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Flat-tipped sensillum in Baetidae (Ephemeroptera): a microcharacter for taxonomic and phylogenetic considerations

Elda Gaino[a] and Manuela Rebora

Institute of Zoology, University of Perugia, 06123 Perugia, Italy

Abstract. In some mayfly nymphs, family Baetidae, flat-tipped sensilla occur on antennal segments and on other body parts (tergal borders and cerci). These fine, flat-tipped setae have an apical pore, a basal tubular body, and a branched dendrite along the shaft, an organization consistent with a chemo-mechanosensory function. Phylogenetic considerations are presented on the basis of a survey on the presence or absence of flat-tipped sensilla in some members of the Baetidae. The results of this investigation, and those of other authors, support the hypothesis that this sensillum is an apomorphic character.

Additional key words: mayflies, sensilla, phylogeny, taxonomy

Insect sensilla have mainly been studied from two different perspectives: first, from the morphological and electrophysiological point of view, to examine their specific function (Crnjar & Prokopy 1982; Kapoor & Zachariah 1983; Keil & Steinbrecht 1984; McIver 1985; Zacharuk 1985; Solinas et al. 1987; Städler et al. 1994; Isidoro et al. 1996), and second, for the taxonomic relevance assigned to these structures, including setae, scales, and spines.

In the Ephemeroptera, cuticular armatures represent a useful set of characteristics that taxonomists have considered in comparing different species. Scanning electron microscopy (SEM) has allowed new microcharacters to be highlighted. In this regard, Morihara & McCafferty (1979) used SEM in the taxonomic revision of the North American genus *Baetis* to examine and analyze the setae, scales, and spines of the nymphs. Among the three main types of setae studied by these authors, hairlike or bristlelike fine setae that were distinctly clubbed apically were considered to be phylogenetically important traits. This kind of fine setae has been described in the antennae of *Baetis rhodani* (Baetidae) and of *Rhithrogena loyolaea* and *Epeorus sylvicola* (Heptageniidae) (Gaino & Rebora 1996). The location of this setal type and the presence of an apical pore have supported the hypothesis that it could be a sensillum, defined as a flat-tipped sensillum (Gaino & Rebora 1996). Ultrastructural serial sections showed the presence of both a tubular body and the dendrites extending along the shaft, further supporting the hypothesis of a chemo-mechanosensory function (Gaino & Rebora 1998).

Considering the possible systematic relevance of this sensillum to systematics, we have documented its presence (or absence) and its distribution in species representing almost all the European genera of the family Baetidae: *Acentrella, Alainites, Baetis, Centroptilum, Cloeon, Labiobaetis, Nigrobaetis, Procloeon,* and *Pseudocentroptilum.*

For comparison, we examined the antennae of the New Zealand mayfly *Siphlaenigma janae,* belonging to Siphlaenigmatidae, generally regarded as the sister group of Baetidae.

