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ULTRASTRUCTURE OF THE ANTENNAL SENSILLA OF THE MAYFLY *BAETIS RHODANI* (PICTET) (EPHEMEROPTERA : BAETIDAE)

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Abstract—The distribution and fine morphology of antennal sensilla of nymphal and adult mayfly, *Baetis rhodani* (Ephemeroptera : Baetidae), were examined. In the nymph, various kinds of sensilla (chaetica, basiconica, coeloconica and cuticular pits) are differently arranged on the antennal segments, whereas sensilla campaniformia delimit the distal border of the pedicel. A peculiar kind of sensillum basiconicum, named flat-tipped sensillum, is present along the entire antenna, even though in the flagellum it has a regular arrangement between the cuticular lobes that delimit the distal border of each article. In the *subimago* the scape and pedicel are profusely covered with microtrichia and scattered sensilla trichodea, whereas the flagellum shows cuticular ribs. Sensilla coeloconica are present along the ventral side of the flagellum. In the *imago*, the antenna is completely decorated with scales among which sensilla trichodea and sensilla coeloconica occasionally occur. As in the nymph, adult mayflies have a ring of sensilla campaniformia along the distal border of the pedicel. When compared with nymphal antennae, those of adults have fewer types of sensilla, presumably in relation to the short, non-feeding terrestrial life. © 1998 Elsevier Science Ltd. All rights reserved.

Index descriptors (in addition to those in the title): fine structure; sensory system; SEM; TEM.

INTRODUCTION

Mayfly adults are short-lived with aquatic immature stages dominating the life cycle. *B. rhodani* is a typical univoltine species with considerable flexibility in its life cycle (Brittain, 1982), which is probably due to temperature variations (Humpesh, 1979). Because mayflies live in different habitats, they require specialized sensory mechanisms adapted to work in different environmental conditions.

Insect sensilla have been the subject of exhaustive reviews (Schneider, 1964; Altner and Prillinger, 1980; Keil and Steinbrecht, 1984; McIver, 1985; Zacharuk, 1985; Altner and Loftus, 1985; Zacharuk and Shields, 1991). Ultrastructural studies on mayfly antennal sensilla are limited, at present, to some details in the ephemerellid *Vietnamella dabieshanensis* (Su and Cai, 1994) and to the description of mechanoreceptors in the pedicel of several species of mayflies (Schmidt, 1974). The occurrence of a peculiar type of flat-tipped sensillum with an apical pore has recently been shown by scanning electron microscopy in *Baetis rhodani*, *Epeorus sylvicola* and *Rhithrogena loyolaea* (Gaino and Rebora, 1996).

When compared with the other 2 species, the antennal sensilla of *B. rhodani* present a major diversification. In order to understand how this species senses its environment, we have extended our studies on such receptors in nymphs and adults (*subimago* and *imago*). The categorization of sensilla has been based on morphological and ultrastructural characteristics observed by scanning and transmission electron microscopy.

MATERIALS AND METHODS

Nymphs and adults of *B. rhodani* were collected in the Scrivia River (Casella, Liguria). Some nymphs with dark wing-pads were about to emerge.

For scanning electron microscopy (SEM), excized antennae were fixed in Karnovsky's medium (Karnovsky, 1965) in cacodylate buffer, pH 7.2 and repeatedly rinsed in the same buffer. After dehydration in the graded ethanol series, the antennae were critical point-dried using a CO_2 Pabisch CPD 750 apparatus, mounted on stubs with silver conducting paint, and coated with gold palladium (20 nm) in a Balzers Union evaporator. Specimens were observed with a Philips EM 515 scanning electron microscope, at an accelerating voltage of 18 kV.

