The sensilla on the labial and maxillary palps of the nymph of *Baetis rhodani* (Ephemeroptera: Baetidae)

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Abstract

The nymph of *Baetis rhodani* shows an orthognathous head in which only the tips of the maxillary and labial palps contact the substrate. In these areas many setae are located. These setae have been investigated under scanning and transmission electron microscopy (SEM, TEM) in order to disclose their sensory function. The labial palps consist of three segments. The most represented hairs on the labial palps are constituted of flat-tipped sensilla (a peculiar kind of uniporous basiconic sensilla with their apical flange adhering to the cuticle), and uniporous basiconic sensilla without apical flange. Both kinds of sensilla show the same internal features, thus revealing their chemo-mechanosensory function. Many bristles (short and long) are located on the distal-ventral side of the labial palps. These bristles are mechanosensory structures probably involved in sensing and sweeping the substrate. The maxillary palps are constituted of two segments. As in the labial palps, most of the hairs are located on the distal segment and are chemo-mechanoreceptors represented by flat-tipped and uniporous basiconic sensilla. At the apex of the distal segment, a big mechanosensory spine is located, and just below this, two gustatory coeloconic sensilla and a proprioreceptor are positioned.

Before feeding, *Baetis rhodani* spends some time touching repeatedly the substrate with its maxillary palps. Considering these behavioural observations and the morphological results, it emerges that the maxillary palps are mainly involved in searching and selecting food. The labial palps seem to be involved mainly in a mechanical survey of the substrate.

Keywords: mayfly, sensory structures, chemo-mechanoreceptors, mouthparts, aquatic insects.

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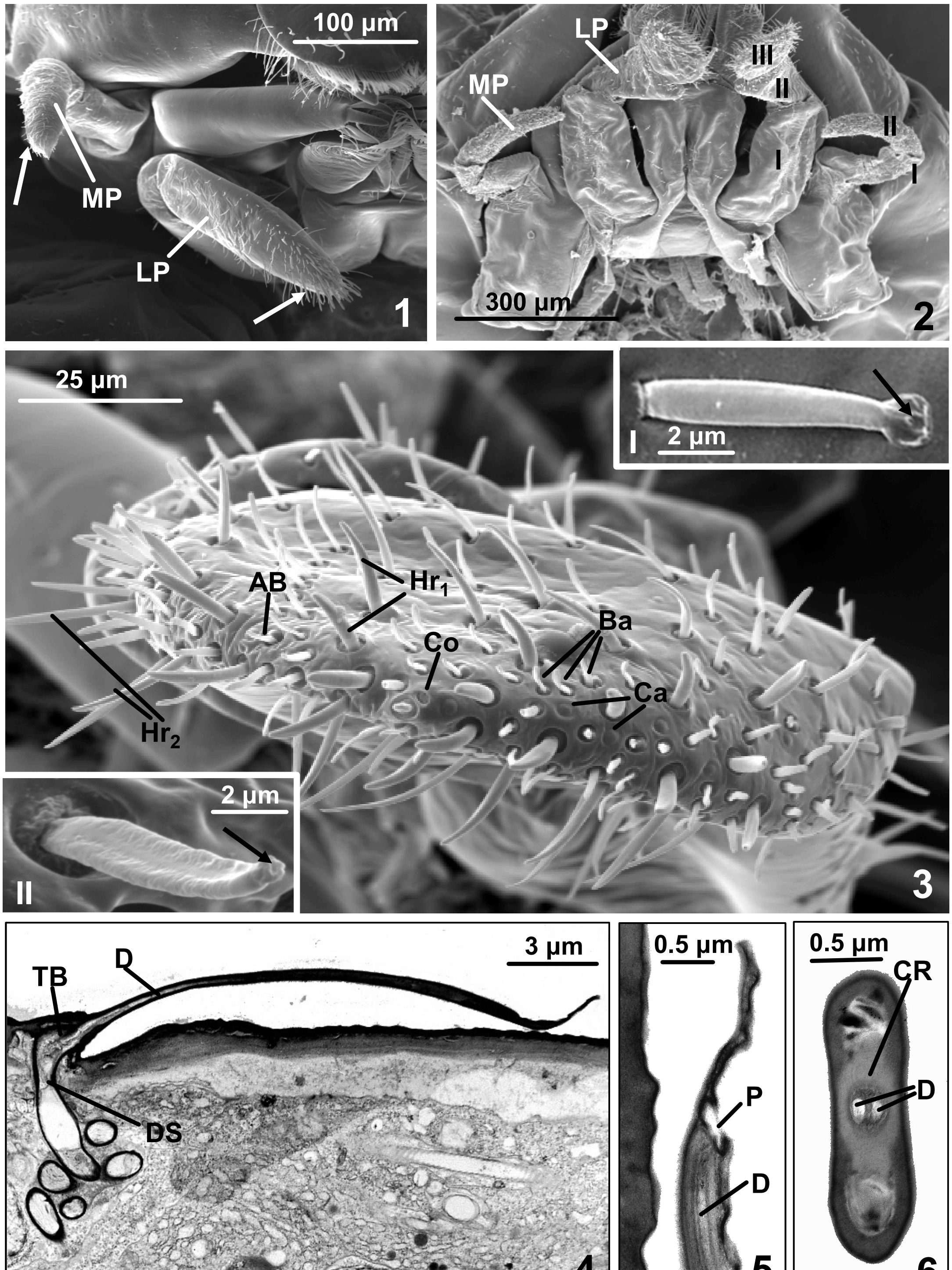
Introduction