In this paper we report that flat-tipped sensilla are a

[a] Author for correspondence. E-mail: gaino@unipg.it

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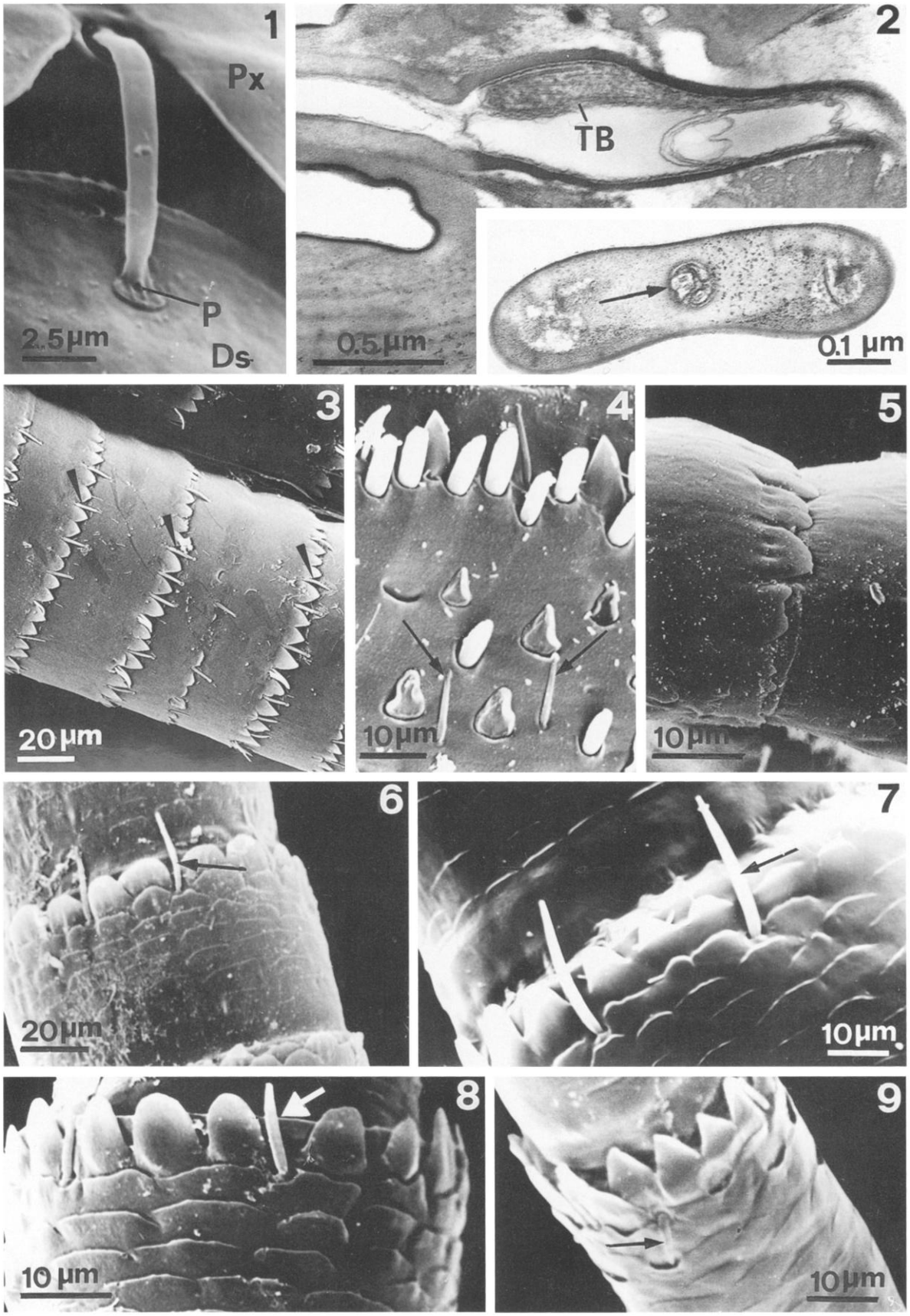
Figs. 1–4. Flat-tipped sensilla in *Baetis rhodani.* **Fig. 1.** The sensillum connects two consecutive antennal articles. Note the pore (P). Proximal article (Px); distal article (Ds). **Fig. 2.** Basal region of a flat-tipped sensillum. Note the tubular body (TB) in longitudinal section. Inset: distal portion of the sensillum in cross section, with dendrites in the middle (arrow). **Fig. 3.** Numerous sensilla (arrowheads) located on a cercus. **Fig. 4.** Sensilla on a urotergite (arrows).