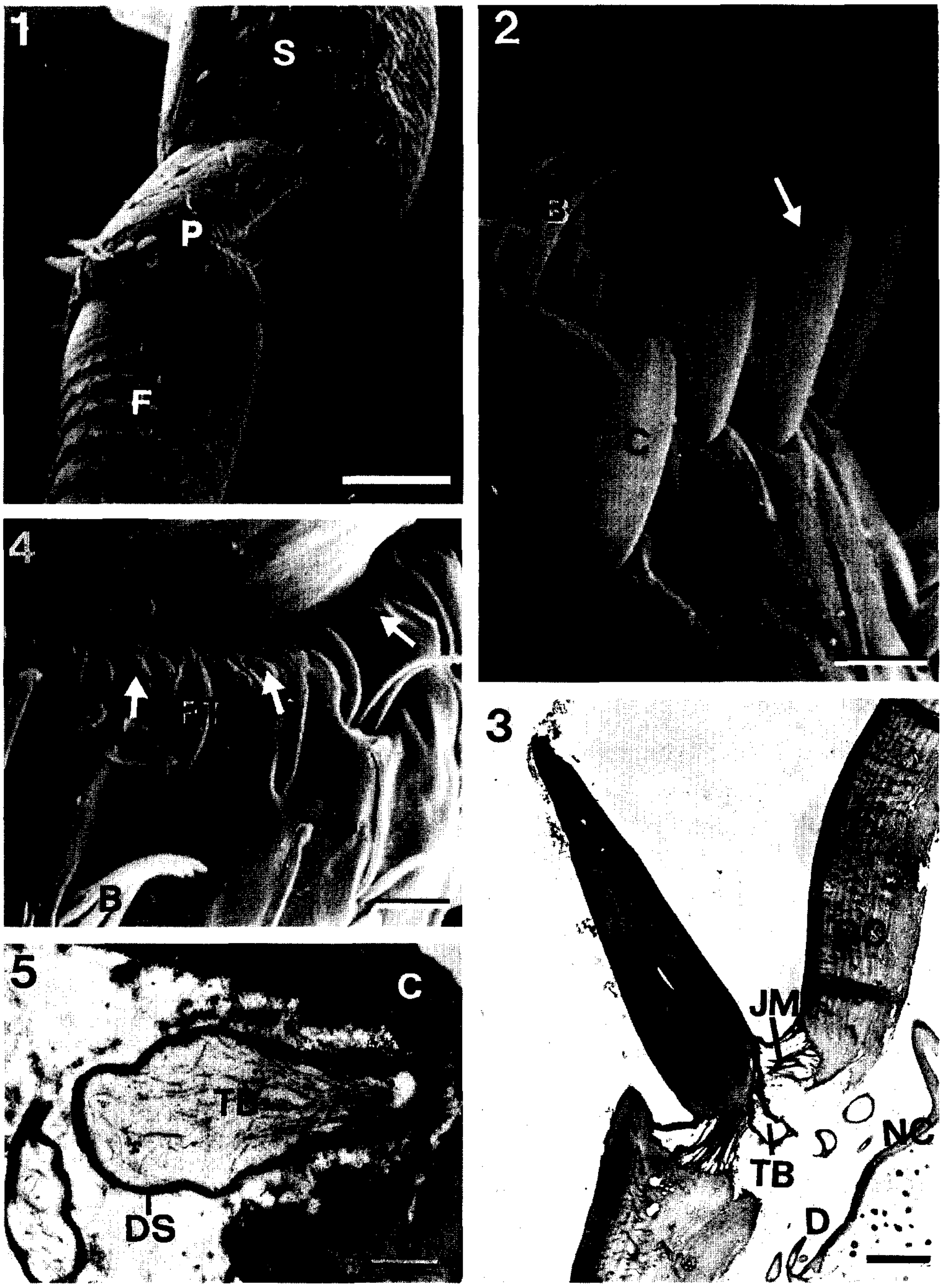
For transmission electron microscopy (TEM), antennae were postfixed for 1 hr in osmium tetroxide, rinsed in cacodylate buffer, dehydrated in the graded ethanol series, and embedded in Epon–Araldite mixture resin. Thin sections, obtained with a Reichert ultramicrotome, were collected on formvar-coated copper grids, stained with uranyl acetate and lead citrate, and observed with a Philips EM 400 electron microscope. The terminology of McIver (1985) and Zacharuk (1985) was followed.

