details (a) and reconstruction (b) of a spine of the coniform sensillar complex. SEM view of a coeloconic sensillum of the flagellum (c). **a**: Longitudinal section of a spine showing the two dendrites (D) ensheathed by the dendritic sheath (DS) and the microvillated (MV) inner accessory cell (A1); **b**: Arrangement of the cellular components (A1, A2, A3 = accessory cells, N = neurons) of a single spine (SP). Note the naked perikaria of the neurons (N). C = cuticle, P = pores; **c**: Uniporous

coeloconic peg. a,b modified from Kapoor NN. 1987. Fine structure of the coniform sensillar complex on the antennal flagellum of the stonefly nymph *Paragnetina media* (Plecoptera: Perlidae). *Can J Zool* 65:1827–1832. with permission of the National Research Council of Canada; c modified from Kapoor NN. 1985. External morphology and distribution of the antennal sensilla of the stonefly, *Paragnetina media* (Walker) (Plecoptera: Perlidae). *Int J Insect Morphol & Embryol* 14:273–280.

changes in the position of the flagellum in relation to the pedicel. Another type is constituted by oblong plates, surrounded by low and raised rims. Four to five of these sensilla are present around the distal margin of flagellar segments 2 onwards, and are supposed to be involved in monitor from induced stress on the flagellar segment. In both cases, sensilla campaniformia show a similar internal structure and present the general features described in other insects for these sensilla: (1) a bipolar neuron ending with a tubular body, (2) a dendritic sheath attached to the cap, and (3) two accessory cells.

Kapoor (1985) linked the exclusiveness of all the above-reported kinds of sensilla on the antenna of the nymph to its behavior in the stream. Indeed, in *P. media* a remarkable variety of sensilla distinguishes the antenna of the nymph from that of the adult, which shows sensilla trichodea only. Like Ephemeroptera, the adult of this stonefly species does not feed and lives for a few days only. Nymphs are strictly aquatic and live under stones in running water. This way of life explains the differentiation of various kinds of sensilla in order to acquire information regarding temperature, water flow characteristics, and chemical composition.

**ULTRASTRUCTURAL ORGANISATION
OF LARVAL SENSILLA IN AQUATIC
HOLOMETABOLOUS INSECTS
Diptera**

Ultrastructural investigation has almost exclusively regarded the sensilla located on the antennae of larval Diptera Nematocera (i.e., Culicidae, Simuliidae, Chaoboridae, Psychodidae) (Fig. 6).

In the 1970s, three detailed papers on *Aedes aegypti* represented a breakthrough in the knowledge on antennal sensilla of water-living insects (Zacharuk et al., 1971; Zacharuk and Blue, 1971a,b). Since then, various authors have described the larval antennae of other Diptera Nematocera differing in diet and antennal morphology, by outlining their possible homologies (Craig and Batz, 1982; Jez and McIver, 1980; Nicastro et al., 1995, 1998; Seifert et al., 1990).

A general profile of the antennae of Diptera Nematocera described by these authors is briefly reported along with their related sensilla. The ultrastructure of some of the more interesting sensilla is illustrated with particular attention to their possible homologies.

Antennal Morphology and Kind of Sensilla

Culicidae (Fig. 6a–c). In the filter-feeding larva of *Aedes aegypti*, the antenna consists of a single tubular segment (bearing a single hair at about the middle), which ends in a terminal membranous region where the remaining sensilla are located (Fig. 6a). Among these, the dorsomedial cone organ is the largest one. The other sensilla are represented by a basiconic-like peg organ; five innervated hairs (differing in length and position); a thin sinusoidal peg. Within the antennal cavity a chordotonal organ is suspended (Fig. 8c).

The larval antenna of the culicid predator *Toxorhynchites brevipalpis* is a simple cylinder bearing five types of sensilla located at the apex, in the middle, and at its base (Jez and McIver, 1980). At the antennal tip are a cone sensillum with a protuberance, a basiconic peg sensillum, and an unbranched hair sensillum (Fig. 6b). In the midregion, two other unbranched hair sensilla and a campaniform sensillum are located (Fig. 6c), together with a branched hair sensillum. Another campaniform sensillum is at the base of the antenna.

Simuliidae (Fig. 6d and inset). The antenna of a larval simuliid (*Simulium vittatum*, *Cnephia dacotensis*, *Prosimulium mixtum/fuscum*) is tubular with a domed, membranous base and is constituted of two articles, the first of which is annulated (Craig and Batz, 1982) (Fig. 6d). Five kinds of sensilla are present. A single uniporous cone sensillum (Fig. 6d and inset) is on the antennal apex. Two multiporous peg sensilla are at the base of the distal antennal article. On the membranous antennal base is a single trichoid sensillum and two small uniporous peg sensilla. At the base of the antennal articles, a bacteria-covered multiporous sensillum closely accompanied by a sensillum trichodeum is present.

Chaoboridae (Fig. 6e). The highly modified prehensile antenna of the carnivorous “phantom” larva of *Chaoborus crystallinus* described by Nicastro et al. (1995) is constituted of an extended article showing spines and (or) sensilla in the proximal, middle, and distal regions (Fig. 6e). Seven kinds of sensilla are present. The distal region is the most complex portion:

it bears five long catching hairs (two of them are sensilla), a short hair, and three sensilla lacking external cuticular specialisation. Adjacent to the proximal articulation of the antennae is a single campaniform sensillum and in the middle region a sensillum trichodeum is present (Fig. 6e).

Psychodidae (Fig. 6f). A pair of multimodal receptor fields has been described on the front part of the larval head of *Psychoda cinerea* by Seifert et al. (1990). Each receptor field contains eight morphologically different types of sensilla including a mushroom-shaped globular sensillum, sensilla basiconica, clavate sensilla, a tuft-shaped mechanoreceptor, sensilla coeloconica, and campaniformia.

Fine Organisation of the More Interesting Sensilla. The architecture of sensilla, beyond allowing authors to deduce their possible function, constitutes the prevalent feature to define homologies.

Composite Sensilla. According to Zacharuk (1985), composite sensilla may have evolved from a cluster of sensilla whose cuticle fused in a continuous covering.

The cone-organ of the larval antenna of *Aedes aegypti* (Fig. 6a) is ascribed to composite sensilla. It is innervated by 12–13 bipolar neurons whose dendrites are enveloped and grouped into six units. The dendritic sheath ends at the middle region of the cone where the dendrites begin to branch. The branched dendrites terminate at or near the cuticle of the distal half of the cone (Fig. 7a). In this region, the cuticle consists of epicuticular layers only and does not show pores. Nevertheless, the structure of the cone sensillum is suggestive of a chemosensory function. Zacharuk et al. (1971) related the lack of pores and of a pore tubule system of this sensillum to the aquatic habitat. Indeed, the authors hypothesised that in the absence of pores, the molecules of the stimulating chemical diffuse primarily through the thin covering cuticle and reach the dendrites through a narrow underlying liquor-filled space. In the aquatic environment, the lack of a pore tubule system, which in terrestrial insects reduces moisture loss, would not be necessary.

A surprising feature of the antennal sensory cone is that the accessory cells invest only a portion of the proximal dendrite in such a way that in the antennal sinus the remaining dendrite together with the perikaryon and the axon of each neuron are naked in the hemolymph (Fig. 6a).

