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Fine structure of flat-tipped antennal sensilla in three species of mayflies (Ephemeroptera)

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Abstract. A flat-tipped type of sensillum was studied with the scanning electron microscope in three species of mayflies (Ephemeroptera): *Baetis rhodani, Rhithrogena loyolaea,* and *Epeorus sylvicola.* This sensillum bears an apical pore and its slightly enlarged tip forms a flange. In *B. rhodani,* flat-tipped sensilla are distributed over the surface of the antennal articles, except on the flagellum, where they are arranged along the distal border of each article to form bridges between consecutive articles. In *R. loyolaea* and *E. sylvicola,* flat-tipped sensilla show a characteristic arrowhead shape, emerge farther from the distal border of each flagellar article, and are not present on scape and pedicel. They are irregularly distributed, fewer than those of *B. rhodani,* and rarely reach the adjoining antennal article. On the basis of morphology and location, we hypothesize that flat-tipped sensilla function both as mechano- and chemoreceptors.

Additional key words: integumentary structures, Baetidae, Heptageniidae

Insect sensilla are involved in monitoring the external environment. An exhaustive review of sensilla, including their categorization, organization, and function, has been published by Zacharuk (1985). Likewise, McIver (1985) has given a detailed description of a variety of structures involved in mechanoreception.

Among the wide range of sensilla, particular interest has been paid to antennal receptors (McIver 1973, 1974; McIver & Siemicki 1979; Barlin et al. 1981; Solinas et al. 1987; Catalá & Schofield 1994; Schmidt & Berg 1994; Nicastro et al. 1995).

Sensilla of aquatic insects have been the subject of several studies (Rupprecht 1969; Kapoor & Zachariah 1983; Kapoor 1985a,b, 1986, 1988, 1991; McIver & Beech 1986; Jensen & Zacharuk 1991, 1992).

In mayflies (Ephemeroptera), the distribution of sensilla has been used as a taxonomic trait, but sensillum morphology has not attracted much attention. The first description of ephemeropteran sensilla, on gills of *Caenis,* was by Eastham (1936). As for antennal sensilla, a general account of those in Baetidae can be found in Müller-Liebenau (1969), but ultrastructural investigation is limited at present to the description of mechanoreceptors of the pedicel in a few species (Schmidt 1974).

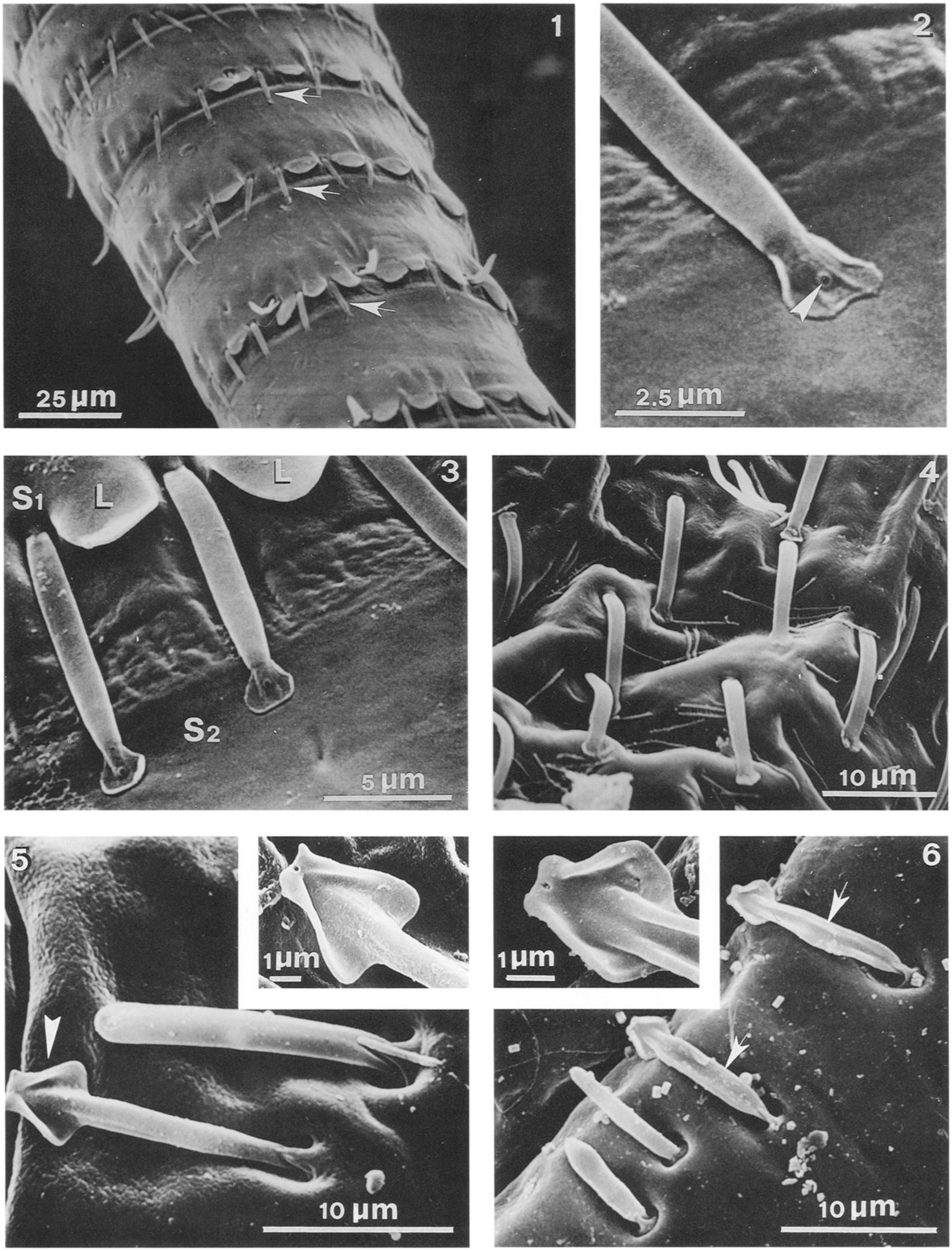
Adult mayflies are short lived and the aquatic immature stages, or nymphs, dominate the life cycle. As a consequence, most behavioral activity depends on the sensory mechanisms of immature stages, as in other insect groups characterized by a short adult phase (Kapoor 1985a).