RESULTS

The antennae of the nymphs of *B. rhodani*, are composed basally of scape and pedicel, and of a long flagellum (Fig. 1), the latter consisting of about 42–45 segments. All antennal segments bear sensilla, which vary in morphology and location.

Scape and pedicel show various types of sensilla:

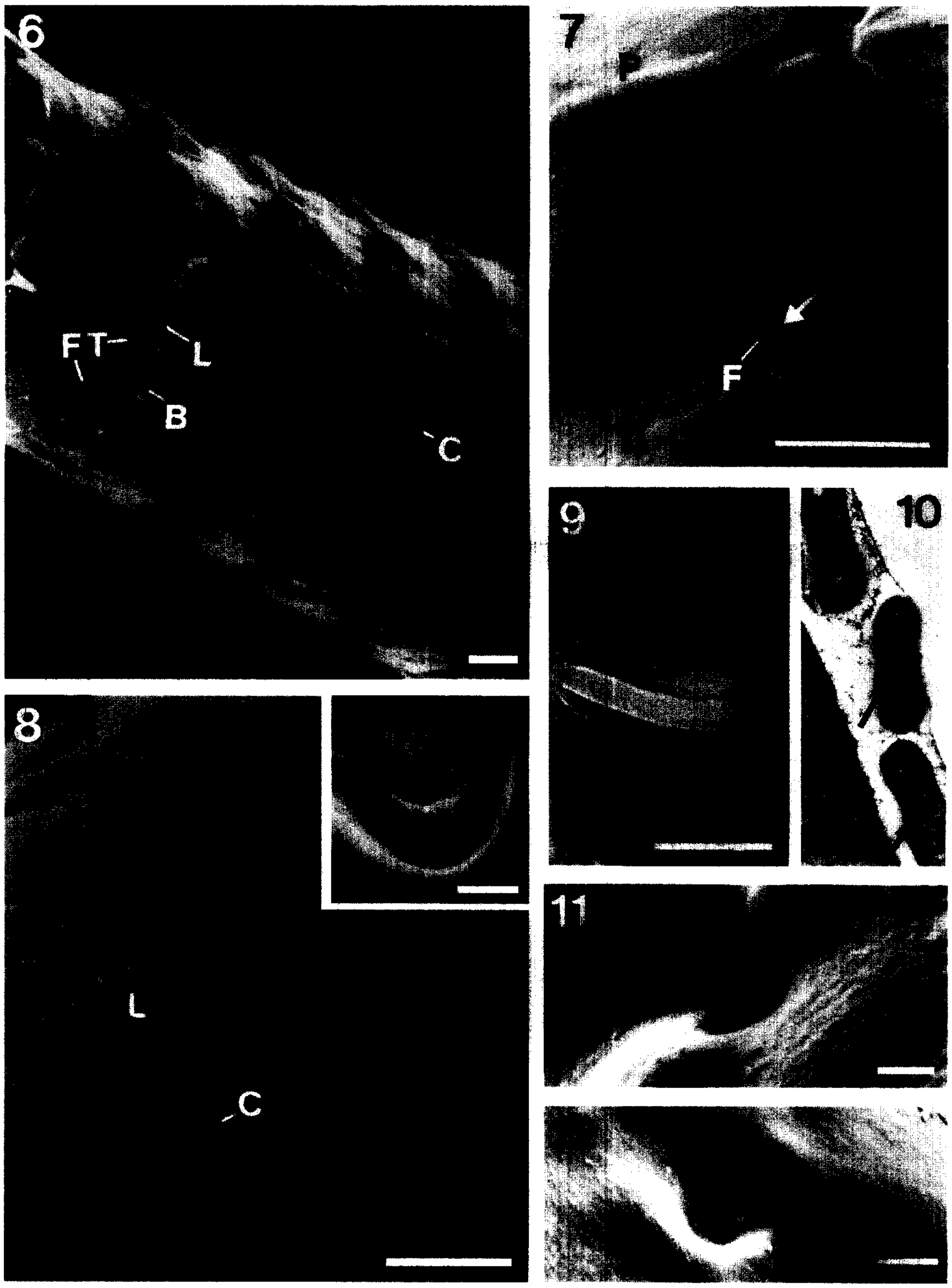
- *Sensilla chaetica* (Figs 2 and 3), in the form of thick bristles set in a socket, which emerge only from the dorsal surface of both segments. They measure 14–15 μm in length and are delimited by an indented border (Fig. 2). In sections, a tubular body and a joint