Figs. 5–9. Distal border of antennal articles in several species that lack flat-tipped sensilla but which may present other kinds of fine setae (arrows). **Fig. 5.** *Siphlaenigma janae.* **Fig. 6.** *Centroptilum luteolum.* **Fig. 7.** *Cloeon dipterum.* **Fig. 8.** *Procloeon bifidum.* **Fig. 9.** *Pseudocentroptilum pennulatum.*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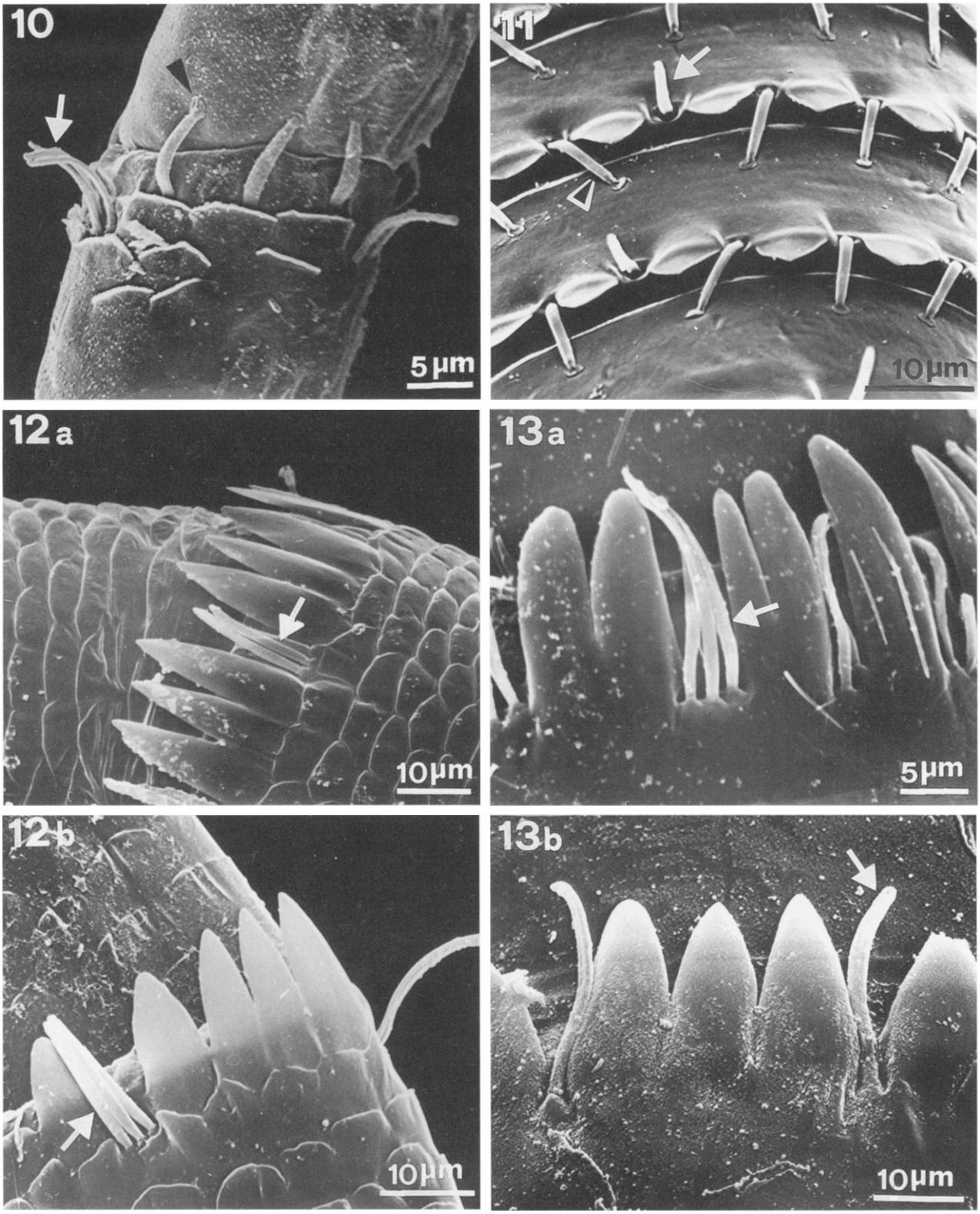


Figs. 10–13. Details of consecutive antennal segments showing the arrangement of flat-tipped sensilla (arrowheads in 10, 11) and other setae (arrows in 12a,b, 13a,b) in different baetids. **Fig. 10.** *Acentrella sinaica.* **Fig. 11.** *Baetis rhodani.* **Fig. 12. a,** *Alainites muticus;* **b,** *A. navasi.* **Fig. 13. a,** *Nigrobaetis digitatus;* **b,** *N. niger.*

Table 1. Absence or presence of flat-tipped sensilla in nymphs of Baetidae.