Food selection and gustation are of primary importance to sustain growth and development of insect voracious immature stages (Zacharuk and Shields, 1991). In this respect, among aquatic insects, some attention has been paid to mouthpart sensilla in larvae of Odonata (Pritchard, 1965; Bassemir and Hansen, 1980; Wazalwar and Tembhare, 1999), Plecoptera (Kapoor, 1989), Diptera Simuliidae (Craig and Borkent, 1980) and Culicidae (McIver and Beech, 1986), involved in prey finding behaviour. Since adult Ephemeroptera are unable to feed, the long-lasting aquatic stages are the only trophic ones. Whereas the gross morphology of the mouthparts in mayfly nymphs has long served as a basis for classification, their functional morphology has been investigated in consideration of the relevance of these structures in understanding ecological and evolutionary relationships (McCafferty and Provonsha, 1986; McShaffrey and McCafferty, 1988, 1990; Elpers and Tomka, 1994; McShaffrey, 1995; Staniczek, 2000). In particular, setae located on mayfly mouthparts have been described in relation to their role in taking food particles, but so far no ultrastructural data are available on their internal organisation in order to ascertain a possible sensory function. *Baetis rhodani* (PICTET, 1843) is a mayfly commonly present in various freshwater habitats. The external morphology and functioning of its mouthparts have been described under light microscope by Brown (1961). The antennal sensilla of the nymph of this species have been documented by scanning and transmission electron microscopy investigations (Gaino and Rebora, 1996, 1998, 1999a, b). The aim of the present work is to highlight at ultrastructural level (SEM, TEM) the main sensilla of the labial and maxillary palps of the nymph of *B. rhodani*.

Material and Methods

Mature nymphs of *Baetis rhodani* were collected in the Ierna brook (a left tributary of the Nestore River), close to Tavernelle (Perugia, Umbria Region, Italy) and in the Nera River, tributary of Lake Piediluco (13 km far from Terni, Umbria Region, Italy), in autumn 2000. The mouthparts of 6 nymphs (4 females and two males) were dissected under a stereomicroscope. The palps were fixed in 2.5% glutaraldehyde buffered in cacodylate, pH 7.2, for 1 h, repeatedly rinsed in the same buffer, and postfixed in 1% osmium tetroxide for 1 h. For scanning electron