This feature is quite uncommon in insect sensilla. Jensen and Zacharuk (1991) described neuronal perikarya unshathed and exposed to the hemolymph in the multiporous sensilla of the diving beetle *Graphoderus occidentalis*. The authors stress the similarity between the sheath cell organisation in this beetle and the situation present in the crustacean aesthetasc sensilla where sheath cell somata terminate in a cluster at the level of neuronal perikarya (Grünert and Ache, 1988). Jensen and Zacharuk (1991) speculate that this feature could be a peculiarity of some aquatic arthropods and stress the importance of this aspect in consideration of the physiological implications related to nerve impulse generation and conduction. Among the insects reported in this review, naked perikarya were described only in *Aedes aegypti* and illustrated in a diagrammatic drawing of the coniform sensillar complex in a stonefly nymph. Among terrestrial insects,

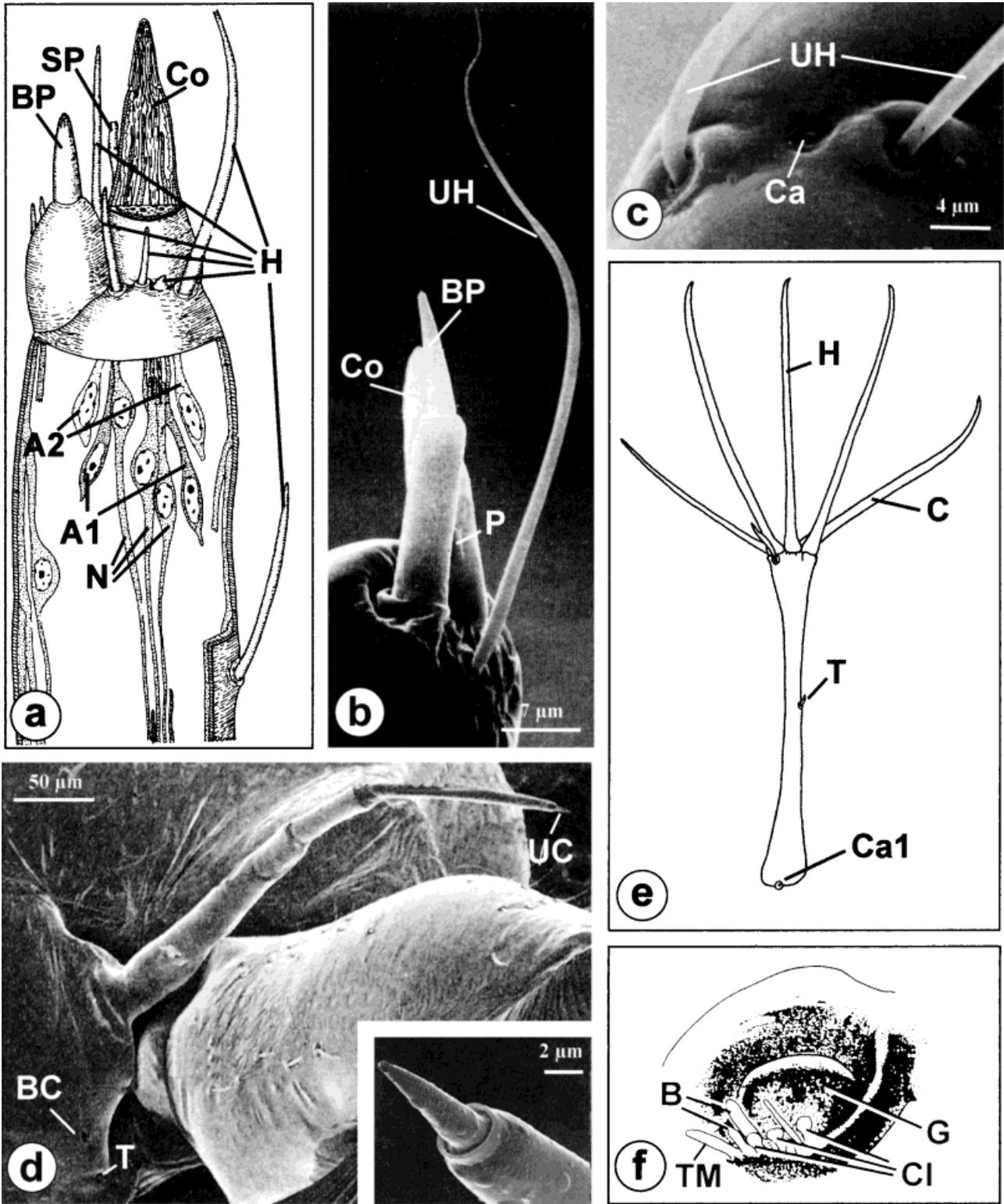


Fig. 6.

naked axons have been reported in the antennal receptors of the bed bug *Cimex lectularius* by Steinbrecht and Müller (1976). In plant-feeding insects, the ionic composition of the hemolymph would not permit normal function of nerve fibers unless they were protected by a perineurium providing the proper extracellular milieu (Steinbrecht and Müller, 1976; Treherne and Pichon, 1972). In the blood-feeding bed bug such a nerve sheath could be dispensable due to its specialised nutrition (Steinbrecht and Müller, 1976). So far, the physiological significance of naked perikarya in aquatic insects is unknown and any hypothesis is merely speculative.

The homologue cone sensillum in *Toxorhynchites brevipalpis* is located in the apical region of the antenna (Figs. 6b, 7b). This composite sensillum is poreless and innervated by 11 neurons subdivided into six groups, each consisting of 1–3 neurons (Jez and McIver, 1980).

A distal uniporous cone sensillum is located at the apex of the second antennal article (Fig. 6d and inset) of some larval simuliids. It is innervated by five dendrites invested in a common dendritic sheath (Fig. 7c). Craig and Batz (1982), in speculating its homology with the apical cone sensillum of culicid larval antennae described by Zacharuk et al. (1971) and Jez and McIver (1980), are aware that such a homology can be proved only with additional investigation. As a matter of fact, the sole sensillar section (Fig. 7c) included in the paper differs remarkably from the typical structure of a composite sensillum.

One of the long catching hairs present on the antenna of *Chaoborus crystallinus* (Fig. 6e) is a composite chemosensory sensillum (Fig. 7d). It is considered a homologue of the cone sensillum of the above-reported culicid larvae (Nicastro et al., 1995) from which it

differs because of the neuron arrangement and the cuticle, which is provided with pores and pore tubules. Nicastro et al. (1995) relate the occurrence of pores in this sensillum to the relatively thick cuticle that would prevent the stimulating molecules from the diffusion through it.

In the multimodal receptor field of *Psychoda cinerea* (Seifert et al., 1990) one of the sensilla is a composite sensillum (Fig. 6f). It has a globular shape and has a mushroom-like appearance. It consists of 21 neurons whose dendrites are subdivided by the dendritic sheath into 7 groups of 3 elements each. The dendrites branch in the sensillar sinus and end under the cuticle. This sensillum is considered to be olfactory and differs from the cone sensillum described in *Aedes aegypti* and in *Toxorhynchites brevipalpis* by the number of dendrites and by the cuticle that bears pores.

The cone sensillum is very common in Diptera. Nicastro et al. (1998) in their comparative analysis of the antennae of larval midges and flies report the presence of the sensory cone in all the 64 species analysed belonging to 41 families, but Simuliidae. Composite sensilla are described in some species of cecidomyiids (Solinas et al., 1987), in the Brachycera *Musca domestica* (Chu-Wang and Axtell, 1971) and *Callifora vicina* (Richter, 1962), and in the Coleoptera Elateridae *Ctenicera destructor* (Scott and Zacharuk, 1971).