We compared the antennal sensilla of mayfly nymphs with those described in the literature for other insects. Our studies have revealed a sensillum with an unusual morphology.

Methods

Nymphs of *Baetis rhodani* (Pictet 1843–45), family Baetidae, and of *Rhithrogena loyolaea* Navás 1922 and *Epeorus sylvicola* (Pictet 1865), family Heptageniidae, were collected in the Scrivia River (Casella, Liguria) and in the Verde Stream (Isoverde, Liguria). Specimens studied numbered 20 of *B. rhodani,* 15 of *R. loyolaea,* and 10 of *E. sylvicola.* We measured 50 sensilla of each type. The terminology of McIver (1985) and Zacharuk (1985) was followed.

For scanning electron microscopy (SEM), antennae were dissected from anesthetized specimens. Selected material was immersed in 5% glutaraldehyde and 4% paraformaldehyde in 0.1 M cacodylate buffer, pH 7.2 (Karnovsky 1965) and in osmium tetroxide buffered with 0.1 M sodium cacodylate at pH 7.2 for 1 hour at 4° C. After dehydration in a 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 ob-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served with a Philips EM 515 scanning electron microscope, at an accelerating voltage of 18 kv.

Results

Antennal flagellar articles of *Baetis rhodani* are characterized by a distal border of triangular-shaped lobes among which sensilla protrude (Fig. 1). The lobes are particularly evident ventrally, less evident dorsally. Among various types of basiconic sensilla, the most common are flat-tipped sensilla. Each bears a pore at the tip, where it enlarges, giving rise to a thin flange about 2 μm wide (Fig. 2). These spatulate bristles are from 8.0 μm to 11.6 μm long (mean 9.8 μm ± SD 1.1), including the flange, and from 1.0 μm to 1.6 μm wide (mean 1.2 μm ± SD 0.2). The values for width do not include the flange (about 2 μm in its widest region). The spatulate bristles emerge from a well-defined socket located among the distal lobes of each antennal article (Fig. 3) and bend toward the antennal axis, contacting the next adjacent article to form a bridge connecting the two (Fig. 3). The flange is closely apposed to the cuticular surface (Figs. 2, 3). This type of sensillum is also evenly distributed on the coarse surface of scape and pedicel, where the typical bent configuration is also seen (Fig. 4). Sensilla are oriented from the basal region to the tip of the antenna.