Figs. 1-6 - Palps of the nymph of *Baetis rhodani* under SEM (Figs. 1-3 and insets I and II) and flat-tipped uniporous basiconic sensillum under TEM (Figs. 4-6). Fig. 1. Frontal view of the head. Note the richness of hairs (arrows) on the distal portion of the labial (LP) and maxillary palps (MP); Fig. 2. Ventral view of the head. Note the labial palp (LP) constituted of three segments (I-III) and the maxillary palp (MP) constituted of two segments (I-II); Fig. 3. Detail of the ventral side of the 3rd segments of the labial palp. Note the short (Hr_1) and long (Hr_2) hairs, the uniporous basiconic sensilla (Ba, detailed in the inset II), the campaniform sensilla (Ca). Inset I shows the flat-tipped uniporous basiconic sensilla mainly located on the proximal portion of the third segment of the labial palps. Arrows point out the apical pore; Fig. 4. Flat-tipped uniporous basiconic sensillum in longitudinal section. D, dendrites; DS, dendritic sheath; TB, tubular body; Fig. 5. Apical portion of flat-tipped sensillum in longitudinal section. Note the apical pore (P) and the dendrites (D); Fig. 6. Cross section of a flat-tipped sensillum. Note the two dendrites (D) inside a cuticular ring (CR).

microscopy (SEM) observations, the specimens were dehydrated by using ethanol gradients, followed by critical-point drying in a CO_2 Pabisch CPD apparatus. Specimens were mounted on stubs with silver conducting paint, sputter-coated with gold-palladium in a Balzers Union Evaporator, observed and photographed in a Philips EM XL30 of the Electron Microscopy Centre of the University of Perugia.

For transmission electron microscopy (TEM) analysis, selected material was dehydrated in the graded ethanol series and embedded in Epon-Araldite mixture resin. Thin sections, cut on a Reichert ultramicrotome, were collected on formvar-coated copper grids and stained with uranyl acetate and lead citrate. The thin sections were examined with Philips EM 400 and EM 208 transmission electron microscopes of the Electron Microscopy Centre of the University of Perugia.

In order to collect data on the involvement of the labial and maxillary palps during feeding, three female nymphs grazing on diatoms, which had settled on algal filaments, were observed under the stereomicroscope (Leika MZ6).

Results

The nymph of *Baetis rhodani* has an orthognathous head, a position that allows only the tips of the labial and maxillary palps to contact the substrate. This feature is consistent with the occurrence of a large number of setae on the distal portion of labial and maxillary palps (Figs. 1, 2).