Basiconic-like peg organ. The basiconic-like peg organ (Fig. 6a) in the larva of *Aedes aegypti* has no distinct socket and is thick, straight, and tapered to the tip. It is innervated by four unbranched dendrites extending through the lumen to the tip of the peg wrapped by the dendritic sheath (Fig. 8a). This sensillum bears, in its apical part, six short slits, which probably function as chemosensory channels, thus providing easy passage for substances from the exterior to the dendrites. As for the cone sensillum, the proximal part of the dendritic processes together with the perikarya and the axons are naked in the antennal sinus. On the base of its morphology, the peg organ is supposed to be a chemosensory sensillum (Zacharuk and Blue, 1971a) in which one of the dendrites, owing to its additional close apposition and association with the dendritic sheath, may have a mechanoreceptive function.

In *Toxorhynchites brevipalpis* the homologous sensillum is situated in a bulbous extension of the antennal tip (Fig. 6b). Its cuticle shows no perforation. Three of the dendrites run to the tip of the peg, while the fourth ends subterminally without any tubular body-like structure. Jez and McIver (1980) suggested for this sensillum a chemosensory function and related the lack of pores with the possibility of molecules to penetrate the cuticle with no need for physical perforations.

Hairs. The five hair sensilla at the apex of the antenna of *Aedes aegypti* (Fig. 6a) have different lengths; two of them are very short and one is often reduced to a tubercle. Three long hairs are innervated by two neurons, one ending at the base with the tubular body and one extending naked along the shaft (Fig. 8b). Minute and hardly visible pores are present in the distal portion of the hair cuticle. A double chemo-mechanosensory function is presumed (Zacharuk and Blue, 1971a). The remaining hairs (the short and the tubercle) to-

Fig. 6. Antennae and related sensilla of different Diptera Nematocera Culicomorpha (a–e) and Psychodidae (f). **a:** Schematic reconstruction of right antenna of *Aedes aegypti*. Ventral view showing the position of the different sensilla and the cellular component of the cone sensillum (Co). Note that the accessory cells (A1, A2) do not ensheath the neurons (N). BP = basiconic peg, H = hairs, SP = sinusoidal peg; **b:** Scanning electron micrograph (SEM) of tip of antenna of *Toxorhynchites brevipalpis* showing cone (Co) with protuberance (P) and unbranched hair sensillum (UH); BP = basiconic peg. **c:** SEM of midregion of antenna of *T. brevipalpis* showing basal portion of 2 unbranched hair sensilla (UH) and a campaniform sensillum (Ca); **d:** SEM of right antenna of *Simulium vittatum* showing position of uniporous cone sensillum (UC) detailed in the inset, and bacteria-covered multiporous sensillum (BC). T = trichoid sensillum. **e:** Sketchy representation of the prehensile antenna of *Chaoborus crystallinus*. Dorsal view showing the position of the chemosensory hair (C), the mechanosensory long hair (H), a campaniform sensillum (Ca1), and the trichoid sensillum (T) located in the middle of the antenna; **f:** Schematic drawing of one of the multimodal receptor fields on the frontal head of *Psychoda cinerea*. G = globular sensillum, B = sensilla basiconica, Cl = clavate sensilla, TM = tuft-shaped mechanoreceptor sensillum, a modified from Zacharuk RY, Yin R-S L, Blue S, 1971. Fine structure of the antenna and its sensory cone in larvae of *Aedes aegypti* (L.). *J Morphol* 135:273–298. b and c modified from Jez DH, McIver S. 1980. Fine structure of antennal sensilla of larval *Toxorhynchites brevipalpis* Theobald (Diptera: Culicidae). *Int J Insect Morphol & Embryol* 9:147–159. d and inset modified from Craig D, Batz H. 1982. Innervation and fine structure of antennal sensilla of Simuliidae larvae (Diptera: Culicomorpha). *Can J Zool* 60:696–711. with permission of the National Research Council of Canada; f modified from Seifert P, von Perger M, Smola U. 1990. Multimodale Rezeptorfelder am Larvenkopf von *Psychoda cinerea* (Banks) (Diptera, Psychodidae). *Mitt Dtsch Ges Allg Angew Ent* 7:492–498.

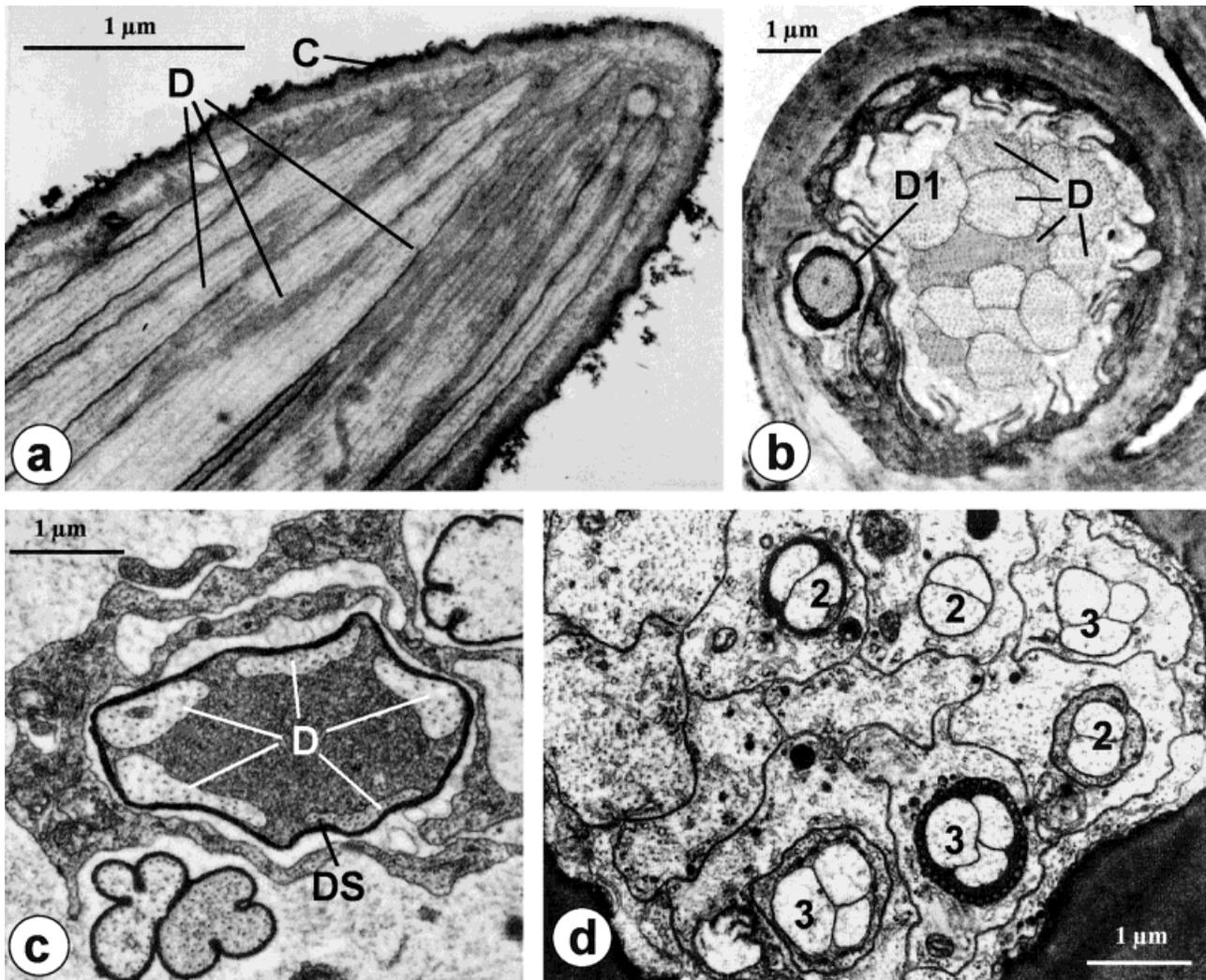


Fig. 7. Transmission electron micrographs of composite sensilla in *Aedes aegypti* (a), *Toxorhynchites brevipalpis* (b), *Chaoborus crystallinus* (c), and of the uniporous cone sensillum of *Simulium vittatum* (d). **a:** Longitudinal section of the dendrites (D) under the thin cuticle (C); **b:** Cross-section through the base of cone sensillum showing bundle of 11 dendrites (D), and the unbranched dendrite of protuberance (D1); **c:** Cross-section showing the five distal dendrites (D) ensheathed by the dendritic sheath (DS) of the uniporous cone sensillum; **d:** Cross-section at the level of the outer dendritic segment of the chemoreceptor hair. Six sensory units containing 2 or 3 dendrites are visible. a modified from Zacharuk RY, Yin R-S L, Blue S. 1971. Fine structure of