Sensilla with similar morphology have also been found in *Rhithrogena loyolaea* (Fig. 5) and *Epeorus sylvicola* (Fig. 6). The flat-tipped sensilla of these two species are limited to the antennal flagellum. The terminal expansion shows a characteristic arrowhead shape, consisting of two thin wings that include the apical pore where they join together (inset of Figs. 5, 6).

These sensilla are found together with a uniporous basiconic type similar in arrangement and size to the flat-tipped sensilla (Figs. 5, 6).

In *R. loyolaea* and *E. sylvicola* (in contrast to *B. rhodani*), the distal region of each flagellar article has an unlobated border; sensilla are inserted proximal to the border (Figs. 5, 6). As in the antennal flagellum of *B. rhodani,* sensilla are oriented towards the apical tip of the antenna, but they are irregularly distributed and less numerous than in *B. rhodani.* Only rarely do they protrude beyond the article border and reach the surface of the adjacent article.

In *R. loyolaea,* flat-tipped sensilla are from 15.0 μm to 17.0 μm long (mean 15.9 μm ± SD 0.7), including the flange, and from 0.8 μm to 1.5 μm wide (mean 1.2 μm ± SD 0.2). The arrowhead tip is about 4.5 μm in the widest region.

In the flat-tipped sensilla of *E. sylvicola* (in contrast to *R. loyolaea*), a groove extends from the base to the apex of each sensillum (Fig. 6). Sensilla are from 7.3 μm to 13.5 μm long (mean 10.4 μm ± SD 1.8), including the flange, and from 0.9 μm to 2.5 μm wide (mean 1.7 μm ± SD 0.4). The arrowhead tip is about 3 μm in its widest region.

Discussion

A very uncommon sensillum has been described in the aquatic stages of three mayfly species belonging to different genera, although the fine external morphology of these antennal sensilla differs slightly among the species examined. These structures are peculiar to the aquatic stages and are characterized by an apical pore and a thin expansion at the tip. The occurrence of a pore is consistent with chemoreceptors and makes it possible to include these sensilla among the gustatory type, sensitive to chemicals in solution (Zacharuk 1985). The location of the flat-tipped sensilla, which form "bridges" between adjacent flagellar articles, supports the notion that they could also serve as mechanoreceptors.

Spatulate bristles have been found in males of the mealworm beetle, *Tenebrio molitor,* and are considered to perform a special gustatory function as pheromone receptors (Harbach & Larsen 1977).

We hypothesize that flat-tipped sensilla have a double function, acting as both mechano- and chemore-

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Figs. 1–6. SEM views of flat-tipped sensilla in mayfly nymphs: *Baetis rhodani* (Figs. 1–4), *Rhithrogena loyolaea* (Fig. 5), and *Epeorus sylvicola* (Fig. 6).

Fig. 1. Part of the antennal flagellum showing the arrangement of sensilla (arrows) along the distal border of each article.

Fig. 2. Detail of a sensillum with characteristic spatulate shape and terminal pore (arrowhead).

Fig. 3. Sensilla emerge between distal lobes (L) of each antennal article and form bridges connecting consecutive articles (S_1,S_2).

Fig. 4. Sensilla evenly distributed on the scape surface. Note their bent configuration.

Fig. 5. Arrowhead-shaped apex of a sensillum (arrowhead) and location of the apical pore (inset). In this micrograph the sensillum does not extend to the subsequent article.

Fig. 6. Grooved appearance of arrowhead sensilla (arrows). Note that sensilla protrude slightly beyond the edge of the antennal article. Sensillar tip and pore location (inset).

ceptors. This dual modality has been argued, on the basis of ultrastructural investigation, in the larval stage of a culicid (Jez & McIver 1980) and in several other insect groups (McIver 1975).

Beyond the function they perform during the long-lasting aquatic stages of these insects, flat-tipped sensilla could represent a trait useful for phylogenetic studies. *Rhithrogena* and *Epeorus* are thought to be adelphotaxa (Tomka & Elpers 1991) and this sister-group relationship is also supported by the occurrence of arrowhead sensilla in both genera.

In conclusion, flat-tipped sensilla seem to be specialized structures affecting the relationship between insects and environment. In addition, they may provide a set of microcharacters useful for taxonomic purposes.

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