Figs 1–5. Antennal sensilla of the nymph. Fig. 1. SEM view of the dorsal surface of the antenna showing scape (S), pedicel (P) and a portion of the flagellum (F) (scale bar = 50 μm). The lateral shrinkage of the pedicel is due to the critical point procedure. Fig. 2. SEM view of the dorsal suface of the pedicel (P) showing sensilla chaetica (C) delimited by an indented border (arrow) and a group of sensilla basiconica (B) (scale bar = 5 μm). Fig. 3. Longitudinal section of a sensillum chaeticum in a molting specimen. The tubular body (TB) and the joint membrane (JM) are visible. Note the dendrite (D) in the space between the old (OC) and the new (NC) cuticle (scale bar = 2 μm). Fig. 4. Campaniform sensilla (arrows) along the distal border of the pedicel (P). Note sensilla basiconica (B) and flat-tipped sensilla (FT) protruding towards the flagellum (F) (scale bar = 5 μm). Fig. 5. Longitudinal section of a campaniform sensillum located under the cuticle (C). Note the tubular body (TB) delimited by the dendritic sheath (DS) (scale bar = 1 μm).

membrane, which allows the movements of this bristle, are evident (Fig. 3).

- *Sensilla basiconica* isolated or in groups, are evenly distributed on the scape and pedicel. They vary in length (10–15 μm) and are hair-shaped (Fig. 2).
- *Sensilla campaniformia* are shaped like small circular domes, of 2 μm in diameter. They form a single ring located along the distal border of the pedicel near the articulation with the flagellum (Fig. 4). In longitudinal section, they reveal the presence of a tubular body surrounded by its dendritic sheath (Fig 5).
- *Flat-tipped sensilla* are evenly distributed on scape and pedicel (Fig. 4), showing a regular arrangement on each flagellar segment (Fig. 6). This kind of spatulate bristle is the most common antennal sensillum of this species. On the flagellum, flat-tipped sensilla are