Species	Fig. no.
Flat-tipped sensilla absent	
Alaintes muticus	12a
A. navasi	12b
Centroptilum luteolum	6
Cloeon dipterum	7
Nigrobaetis digitatus	13a
N. niger	13b
Procloeon bifidum	8
Pseudocentroptilum pennulatum	9
Flat-tipped sensilla present: *Baetis* complex	
Acentrella sinaica	10
alpinus group	
B. alpinus	14a
B. maurus	14b
B. melanonyx	14c
B. punicus	14d
fuscatus group	
B. fuscatus	17a
B. scambus	17b
lutheri group	
B. lutheri	15a
B. meridionalis	15b
B. nigrescens	15c
B. vardarensis	15d
rhodani group	
B. gemellus	16
B. rhodani	11
B. buceratus	18
B. liebenauae	19
Labiobaetis tricolor	20

reliable and useful characteristic for distinguishing species groups and for reinforcing generic criteria within the family. This finding is consistent with the proposal of Morihara & McCafferty (1979) that peculiar larval microstructures may lead to an improved understanding of phylogeny.

Methods

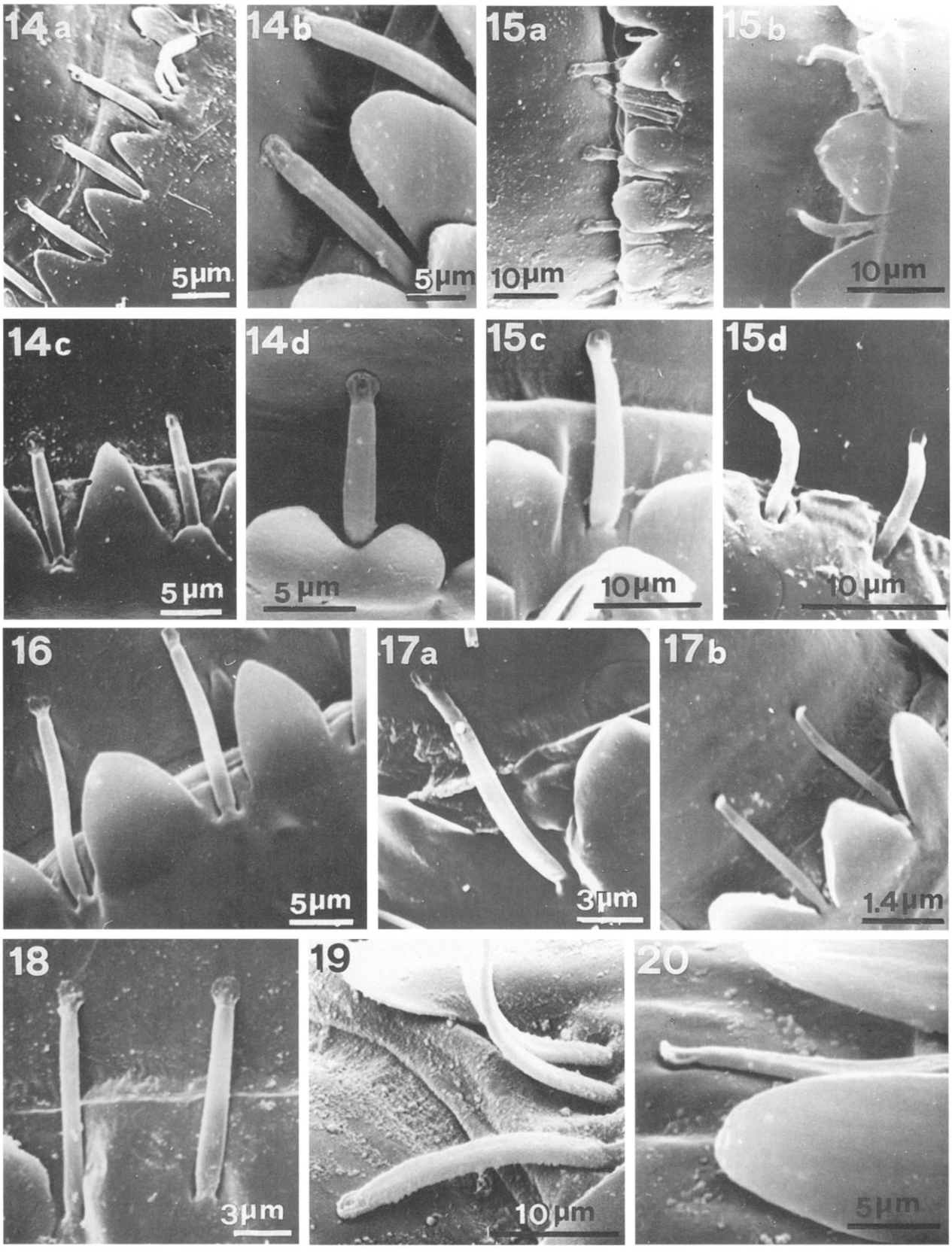
Nymphs of the family Baetidae were examined from various collections and sampling stations here listed:

Acentrella sinaica BOGOESCU 1931, S. Paolo River (Messina, Sicilia), C. Belfiore leg. and det.; *Alainites muticus* (LINNAEUS 1758), Carmen Stream (N. E. Spain), M.A. Puig leg. and det.; *A. navasi* MÜLLER-LIEBENAU 1974, Llanaves de la Reyna (Picos de Europa, Spain), E. Gaino and E. Bongiovanni leg. and det.; *Baetis alpinus* (PICTET 1843), Berlino Stream (Genova, Liguria), E. Bongiovanni leg., E. Gaino det.; *B. buceratus* EATON 1870, Fossalto Stream (Terni, Umbria), C. Belfiore leg. and det.; *B. fuscatus* (LINNAEUS 1761), Alcantara River (Messina, Sicilia), E. Gaino leg. and det.; *B. gemellus* EATON 1885, Molleres Stream (Pyrenees, Spain), M.A. Puig leg. and det.; *B. liebenauae* KEFFERMÜLLER 1974, River Enz (Eutingen, Germany), B. Breitinger leg., A. Staniczek det.; *B. lutheri* MÜLLER-LIEBENAU 1967, Sansobbia Stream (Savona, Liguria), E. Gaino leg. and det.; *B. maurus* KIMMINS 1938, Beixiberri Stream (Pyrenees, Spain), M.A. Puig leg. and det.; *B. melanonyx* (PICTET 1843), Centa River (Savona, Liguria), E. Gaino leg., M. Rebora det.; *B. meridionalis* IKONOMOV 1962, Canovas Stream (Besos basin, Spain), M.A. Puig leg. and det.; *B. nigrescens* NAVÁS 1932, Llobregat Stream (Pont de Vilumara, Spain), M.A. Puig leg. and det.; *B. punicus* THOMAS, BOUMAIZA, SOLDAN 1983, Nere Stream (Viella, Spain), M.A. Puig leg. and det.; *B. rhodani* (PICTET 1843), Lemme Stream (Alessandria, Piemonte), M. Rebora leg. and det.; *B. scambus* EATON 1870, Tietar Stream (Tajo basin, Spain), M.A. Puig leg. and det.; *B. vardarensis* IKONOMOV 1962, Sansobbia Stream (Savona, Liguria), Gaino leg. and det.; *Centroptilum luteolum* (MÜLLER 1776), Vara Stream (La Spezia, Liguria), E Gaino leg. and det.; *Cloeon dipterum* (LINNEO 1761), Erro Stream (Acqui, Piemonte), E. Gaino leg., M. Rebora det.; *Labiobaetis tricolor* (TSHERNOVA 1928), Segura Stream (Baños de Archena, Spain), M.A. Puig leg. and det.; *Nigrobaetis digitatus* BENGTSSON 1912, Mignone River (Roma, Lazio), C. Belfiore leg. and det.; *N. niger* (LINNAEUS 1761) Neckar River (Tübingen, Germany), A. Staniczek leg. and det.; *Procloeon bifidum* (BENGTSSON 1912), Erro Stream (Acqui, Piemonte), E. Gaino leg., M. Rebora det.; *Pseudocentroptilum pennulatum* (EATON 1870), Berlino Stream (Genova, Liguria), E. Gaino and E. Bongiovanni leg. and det.; *Siphlaenigma janae* PENNIKET 1962 (South Island, Maori Gulli, New Zealand), A. Staniczek leg. and det.