the antenna and its sensory cone in larvae of *Aedes aegypti* (L.). *J Morphol* 135:273–298. b modified from Jez DH, McIver S. 1980. Fine structure of antennal sensilla of larval *Toxorhynchites brevipalpis* Theobald (Diptera: Culicidae). *Int J Insect Morphol & Embryol* 9:147–159. c modified from Craig D, Batz H. 1982. Innervation and fine structure of antennal sensilla of Simuliidae larvae (Diptera: Culicomorpha). *Can J Zool* 60:696–711. with permission of the National Research Council of Canada; d modified from Nicastro D, Smola U, Melzer R. 1995. The antennal sensilla of the carnivorous “phantom” larva of *Chaoborus crystallinus* (De Geer) (Diptera, Nematocera). *Can J Zool* 73:15–26 with permission of the National Research Council of Canada.

gether with the lateral hair extending from the medial wall of the antenna, are innervated by a single neuron ending in a tubular body. They are believed to be typical

mechanoreceptive sensilla, adapted to react to stimulation when the hair is touched or moved. In all five apical hairs, the arrangement of the accessory cells with

Fig. 8. Antennal sensilla of *Aedes aegypti* (a–c) and *Toxorhynchites brevipalpis* (d,e). **a:** Cross-section of the basiconic peg at the level of the base showing the four dendrites (D) and the dendritic sheath; **b:** Longitudinal section at the level of the socket of the double innervated hair showing the chemoreceptive (C) and the mechanoreceptive (M) dendrites. DS = dendritic sheath; **c:** Schematic reconstruction of the right antenna with the sinusoidal peg (SP) and chordotonal organ (C) and their cellular components. A1, A2 = accessory cells, BP = basiconic peg, CC = ciliary constriction, Co = cone, DD = distal dendrite, N = neuron, TS = sensillar sinus; **d:** Protuberance (P) present on the cone sensillum (Co); **e:** Cross-section of lamellated (D1)

and undivided (D2) dendrites innervating the protuberance. a,b modified from Zacharuk RY, Blue S. 1971a. Ultrastructure of the peg and hair sensilla on the antenna of larval *Aedes aegypti* (L.). *J Morphol* 135:433–456. c modified from Zacharuk RY, Blue S. 1971b. Ultrastructure of a chordotonal and a sinusoidal peg organ in the antenna of larval *Aedes aegypti* (L.). *Can J Zool* 49:1223–1229. with permission of the National Research Council of Canada; d and e modified from Jez DH, McIver S. 1980. Fine structure of antennal sensilla of larval *Toxorhynchites brevipalpis* Theobald (Diptera: Culicidae). *Int J Insect Morphol & Embryol* 9:147–159.

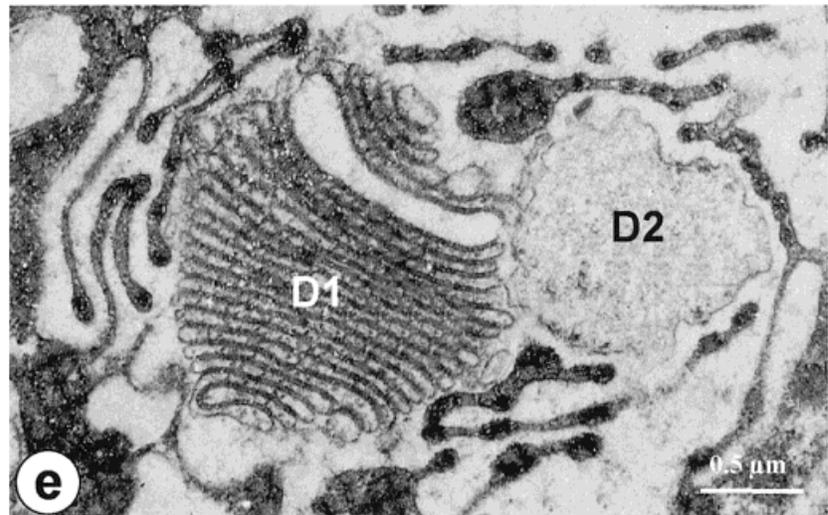
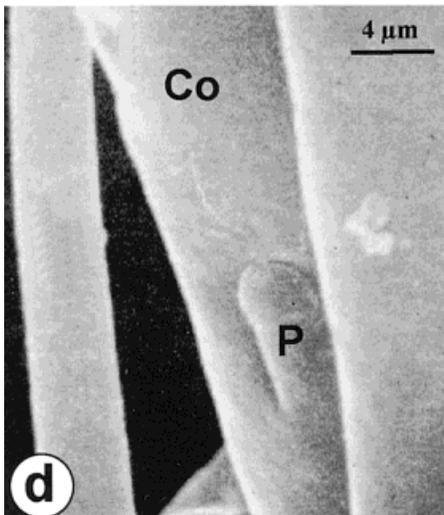
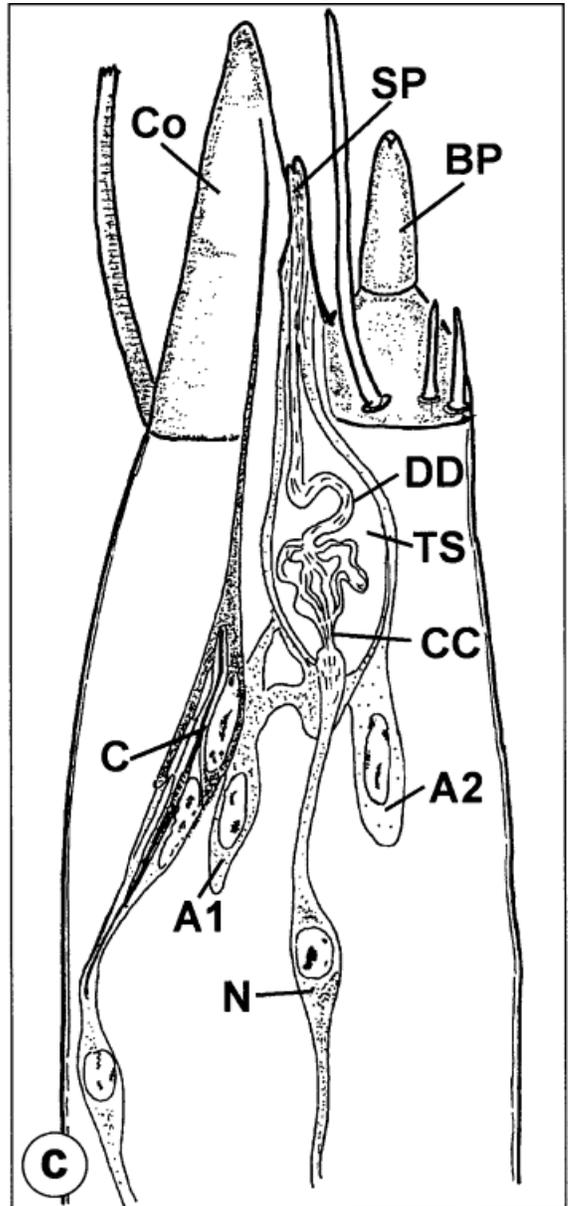
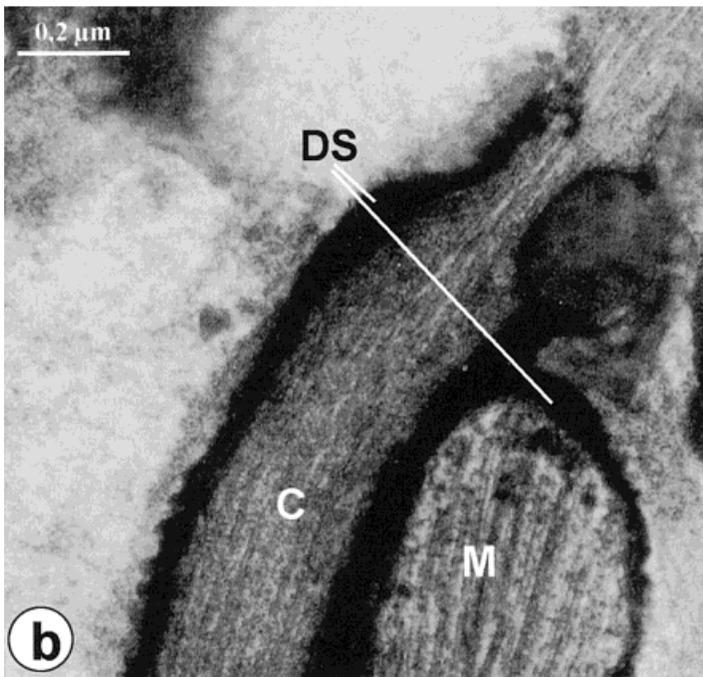
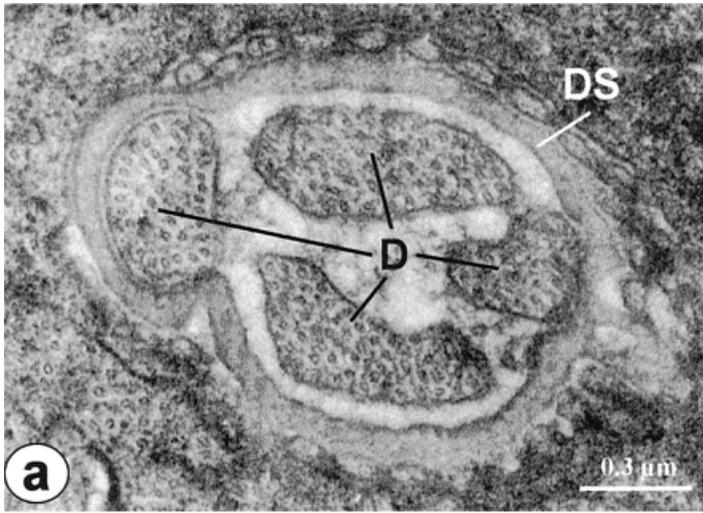