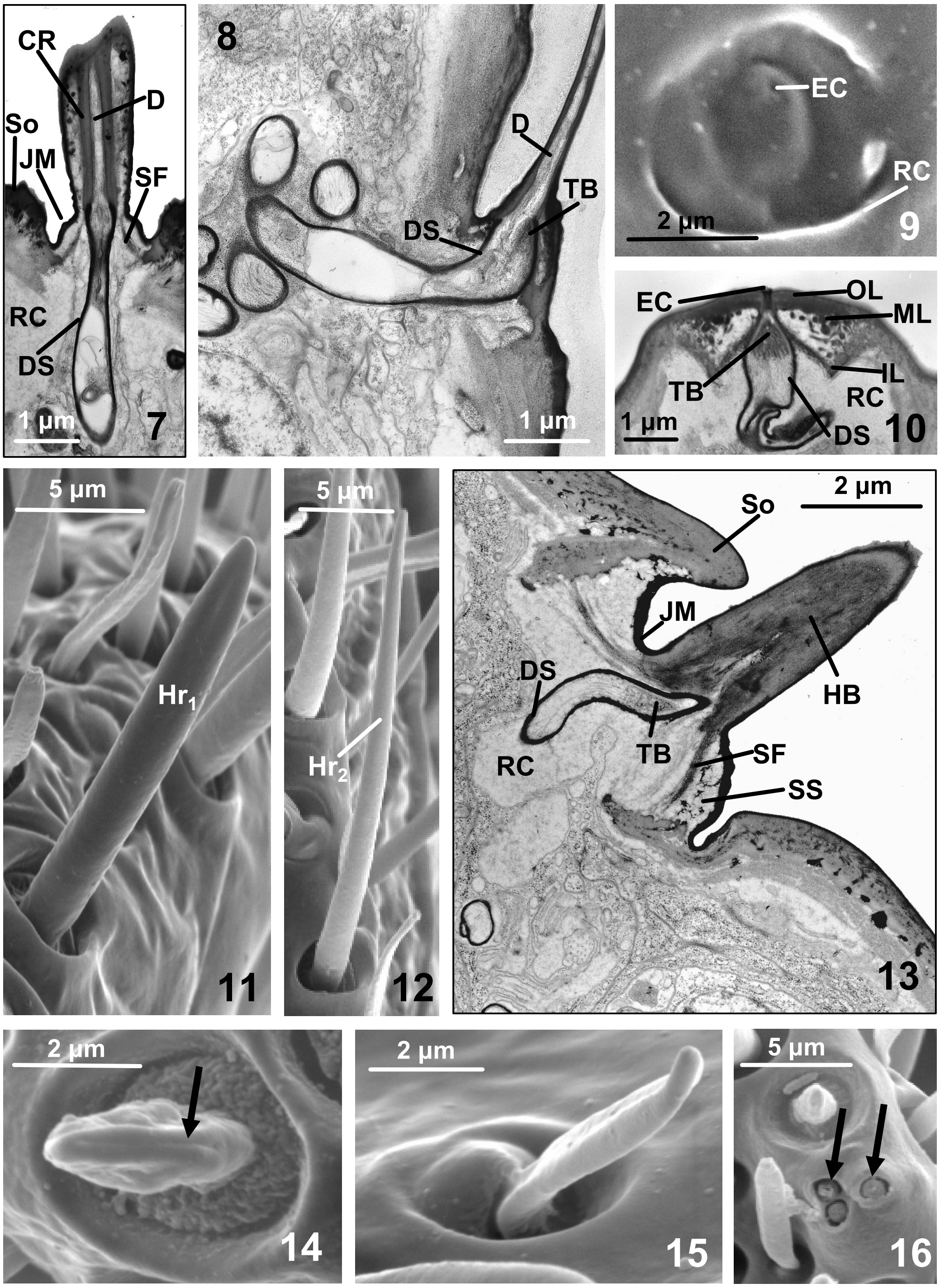
The labial palps consist of three segments (Fig. 2) bearing hairs increasing in number from the proximal to the distal segment. These hairs are restricted only on the ventral side of the palps (Fig. 3). Most of hairs are flat-tipped uniporous basiconic sensilla with their apical flange adhering to the cuticle (Fig. 3, inset I), and uniporous basiconic sensilla without apical flange (Fig. 3, inset II). Flat-tipped sensilla are prevalent on the proximal segments of both palps, while uniporous basiconic sensilla without apical flange are mostly distributed on the distal segments. These two types of sensilla share similar ultrastructural features (Figs. 4-8). They show three dendrites: one ends at the base of the shaft with a tubular body (Fig. 8) and two extend along the shaft up to the apical pore (Figs. 5-8). The tubular body and the two dendrites are wrapped with a dendritic sheath (Figs. 7, 8), which merges into a characteristic cuticular ring surrounding the dendrites along the shaft (Figs. 6-8). The shaft is flattened and, in cross section, slightly biconcave in shape (Fig. 6). In the area of the socket, the basal cuticle of the hair extends to the tubular body (Fig. 8).

On the third segment of the labial palps, amidst the basiconic sensilla, campaniform sensilla (Fig. 3), and two types of hairs (Fig. 3, Hr_1 and Hr_2) are distributed.