Microscopy

The arrangement, distribution, and structure of the flat-tipped sensilla were investigated with scanning electron microscopy (SEM) and transmission electron microscopy (TEM).

For SEM, the specimens were dehydrated in graded ethanol series, critical point dried using a CO_2 Pabisch CPD apparatus, mounted on stubs with silver conducting paint, coated with gold palladium in a Balzers Union Evaporator, and observed with a Philips EM 515 SEM. For TEM analysis, excized antennae were fixed in Karnovsky's medium (Karnovsky 1965) in cacodylate buffer, pH 7.2, repeatedly rinsed in the same buffer, and postfixed for 1 h in osmium tetroxide. They were then rinsed in cacodylate buffer, dehydrated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3µm
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20
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in graded ethanol series, and embedded in Epon-Araldite mixture resin. Thin sections, cut on a Reichert ultramicrotome, were collected on formvar-coated copper grids, stained with uranyl acetate and lead citrate, and observed with a Philips EM 400 TEM.

Results

The flat-tipped sensilla are basiconic sensilla with a pore in the middle of the flattened tip, shaped like a blunt spatula (Fig. 1). These bristles are 8.0–11.6 μm long including the apical flange, and 1.0–1.6 μm wide. Each bristle arises from a socket located among the distal lobes of each antennal article and is bent towards the next adjacent article to form a bridge connecting the two (Fig. 1). The flange is closely apposed to the cuticular surface. Sensilla are oriented from the basal region to the tip of the antenna.

A tubular body is present at the base of the seta (Fig. 2), together with 2 or 3 dendrites extending along the shaft (inset of Fig. 2). This organization is consistent with a mechano-chemical function (Gaino & Rebora 1998).

On the larval body, this type of sensillum, if present on the antennae, always occurs also on the surface of cerci (Fig. 3) and urotergites (Fig. 4). In contrast with antennae and cerci, where the sensilla are located along the distal border of each article, in the urotergites the sensilla are evenly distributed. In this case also, the flange adheres to the cuticle.

Other fine setae similar to the flat-tipped sensilla are located on the distal margin of each antennal article. They are apically acute or blunt and occur on the representatives of all the genera of Baetidae analyzed here. They can be easily distinguished from the flat-tipped sensilla since no pores are visible under SEM. On occasion, they touch the surface of the adjacent antennal article. These setae can be single as in *Centroptilum* (Fig. 6), *Cloeon* (Fig. 7), *Procloeon* (Fig. 8), and *Pseudocentroptilum* (Fig. 9), or in groups of 2 or 3, as in *Acentrella* (Fig. 10), *Baetis* (Fig. 11), *Alainites* (Fig. 12a,b), *Nigrobaetis* (Fig. 13a,b), and *Labiobaetis*. In addition to these other setae, the porous flat-tipped sensilla are present in *Acentrella sinaica* and *Labiobaetis tricolor* along with the other species belonging to the genus *Baetis* examined here (Figs. 10, 11, 14–20).