Fig. 8.

respect to dendrites, perikarya, and axons is similar to that observed in the cone sensillum.

Sinusoidal peg. In the larval antenna of *Aedes aegypti*, the thin sinusoidal peg (Figs. 6a, 8c) is a small cuticular peg emerging from the mediodorsal wall of the antennal cone near its base. It has no basal socket and is innervated by a single bipolar neuron. A representation of this sensillum is given in Figure 8c. This sensillum is innervated by a single bipolar neuron branching in its apical portion where the cuticle bears at least one cleft, which is considered a possible channel for stimulation. The outer dendritic segment is enclosed in a wide sensillar sinus where it shows a very peculiar organisation: it is characterised distally by a concentric structure; proximally it divides into several branches that merge to a single process at the ciliary constriction. On the basis of the structure of the sinusoidal peg organ, Zacharuk and Blue (1971b) suggested a possible function as an osmoreceptor allowing salts from the aquatic environment to reach the dendritic tip through the apical pores and change pressure inside the sensillar sinus, thus inciting receptor stimulation. As in the other sensilla of *Aedes aegypti*, the proximal half of the proximal dendrite process together with the neuronal perikaryon and the axon are not ensheathed within the antennal hem sinus.

In *Toxorhynchites brevipalpis*, a similar sensillum is represented by a small protuberance located on the medial side of the cone (Figs. 6b, 8d). In section, it shows two associated neurons, one ending below the base of the cone and divided in numerous lamellae in its terminal part; the other undivided, extending into the protuberance (Fig. 8e). No apical pore is visible. The function of this sensillum is uncertain (Jez and McIver, 1980). A possibility is that the neuron extending into the protuberance could have a chemoreceptive function notwithstanding the lack of pores. Alternatively the lamellated dendrite could have a thermoreceptive function, as reported for the lamellated dendrites of numerous insect sensilla (Altner et al., 1978, 1981, 1983; Becker, 1978; Steinbrecht and Müller, 1991).

In Simuliidae, the homologous of both the sinusoidal peg sensillum of *Aedes aegypti* and the cone protuberance of *Toxorhynchites brevipalpis* is represented by two small uniporous peg sensilla (Craig and Batz, 1982). Each of them is innervated by three neurons with lamellated distal dendritic segments. Craig and Batz (1982) devoted much attention to the lamellated dendrites whose septate junctions and associated mitochondria have been advocated to support an absorptive-secretory role. Notwithstanding the lack of electrophysiological investigations, in consideration of the importance of hydrochemistry in habitat selection for simuliid larvae, Craig and Batz (1982) surmise that the uniporous peg sensilla and the sinusoidal peg sensillum of culicid larvae could be used to monitor the ionic composition of water.

Bacteria-covered sensillum. The external and internal similarities between the chloride-absorbing cells and sensilla have often led to confusion of these two structures (Kapoor, 1976; Komnick, 1977). Kapoor and Zachariah (1978) noticed that chloride absorbing cells on gills of some Plecoptera nymphs had numerous characteristics in common with sensilla. In this respect, Kapoor and Zachariah hypothesised that chloride cells

could have originally been chemosensilla that have lost their innervation and have acquired a mere osmoregulatory role. In consideration of the structural similarities between these two devices, Craig and Batz (1982) described in larvae of some species of Simuliidae a bacteria-covered sensillum whose external features were similar to those of chloride absorbing cells of other aquatic insects (Komnick, 1977).

The bacteria-covered multiporous sensillum (Fig. 9a) is located ventrally on the antennal base (Fig. 6d). It is sunken below, or at the same level as, the surface cuticle and is included in a heavily cuticularised capsule. Externally, the capsule has a rounded or oval shape and is always covered with bacteria (Fig. 9a). This sensillum is innervated by five neurons with the perikarya in the antennal nerve, each wrapped by sheath cells. A peculiar highly fenestrated dendritic sheath separates each of the outer dendritic segments. They enter the capsule where the dendritic sheath becomes thinner and the dendrites branch end under the porous cuticle (Fig. 9b,c). The pores are numerous and show pore tubules (Fig. 9c and inset). Craig and Batz (1982) concluded that the bacteria-covered sensillum is very similar to the multiporous sensillum with a pitted surface, termed "MPP" by Zacharuk (1980). X-ray spectrometry tests showed that there was no significant statistical difference in the chlorine concentration of this kind of sensillum and another multiporous sensillum on mouthparts. In this respect, Craig and Batz (1982) stressed the difficulties in discriminating chloride cells in aquatic insects by assaying the concentration of chloride present in such structures. Indeed, chloride is normally necessary for neuron function and is present in numerous sensilla. The occurrence of bacteria, both on the outer surface of the sensillum and on chloride-absorbing cells, is still intriguing. It was hypothesised that bacteria could uptake the exudates from sensillar sinus fluid, in part derived from the moulting space fluid (Craig and Batz, 1982). Bacteria-covered sensilla have no homology with sensilla described for the larval antennae of other Culicomorpha families.