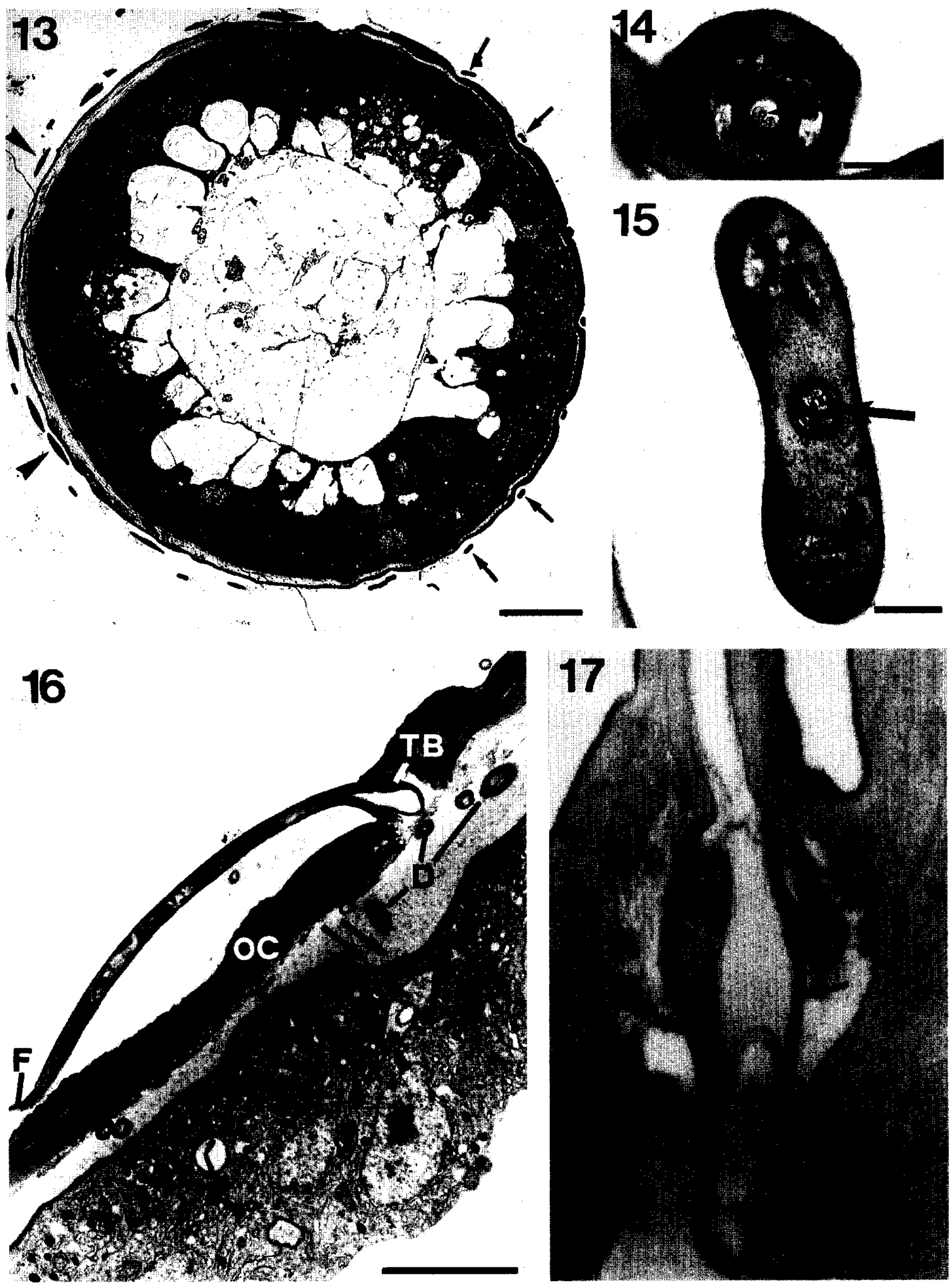


Figs 6–12. Antennal sensilla of the nymph. Fig. 6. SEM view of the flagellum showing different kinds of sensilla: flat-tipped sensilla (FT) located between cuticular lobes (L). Note several groups of sensilla basiconica (B), coeloconic sensilla (C) and cuticular pits (arrows) (scale bar = 10 μm). Fig. 7. SEM view of a flat-tipped sensillum emerging from adjacent cuticular lobes (L). Note the terminal flange (F) with the pore (arrow). P = proximal article, D = distal article (scale bar = 5 μm). Fig. 8. Sensilla coeloconica (C) between the cuticular lobes (L) (scale bar = 5 μm). In the inset (scale bar = 1 μm) a zoomed view of one of them, showing a depression in the middle. Fig. 9. A group of sensilla basiconica (scale bar = 5 μm). Fig. 10. Cross section of a group of sensilla basiconica. Note the dendrites in the middle (arrows) (scale bar = 0.5 μm). Fig. 11. Coeloconic-like sensillum (scale bar = 1 μm). Fig. 12. Detail of a cuticular pit on the lateral surface of the flagellum (scale bar = 1 μm).

located between the distal cuticular lobes of each antennal article (Fig. 6), and bend to contact the adjacent article forming a bridge (Fig. 7). This sensillum is a type of sensillum basiconicum, emerging from a well-defined socket and bearing a pore at its tip, where it enlarges, giving rise to a thin flange about 2 μm wide (Fig. 7). This spatulate bristle averages 9–10 μm, including the flange.