No flat-tipped sensilla are present in *Siphlaenigma janae* (Fig. 5).

The presence or absence of flat-tipped sensilla among Baetidae is summarized in Table 1.

Discussion

Flat-tipped sensilla, beyond the function performed during the long-lasting aquatic stages of mayflies, could represent a microcharacter useful for taxonomic and phylogenetic studies. In our study of the family Baetidae, the presence or absence of these specialized setae was of significance in outlining some phylogenetic affinities.

Bogoescu & Tabacaru (1957) proposed two evolutionary lines in the Baetidae, one including genera with a single intercalary venation in the anterior wings (e.g., *Centroptilum, Cloeon, Procloeon,* and *Pseudocentroptilum*) and the other encompassing the remaining genera (*Acentrella, Baetis,* and *Pseudocloeon*) with two intercalary venations.

The genus *Baetis* has been the object of numerous revisions and several species are now included in new genera, such as *B. muticus* and *B. navasi* (*muticus*-group) in *Alainites* Waltz & McCafferty 1994 (Waltz et al. 1994); *B. digitatus* and *B. niger* (*niger*-group) in *Nigrobaetis* Novikova & Kluge 1987; *B. tricolor* in *Labiobaetis* Novikova & Kluge 1987.

Nigrobaetis and *Labiobaetis* were initially considered as subgenera of *Baetis* (Novikova & Kluge 1987, 1995) and afterwards raised to generic rank by Waltz et al. (1994) and by McCaffetry & Waltz (1995), respectively.

Waltz & McCafferty (1987) observed a ventral femoral patch of setae, the villopore, in nymphs of numerous genera including *Acentrella, Baetis,* and *Labiobaetis*. This character is a significant synapomorphy uniting a number of genera of Baetidae referred to as the *Baetis* complex (Waltz & McCafferty 1997). As a consequence, the authors considered the species without the villopore not congeneric with *Baetis* or with other genera possessing the patch.

The absence of flat-tipped sensilla in the species not belonging to the *Baetis* complex parallels the absence of villopore, thus supporting the apomorphic status of both villopore and flat-tipped sensilla. In this regard

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Figs. 14–20. Flat-tipped sensilla on the antennal articles of species of *Baetis* (14–19) and *Labiobaetis* (20). **Fig. 14.** *alpinus*-group: **a,** *B. alpinus;* **b,** *B. maurus;* **c,** *B. melanonyx;* **d,** *B. punicus.* **Fig. 15.** *lutheri*-group: **a,** *B. lutheri;* **b,** *B. meridionalis;* **c,** *B. nigrescens;* **d,** *B. vardarensis.* **Fig. 16.** *B. gemellus.* **Fig. 17.** *fuscatus*-group: **a,** *B. fuscatus;* **b,** *B. scambus.* **Fig. 18.** *B. buceratus.* **Fig. 19.** *B. liebenauae.* **Fig. 20.** *Labiobaetis tricolor.*

our new character supports the concept of the *Baetis* complex.

The lack of flat-tipped sensilla in *Alainites, Centroptilum, Cloeon, Nigrobaetis, Procloeon,* and *Pseudocentroptilum* has to be considered as an ancestral condition. This conclusion is also supported by the lack of flat-tipped sensilla in *Siphlaenigma janae,* a species discovered by Penniket (1962) and placed in the new family Siphlaenigmatidae. This family is generally regarded as the sister group of Baetidae (see reference in Staniczek 1997), having retained numerous plesiomorphic characters in contrast with the numerous derived characters evolved in Baetidae (Staniczek 1997).

Further comparative studies on the presence or absence of flat-tipped sensilla in other representatives of the genera, besides those examined in this paper, might clarify relationships within the Baetidae. Furthermore, since progress in taxonomy can be made by combining a wide variety of approaches, new microcharacters could bring further opportunities to enhance our knowledge on the phylogeny of this ancient insect group.

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