Trichoptera

In the antenna of *Nectopsyche albida*, Tozer (1982) observed in all five larval instars a single, distally located, sensillum trichodeum. As far as we know, this paper represents the only contribution to the knowledge of larval sensilla in caddisflies.

CONCLUDING REMARKS

Larvae of most insect species, no matter what their order or biotope, show many features in common in the sensory equipment of their antennae. A case in point is the homologous set of sensory systems located on the larval antennae of Diptera, the most well-known insects in this respect.

The lack of electrophysiological data concerning the sensilla of aquatic larval insects reflects the difficulty to test the response to specific stimuli, thus making ultrastructural characteristics the main support to extrapolate a putative function, with the risk of overinterpreting the data.

The need to actively sample the environment for chemical stimuli triggered the diversification of com-

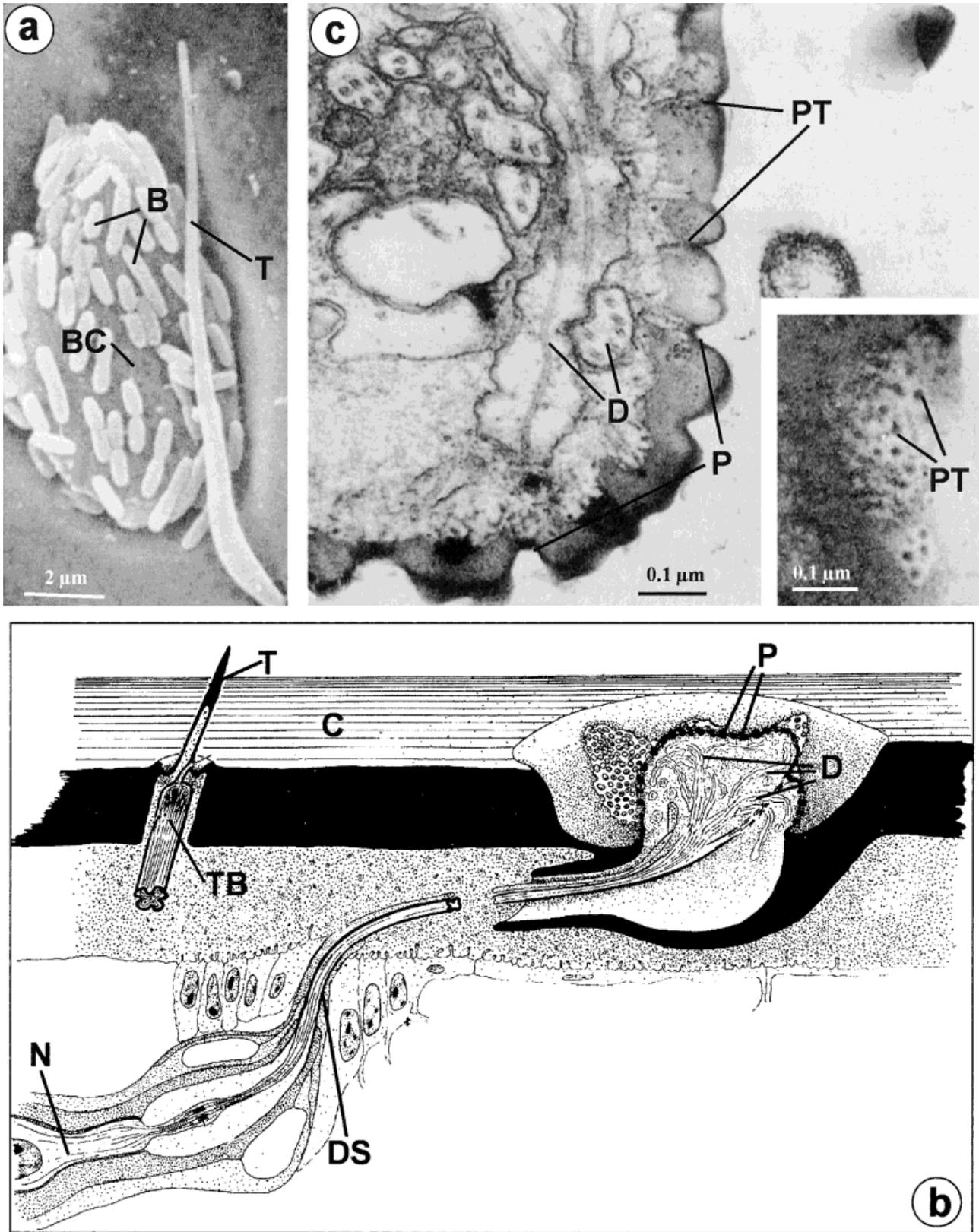


Fig. 9. SEM (a), TEM (c and inset), and diagrammatic reconstruction (b) of the bacteria-covered multiporous sensillum in Simuliidae (a, *Prosimulium mixtum*; b,c, *Simulium vittatum*). a: Bacteria covered-sensillum (BC) with bacteria (B) and the close associated trichoid sensillum (T); b: Cellular components of bacteria-covered multiporous sensillum showing one of the five neurons (N) with its branched distal dendrite (D) wrapped by the dendritic sheath (DS) and the associated

trichoid sensillum (T) with its tubular body (TB). C = cuticle; P = pores. c: Longitudinal section of the bacteria-covered sensillum showing dendrites (D), pores (P) and pore tubules (PT) detailed in the inset. Modified from Craig D, Batz H. 1982. Innervation and fine structure of antennal sensilla of Simuliidae larvae (Diptera: Culicomorpha). Can J Zool 60:696-711 with permission of the National Research Council of Canada.

plex chemosensory organs. Of course, in the aquatic environment the typical distinction between olfaction and taste based on the ability to perceive airborne or water dissolved molecules (Zacharuk, 1980) is absent. Hallberg et al. (1997) indicate the difficulty for aquatic animals to discriminate between olfaction and taste, and report that in an aquatic environment the two senses are better defined by the organismic response they elicit (Atema, 1977). Indeed, olfaction pertains to more complex behaviours, such as social interactions, whereas taste is involved in more reflex-like behaviours, such as food finding. Another possible discrimination between chemosensors could be the long perception of chemicals (low threshold) and close-range chemoreception (relatively high threshold) (Shelton and Laverack, 1970).

A peculiarity correlated to the life in aquatic environment is represented by chemosensory sensilla without pores, a condition allowing molecules to reach the cuticle without any need for physical perforations. Even though additional morphological and electrophysiological investigation is needed to clarify the chemosensory function of the poreless cuticle, it is noteworthy that a thin, poreless cuticle is present in the aesthetascs, the olfactory sensilla of crustaceans (Grünert and Ache, 1988; Hallberg et al., 1997). The aesthetascs have been studied extensively and an olfactory function has been suggested with electrophysiological methods in a number of species (Derby and Atema, 1988; Spencer, 1986). The concept that an absence of pores does not preclude chemosensory function has been also confirmed in the frontal sensory hairs of a branchiopod crustacean of the family Lynceidae (Cash-Clark and Martin, 1994). In accordance with the similar eco-physiological constraints imposed by the freshwater habitat, sensilla of phylogenetically distant animals exhibit the same functional model.