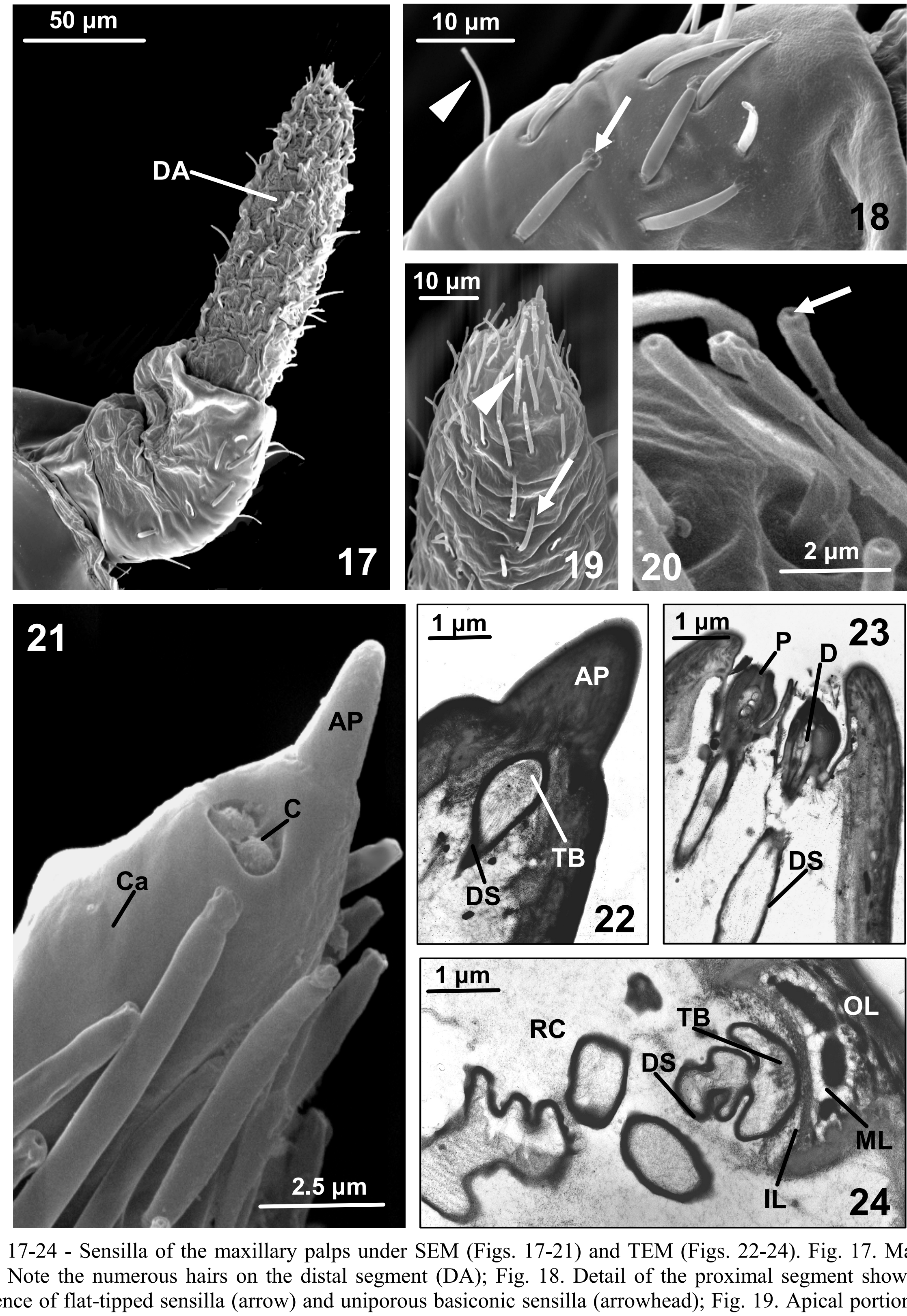
Each campaniform sensillum has a circular dome surrounded by a cuticular rim (Fig. 9). Under TEM the cuticle of the dome is composed of three layers: the outer thick layer, the middle spongy layer and the inner thin fibrous layer (Fig. 10). A pore, representing the ecdysial canal, is visible above the tubular body, which is wrapped with its dendritic sheath (Fig. 10). The hairs on the labial palps are represented by two kinds of sensilla chaetica, thick short hair (20 μm long and 2 μm wide on average) (Fig. 11) and thin long hair (30 μm long and 1.5 μm wide) (Fig. 12). They both emerge from a well-developed socket and are innervated by a single dendrite ending with a tubular body wrapped with the dendritic sheath immersed in the receptor-lymph cavity (Fig. 13). Longitudinal sections at the level of the insertion of the hair base into the socket show a joint membrane, a socket septum and suspension fibres connecting the socket to the hair base (Fig. 13).