In addition to flat-tipped sensilla, other types of sensilla can be observed on the flagellum:

- *Sensilla coeloconica* show a pear-shaped appearance with a depression in the middle (inset of Fig. 8). They average 2.5 μm in length and are occasionally located among the cuticular antennal lobes (Fig. 8).
- *Sensilla basiconica*, in groups of 3 or 4 elements, are closely related to those described in scape and pedicel

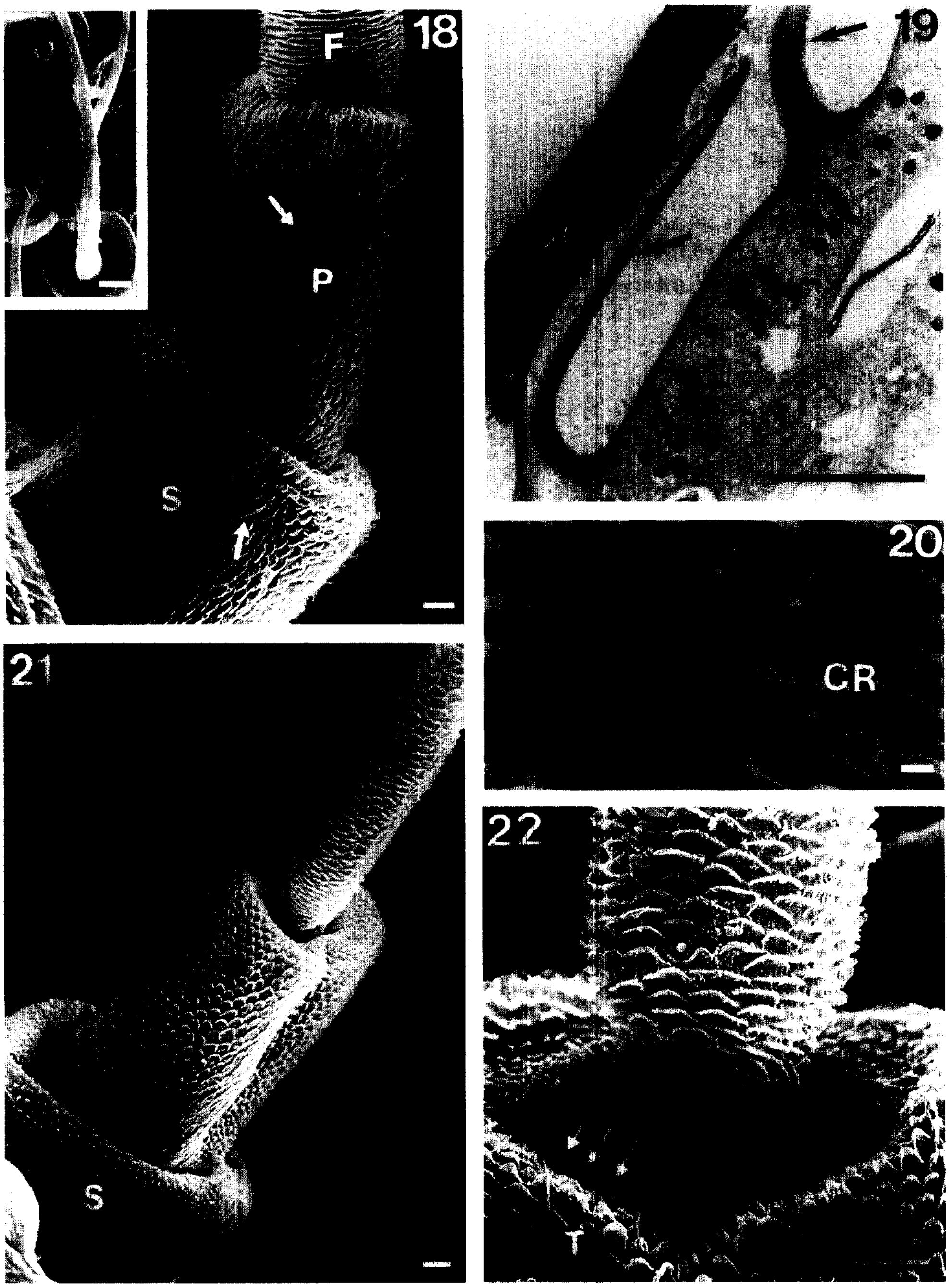


Figs 13–17. Flat-tipped sensilla of the nymph, in section. Fig. 13. General view of a flagellar segment in cross-section. Note the peripheral cuticular lobes (arrowheads) and flat-tipped sensilla (arrows) alternated at regular intervals (scale bar = 9 μm). Fig. 14. Cross section of the basal portion of a flat-tipped sensillum showing two dendrites (arrow) (scale bar = 0.5 μm). Fig. 15. Detail of the distal portion of a flat-tipped sensillum in cross section. Note three dendrites in the middle (arrow) (scale bar = 0.1 μm). Fig. 16. Longitudinal section of a flat-tipped sensillum during the molt. Note the bending of the sensillum and the close apposition of the flange (F) to the cuticle. The tubular body (TB) and the outlines of the dendrites (D) are visible. Old (OC) and new cuticle (arrows) coexist (scale bar = 3 μm). Fig. 17. Longitudinal section of the basal region of a flat-tipped sensillum. Note the tubular body (TB) and the dendritic sheath (arrow) (scale bar = 0.5 μm).

(Fig. 9). They are located on the most distal region of each segment, and, in cross-section, they appear to be innervated by neurons (Fig. 10).

- *Coeloconic-like sensilla* are basiconic pegs (Fig. 11). They are rare and sometimes close to the cuticular lobes. They measure about 1.0–1.5 μm in length and 0.8 μm in diameter at the base. The cuticle is smooth and no pore has been observed.