We hope that some of the issues we have addressed may provide a departure point for more detailed studies on larval antennal sensilla in water-living insects. We expect that these sensory systems will continue to be a source of productive studies in the years to come.

ACKNOWLEDGMENTS

We thank Prof. P. Zwick for having provided us some important papers and Dr. N. Kapoor for kindly sending us original photos. We are very grateful to Dr. G. Salerno for his helpful technical assistance and to Dr. C. Pesce for his critical revision of the text. We are greatly indebted to Prof. A. Steinbrecht and Prof. E. Hallberg for their patience and cooperation. This review has been supported by the grant "Progetto di Ateneo" of the University of Perugia.

REFERENCES

- Altner H, Tichy H, Altner I. 1978. Lamellated outer dendritic segments of a sensory cell within a poreless thermo- and hygroreceptive sensillum of the insect *Carausius morosus*. *Cell Tissue Res* 191:287–304.
- Altner H, Routil C, Loftus R. 1981. The structure of bimodal chemo-, thermo-, and hygroreceptive sensilla on the antenna of *Locusta migratoria*. *Cell Tissue Res* 215:289–380.
- Altner H, Schaller-Selzer L, Stetter H, Wohlrab I. 1983. Poreless sensilla with inflexible sockets: a comparative study of a fundamental type of insect sensilla probably comprising thermo- and hygroreceptors. *Cell Tissue Res* 234:279–307.
- Atema J. 1977. Functional separation of smell and taste in fish and crustacea. In: LeMagen J, MacLeod P, editors. *Olfaction and taste*, vol. 4. London: Information Retrieval. p 165–174.
- Bassemir U, Hansen K. 1980. Single-pore sensilla of damselfly-larvae: representatives of phylogenetically old contact chemoreceptors? *Cell Tissue Res* 207:307–320.
- Becker D. 1978. Elektrophysiologische Untersuchungen zur Feuchterezeption durch die styloconischen Sensillen bei *Mamestra brassica* L. (Lepidoptera, Noctuidae). Doctoral Dissertation, Fachbereichs Biologie und Vorklinische Medizin der Universität Regensburg.
- Behrend K. 1971. Riechen in Wasser und in Luft bei *Dytiscus marginalis* L. *Z Vergl Physiol* 75:108–122.
- Cash-Clark CE, Martin JW. 1994. Ultrastructure of the frontal sensory fields in the Lynceidae (Crustacea, Branchiopoda, Laevicaudata). *J Morphol* 221:153–160.
- Chapman RF. 1982. Chemoreception: the significance of receptor numbers. In: Berridge MJ, Treherne JE, Wigglesworth VB, editors. *Advances in insect physiology*, vol. 16. London: Academic Press. p 247–355.
- Chu-Wang I, Axtell RC. 1971. Fine structure of the dorsal organ of the house fly larva, *Musca domestica* L. *Z Zellforsch Mikr Anat* 117:17–34.
- Craig D, Batz H. 1982. Innervation and fine structure of antennal sensilla of Simuliidae larvae (Diptera: Culicomorpha). *Can J Zool* 60:696–711.
- Craig D, Borkent A. 1980. Intra- and inter-familial homologies of maxillary palpal sensilla of larval Simuliidae (Diptera: Culicomorpha). *Can J Zool* 58:2264–2279.
- Csoknya M, Halász N. 1972. Ultrastructural investigations of the campaniform sensillae on the tracheal gill of *Palingenia longicauda* Oliv. (Ephemeroptera). *Acta Biol Szeged* 18:145–157.
- Derby CD, Atema J. 1988. Chemoreceptor cells in aquatic invertebrates: peripheral mechanisms of chemical signal processing in decapod crustaceans. In: Atema J, Fay RR, Popper AN, Tavolga WN, editors. *Sensory biology of aquatic animals*. Berlin: Springer-Verlag. p 365–385.
- Eastham LES. 1936. The sensillae and related structures on the gills of nymphs of the genus *Caenis* (Ephemeroptera). *Trans R Ent Soc Lond* 85:401–414.
- Filshie B, Campbell I. 1984. Design of an insect cuticle associated with osmoregulation: the porous plates of chloride cells in a mayfly nymph. *Tissue Cell* 16:178–803.
- Gaino E, Rebora M. 1996. Fine structure of flat-tipped antennal sensilla in three species of mayflies (Ephemeroptera). *Invertebr Biol* 115:145–149.
- Gaino E, Rebora M. 1997. Antennal cuticular sensilla in some mayflies (Ephemeroptera). In: Landolt P, Sartori M, editors. *Ephemeroptera & Plecoptera: biology-ecology-systematics*. Fribourg: MTL. p 317–325.
- Gaino E, Rebora M. 1998. Ultrastructure of the antennal sensilla of the mayfly *Baetis rhodani* (Pictet) (Ephemeroptera: Baetidae). *Int J Insect Morphol Embryol* 27:143–149.
- Gaino E, Rebora M. 1999. Flat-tipped sensillum in Baetidae (Ephemeroptera): a microcharacter for taxonomic and phylogenetic considerations. *Invertebr Biol* 118:68–74.
- Grünert U, Ache B. 1988. Ultrastructure of the aesthetasc (olfactory) sensilla of the spiny lobster, *Panulirus argus*. *Cell Tissue Res* 251:95–103.
- Hallberg E, Kjell UIJ, Wallén R. 1997. Olfactory sensilla in crustaceans: morphology, sexual dimorphism, and distribution patterns. *Int J Insect Morphol Embryol* 26:173–180.
- Jensen JC, Zacharuk RY. 1991. The fine structure of uniporous and nonporous pegs on the distal antennal segment of the diving beetle *Graphoderus occidentalis* Horn (Coleoptera: Dytiscidae). *Can J Zool* 69:334–352.
- Jensen JC, Zacharuk RY. 1992. The fine structure of the multiporous sensilla on the antenna of the diving beetle *Graphoderus occidentalis* Horn (Coleoptera: Dytiscidae). *Can J Zool* 70:825–831.
- Jez DH, McIver S. 1980. Fine structure of antennal sensilla of larval *Toxorhynchites brevialpis* Theobald (Diptera: Culicidae). *Int J Insect Morphol Embryol* 9:147–159.
- Kapoor NN. 1976. The cuticular patterns and structures of plecopteran nymph (Insecta) as revealed by scanning electron microscope. *Scan Electr Microsc* 2:619–622.
- Kapoor NN. 1985. External morphology and distribution of the antennal sensilla of the stonefly, *Paragnetina media* (Walker) (Plecoptera: Perlidae). *Int J Insect Morphol Embryol* 14:273–280.
- Kapoor NN. 1987. Fine structure of the coniform sensillar complex on the antennal flagellum of the stonefly nymph *Paragnetina media* (Plecoptera: Perlidae). *Can J Zool* 65:1827–1832.