Other sensilla scattered sparsely on the third segment of the labial palps are: a) centrally grooved lobe-shaped sensilla (Fig. 14); b) aporous basiconic sensilla (Fig. 15), and c) coeloconic sensilla located in pits, single or in groups of two/three (Fig. 16). At present, we have no data on their internal organisation and no hypothesis can be forwarded on their putative function.

The maxillary palps consist of two segments (Fig. 17). The proximal segment shows few sensilla scattered on it (Fig. 18), while the distal one bears numerous sensilla (Fig. 19). On both segments these sensory structures are mostly flat-tipped sensilla (Fig. 18) and uniporous basiconic sensilla (Fig. 20), similar to those already described on the labial palps. Apex of the distal segment of the maxillary palps bears three kinds of sensilla (Fig. 21): a conical poreless apical peg, two sub-apical coeloconic sensilla and a campaniform sensillum (located at a lower level). The apical peg is innervated by a single neuron ending at the base of the peg (Fig. 22). Each of the two sub-apical coeloconic sensilla shows an apical pore and is innervated by two dendrites extending inside the pegs (Fig. 23).



Figs. 7-15 - Sensilla of the labial palps under TEM (Figs. 7, 8, 10, 13) and SEM (Figs. 9, 11, 12, 14-16). Fig. 7. Flat-tipped sensillum in longitudinal section at the level of the socket (So). The dendritic sheath (DS) in the receptorlymph cavity (RC) merges into the cuticular ring (CR) that surrounds the dendrites (D). Note the joint membrane (JM) and the suspension fibres (SF); Fig. 8. Zoomed view of the flat-tipped sensillum of Fig. 4. Note the tubular body (TB) and the dendrites (D) running along the shaft. DS, dendritic sheath; Fig. 9. Campaniform sensillum surrounded by a ring of raised cuticle (RC). EC, ecdysial canal; Fig. 10. Campaniform sensillum in longitudinal section showing its tubular body (TB) wrapped with the dendritic sheath (DS) immersed in the receptorlymph cavity (RC). Note the outer (OL), the middle (ML) and the inner (IL) cuticular layer. EC, ecdysial canal; Fig. 11. Short and thick hairs (Hr_1); Fig. 12. Long and thin hairs (Hr_2); Fig. 13. Longitudinal section of a short and thick hair base (HB) and the socket (So). Note the tubular body (TB) wrapped with its dendritic sheath (DS) and immersed in the receptorlymph cavity (RC), the joint membrane (JM), the suspension fibres (SF) and the socket septum (SS); Fig. 14. A centrally grooved (arrow) lobe-shaped sensillum; Fig. 15. Aporous basiconic sensillum; Fig. 16. Coeloconic sensilla (arrows) located in pits.



Figs. 17-24 - Sensilla of the maxillary palps under SEM (Figs. 17-21) and TEM (Figs. 22-24). Fig. 17. Maxillary palp. Note the numerous hairs on the distal segment (DA); Fig. 18. Detail of the proximal segment showing the presence of flat-tipped sensilla (arrow) and uniporous basiconic sensilla (arrowhead); Fig. 19. Apical portion of the maxillary palp. Note the flat-tipped sensilla (arrow) and uniporous basiconic sensilla (arrowhead); Fig. 20. Uniporous basiconic sensilla; Fig. 21. Apical peg (AP), sub-apical coeloconic sensilla (C) and campaniform sensillum (Ca); Fig. 22. Apical peg (AP) in longitudinal section showing the single neuron (N) ending at the base of the peg; Fig. 23. Coeloconic sensilla in longitudinal section. Note the dendrites inside the pegs; DS, dendritic sheath; P, pore; Fig. 24. Campaniform sensillum in longitudinal section. Note the tubular body (TB) surrounded with the dendritic sheath (DS) immersed in the receptor-lymph cavity (RC); IL,ML,OL, inner, middle and outer cuticular layer.