- *Cuticular pits* are located on the lateral surface of the flagellum (Fig. 6). They appear as cuticular depressions and typically have one edge that is narrower than the opposite one, which descends more gradually (Fig. 12).

Cross-sections of the flagellum confirm the regular arrangement of the flat-tipped sensilla all around the antennal segments (Fig. 13). Serial sections of one of these sensilla reveal the occurrence of 2 dendrites at the base (Fig. 14). At the upper level 3 dendrites are visible (Fig. 15). Longitudinal sections corroborate the bending



Figs 18–22. Antenna of the *subimago* (18–20) and of the *imago* (21–22). Fig. 18. SEM view showing scape (S), pedicel (P) and a portion of the flagellum (F). Note the large amount of microtrichia on both scape and pedicel. Some sensilla trichodea are visible (arrows) (scale bar = 10 μm). In the inset (scale bar = 1 μm) a zoomed view of a sensillum trichodeum. Fig. 19. Longitudinal section of the pedicel of a nymph about to emerge. Microtrichia (arrows) are evident below the old larval cuticle (OC) (scale bar = 2 μm). Fig. 20. Coeloconic pegs in pairs (arrows) among cuticular ribs (CR) located along the ventral side of the flagellum (scale bar = 1 μm). Fig. 21. SEM view showing scape (S), pedicel (P) and a portion of the flagellum (F) completely covered with scales (scale bar = 10 μm). Fig. 22. Sensilla coeloconica (arrowhead) and trichodea (T). Note the ring of sensilla campaniformia (arrows) on the distal border of the pedicel (scale bar = 10 μm).

of each sensillum on the antennal surface and the close apposition of the flange to the cuticular surface (Fig. 16). A tubular body ends at the base of the bristle (Fig. 17).