- Kapoor NN. 1989. Distribution and innervation of sensilla on the mouthparts of the carnivorous stonefly nymph, *Paragnetina media* (Walker) (Plecoptera: Perlidae). *Can J Zool* 67:831–838.
- Kapoor NN. 1991. Antennal campaniform and coeloconic sensilla of the stonefly nymph, *Paragnetina media* (Walker) (Plecoptera: Perlidae). In: Alba-Tercedor J, Sanchez-Ortega A, editors. Overview and strategies of Ephemeroptera and Plecoptera. Sandhill Crane Press, Inc. p 39–46.
- Kapoor NN, Zachariah K. 1978. The internal morphology of sensilla of the abdominal gills of *Thaumatoperla alpina* (Plecoptera: Eustheniidae; subfamily: Thaumatoperlinae). *Can J Zool* 56:2194–2197.
- Kapoor NN, Zachariah K. 1983. Ultrastructure of the sensilla of the stonefly nymph, *Thaumatoperla alpina* Burns and Neboiss (Plecoptera: Eustheniidae). *Int J Insect Morphol Embryol* 12:157–168.
- Kapoor NN, Zachariah K. 1984. Scanning and transmission electron microscopy of the developmental stages of the flower-shape sensillum of the stonefly nymph *Thaumatoperla alpina* Burns and Neboiss (Plecoptera: Eustheniidae). *Int J Insect Morphol Embryol* 13:177–189.
- Karnovsky MS. 1965. A formaldehyde-glutaraldehyde fixative of high osmolality for use in electron microscopy. *J Cell Biol* 27:137–138.
- Komnick H. 1977. Chloride cells and chloride epithelia of aquatic insects. *Int Rev Cytol* 49:285–329.
- Komnick H, Rhees RW, Abel JH. 1972. The function of ephemerid chloride cells. Histochemical autoradiographic and physiological studies with radioactive chloride in *Callibaetis*. *Cytobiologie* 5:65–82.
- McIver S. 1985. Mechanoreception. In: Kerkut GA, Gilbert LI, editors. *Comprehensive insect physiology, biochemistry and pharmacology*. Vol. VI. Nervous system: sensory. New York: Pergamon Press. p71–132.
- McIver S, Beech M. 1986. Prey finding behavior and mechanosensilla of larval *Toxorhynchites brevipalpis* Theobald (Diptera: Culicidae). *Int J Insect Morphol Embryol* 15:213–225.
- Nicastro D, Smola U, Melzer R. 1995. The antennal sensilla of the carnivorous “phantom” larva of *Chaoborus crystallinus* (De Geer) (Diptera, Nematocera). *Can J Zool* 73:15–26.
- Nicastro D, Melzer R, Hruschka H, Smola U. 1998. Evolution of small sense organs: sensilla on the larval antennae traced back to the origin of the Diptera. *Naturwissenschaften* 88:501–505.
- Pritchard G. 1965. Sense organs in the labrum of *Aeshna interrupta lineata* Walker (Odonata; Anisoptera). *Can J Zool* 43:333–336.
- Richter S. 1962. Unmittelbarer Kontakt der Sinneszellen cuticularer Sinnesorgane mit der Aussenwelt. *Z Morphol Oekol Tiere* 52:171–196.
- Schafer R, Sanchez TV. 1976. The nature and development of sex attractant specificity in cockroaches of genus *Periplaneta*. 1. Sexual dimorphism in distribution of antennal sense organs in five species. *J Morphol* 149:139–158.
- Schmidt K. 1974. Die Mechanorezeptoren im Pedicellus der Eintagsfliegen (Insecta: Ephemeroptera). *Z Morph Tiere* 78:193–220.
- Scott DA, Zacharuk RY. 1971. Fine structure of the antennal sensory appendix in the larva of *Ctenicera destructor* (Brown) (Elateridae: Coleoptera). *Can J Zool* 49:199–210.
- Seifert P, von Perger M, Smola U. 1990. Multimodale Rezeptorfelder am Larvenkopf von *Psychoda cinerea* (Banks) (Diptera, Psychodidae). *Mitt Dtsch Ges Allg Angew Ent* 7:492–498.
- Shelton RGJ, Laverack MS. 1970. Receptor hair structure and function in the lobster *Homarus gammarus* (L.). *J Exp Mar Biol Ecol* 4:201–210.
- Slifer EH. 1960. A rapid and sensitive method for identifying permeable areas in the body wall of insects. *Entomol News* 71:179–182.
- Slifer EH., Sekhon S. 1972. Sense organs on the antennal flagella of damselflies and dragonflies (Odonata). *Int J Insect Morphol Embryol* 1:289–300.
- Solinas M, Nuzzaci G, Isidoro N. 1987. Antennal sensory structures and their ecological-behavioural meaning in Cecidomyiidae (Diptera) larvae. *Entomologica* 22:165–184.
- Spencer M. 1986. The innervation and chemical sensitivity of single aesthetascs hairs. *J Comp Physiol A* 158:59–68.
- Steinbrecht RA, Müller B. 1976. Fine structure of the antennal receptors of the bed bug, *Cimex lectularius* L. *Tissue Cell* 8:615–636.
- Steinbrecht RA, Müller B. 1991. The thermo-/hygrosensitive sensilla of the silkworm, *Bombyx mori*: morphological changes after dry- and moist adaptation. *Cell Tissue Res* 266:441–456.
- Treherne JE, Pichon Y. 1972. The insect blood-brain barrier. *Adv Insect Physiol* 9:257–313.
- Tozer W. 1982. External antennal morphology of the adult and larva of *Nectopsyche albida* (Trichoptera: Leptoceridae). *Freshwater Invertebr Biol* 1:35–43.
- Vogel S. 1981. *Life in moving fluids*. Boston, Massachusetts: Willard Grant Press. p 467.
- Wichard W, Komnick H. 1971. Electron microscopical and histochemical evidence of chloride cells in tracheal gills of mayfly larvae. *Cytobiologie* 3:215–228.
- Wichard W, Komnick H. 1973. Feinstruktur der Chloridzellen von Eintagsfliegenlarven. *Verh Deut Zool Ges* 66:80–83.
- Wichard W, Komnick H, Abel JH. 1972. Typology of ephemerid chloride cells. *Z Zellforsch* 132:533–551.
- Wichard W, Tsui PTP, Dewall AM. 1975. Chloridzellen der Larven von *Caenis diminuta* Walker (Ephemeroptera, Caenidae) bei unterschiedlicher Salinität. *Int Revue Ges Hydrobiol* 60:705–709.
- Wolfe WG, Zimmerman JR. 1984. Sensilla, punctuation, reticulation, and body shape in the Hydroporinae (Coleoptera: Dytiscidae) *Int J Insect Morphol Embryol* 13:373–387.
- Zacharuk RY. 1980. Ultrastructure and function of insect chemosensilla. *Annu Rev Entomol* 25:27–47.
- Zacharuk RY. 1985. Antennae and sensilla. In: Kerkut GA, Gilbert LI, editors. *Comprehensive insect physiology, biochemistry and pharmacology*, vol. VI. Nervous system: sensory. New York: Pergamon Press. p1–69.
- Zacharuk RY, Blue S. 1971a. Ultrastructure of the peg and hair sensilla on the antenna of larval *Aedes aegypti* (L.). *J Morphol* 135:433–456.
- Zacharuk RY, Blue S. 1971b. Ultrastructure of a chordotonal and a sinusoidal peg organ in the antenna of larval *Aedes aegypti* (L.). *Can J Zool* 49:1223–1229.
- Zacharuk RY, Shields VD. 1991. Sensilla of immature insects. *Annu Rev Entomol* 36:331–354.
- Zacharuk RY, Yin R-S L, Blue S. 1971. Fine structure of the antenna and its sensory cone in larvae of *Aedes aegypti* (L.). *J Morphol* 135:273–298.
- Zawarzin A. 1912. Histologische Studien über Insekten. II. Das sensible Nervensystem der Aeshnalarven. *Z Wiss Zool* 100:245–286.