The two dendrites are wrapped with a dendritic sheath (Fig. 23). The campaniform sensillum located under the two coeloconic sensilla is hardly visible under SEM, where it appears as a round depression of the cuticle (Fig. 21). Under TEM (Fig. 24), this sensillum shows a well-developed tubular body wrapped with the dendritic sheath immersed in the receptor-lymph cavity. The tubular body is in close contact with the sensory cuticle, which is constituted of three usual layers (Fig. 24).

No apparent sexual dimorphism was observed by comparing the arrangement of mouthpart sensilla in male and female nymphs.

Observations of the feeding behaviour of *B. rhodani* revealed that the nymph, which feeds on diatoms settled on algal filaments, touches repeatedly the substrate with its maxillary palps before taking up diatoms. In contrast, the labial palps do not show active movements during the grazing.

Discussion

Maxillary and labial palps are considered to be primarily sensory appendages (Zacharuk, 1985). In *B. rhodani* the tip of the palps bears many hairs of sensory structures. Flat-tipped sensilla and uniporous basiconic sensilla are the most common type of sensilla on both palps. Flat-tipped sensilla have been first described on the antennal flagellum of the nymph of this species (Gaino and Rebora, 1996) and their presence has also been reported in scape, pedicel, cerci and urotergites (Gaino and Rebora, 1999a). The occurrence of a tubular body and the dendrites extending along the shaft up to the terminal pore are consistent with the attribution of flat-tipped sensilla and uniporous basiconic sensilla to chemo-mechanosensory functions.

In a review on antennal sensilla of aquatic larvae (Gaino and Rebora, 1999b), these chemo-mechanosensory sensilla in mayflies have been considered relevant sensors in water owing to their dual modality. Indeed, as chemosensitive devices, scattered on different body parts, they should be regarded as specialised systems providing gustatory input; as mechanosensitive devices, they could act as hydrodynamic receptors by which the insect can perceive nearby objects (Gaino and Rebora, 1999b). Behavioural studies on predator-prey interactions between stoneflies and mayflies nymphs highlighted in *Baetis bicaudatus* the importance of chemical and hydrodynamic stimuli for the detection of predaceous stoneflies (Peckarsky, 1980; Peckarsky and Penton, 1989). The interaction between chemical and tactile cues in mayflies for detecting stoneflies has been hypothesised by Ode and Wissinger (1993) in a leptophlebiid. We have also described chemo-mechanosensory hairs on the antenna of the mayfly nymph of *Choroterpes picteti* (Gaino and Rebora, 1997).

On the labial palps, numerous hairs of various sizes, belonging to sensilla chaetica and involved with mechanosensory function, are present. Campaniform sensilla, scattered among hairs, are proprioreceptors sensing the stretching of the cuticle (McIver, 1985).

The apical peg of the maxillary palps of the nymph of *B. rhodani* is a sensillum probably acting as thermal or mechanoreceptor. This finding emphasises the relevance of this structure, which was considered an important taxonomic character (Müller-Liebenau, 1969). Just under the apical peg, the two uniporous coeloconic sensilla show a morphology consistent with their attribution to gustatory sensilla.

This morphological investigation on the palps of the nymph of *B. rhodani*, and observations on its feeding behaviour, allow us to hypothesise the following conclusions:

1) Chemo-mechanosensory sensilla seem to be very common in Ephemeroptera. Owing to their presence on different body parts, they do not represent specialised sensilla of mouthparts. They could have an important role in sensing general mechanical and gustatory cues from the external environment.

2) The labial palp surface with its array of mechanosensory hairs could function to sense the mechanical nature of the substrate; in addition, the extension of these hairs from the palp could allow the "sweeping" of the substrate to collect diatoms.

3) The mechanosensory and gustatory sensilla located at the tip of the maxillary palps could function as a "touch and taste" device, performing an essential role in the search and selection of food.

Additional morphological, behavioural and neurophysiological recordings are necessary, in future research, to understand how variable are mouthpart sensilla among individuals, and what sensory cues do these insects respond.

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