In the *subimago*, scape and pedicel are uniformly covered with microtrichia and also show rare sensilla trichodea (Fig. 18), thus differing from the flagellum, which has a typically ribbed surface (Fig. 18). Sensilla trichodea are about 20 μm long, have a diameter of 1 μm at the base and decrease progressively towards the pointed tip. Their cuticle is smooth and their bases are inserted into a well-defined socket (inset of Fig. 18). No pore was observed. Microtrichia are placed obliquely with respect to the longitudinal axis of the antenna. Longitudinal sections of scape and pedicel of a specimen which was about to emerge, reveal the occurrence of newly formed microtrichia immediately underneath the old nymphal cuticle

(Fig. 19). The flagellar cuticular ribs are arranged in parallel rings (Figs 18 and 20). Sensilla coeloconica are normally positioned among ribs (Fig. 20).

In the *imago* (Fig. 21), all the antennal segments are profusely covered with cuticular scales, among which sensilla trichodea and coeloconica are occasionally visible (Fig. 22). As in the nymph, a ring of sensilla campaniformia delimits the distal border of the pedicel (Fig. 22).

No distinct sexual dimorphism was observed in adults.

DISCUSSION

There is a vast variety of insect sensilla with conspicuous differences in shape (McIver, 1973, 1974; Kapoor and Zachariah, 1983; Solinas *et al.*, 1987; Ross, 1992; Catalá and Schofield, 1994; Mellor and Anderson, 1995; Rani and Madhavendra, 1995; Wcislo, 1995; Kim and Yamasaki, 1996). In contrast to most insects, in which immature stages show more limited sensory requirements (Zacharuk and Shields, 1991), mayfly nymphs of *B. rhodani* present types of sensilla that are diverse from those of adults. This agrees with observations made on insects with a short adult phase (Kapoor, 1985).

The characterization of the sensilla of *B. rhodani* has been essentially based on external morphology and on some ultrastructural details of their organization. These characteristics, coupled with previous studies on insect sensilla, allowed us to assign a putative function to some of them.

The mechanical function of sensilla campaniformia in insects is well-established. Located at the main joint areas of the antenna, these sensilla act as proprioreceptors in response to cuticle deformation. In this site, they sense the movements of the flagellum over the pedicel.

The presence of a tubular body and of a joint membrane in sensilla chaetica supports the view that these sensilla have a mechanical function. During the molt, the functional continuity of this receptor is guaranteed by the connection between sensory cells and the old cuticular apparatus. This continuity is associated with elongation of the dendrites, as seen in the filiform hairs of the caterpillar *Barathra brassicae* (Gnatzy and Tautz, 1977).

Adult mayflies do not feed, but utilize reserves stored during their long-lasting aquatic stages (Gaino and Mazzini, 1995). Among a wide variety of mechanisms evolved for a successful adaptation of these water-dweller organisms, chemosensitive devices should be regarded as specialized structures providing gustatory inputs for the reception of food resource information. Sensilla basiconica should be regarded as a system for tasting because of the presence of dendrites extending along the shaft. This gustatory function could be also performed by flat-tipped sensilla. In *B. rhodani*, these sensilla constitute a very uncommon type of antennal receptor and have the ultrastructural characteristic of chemo-mechanoreceptors. The hypothesis of this double function was forwarded on the basis of both the apical pore and the location of sensilla to form "bridges" between adjacent flagellar articles (Gaino and Rebora, 1996). This dual modality has been corroborated in our experience by means of fine internal organizational details.

In adults, the sensory system drastically changes. Different equipment is acquired for monitoring the subaerial environment. It is quite surprising that aerial forms (*subimago* and *imago*) have an enterely different antennal surface. Nevertheless, in both adult stages, in spite of the different cuticular covering such as microtrichia and scale-like structures, sensilla trichodea and coeloconica are present. These latter could function as a hygrosensory system working to prevent dehydration. These receptors may have a pivotal role in the selection of suitable environment for molting, mating and oviposition. In addition, even the densely packed, scale-like structures of the *imago* could be sensory devices, structured as squamiformia sensilla so far unidentified in immature insects (Zacharuk and Shields, 1991).

In conclusion, these morphological data represent a starting point for further studies on the sensory system of mayflies with a view to elucidate the exact function of sensilla and of the other cuticular coverings described.

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