# Sensory Receptors on the Forceps of the Male *imago* and *subimago* of *Ecdyonurus venosus* (Ephemeroptera: Heptageniidae)

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**ABSTRACT** The ultrastructure of the globular papillae located along the inner side of the forceps of the two flying stages, *imago* and *subimago*, of male mayflies belonging to *Ecdyonurus venosus* (F.) is described. The external shape of the globular papillae varies with their position on the forceps' segments and the stage. In longitudinal section, the globular papillae of the *imago* and *subimago* have a single dendrite ending in a tubular body, a feature consistent with their function as mechanosensory sensilla. During mating, the mechanical pressure on the forceps may be sensed by the cuticular surface of the papillae and transmitted to the tubular body. In the *subimago*, the globular papillae have a pointed apex and are less numerous. Another kind of sensillum, hairs having the typical organization of mechanoreceptive bristles, are scattered on the forceps of both *imago* and *subimago*.

**KEY WORDS** mayfly, mating apparatus, sensilla, mechanoreceptors, ultrastructure

AN IMPORTANT PART of the mating habits of many mayfly species consists of an aerial performance, a feature well known as a sort of wedding-dance (Brinck 1957), which differs among species (Fisher 1991). Typically, copulation takes place in flight (Verrier 1956, Grandi 1973) with the male *imago* flying to a female from below and stretching the forelegs upward in such a way that the tarsi catch the wing base of the female. The forceps grasp the abdominal apex of the female, who curves her abdomen, thereby allowing insemination (Brinck 1957).

Mayfly forceps exhibit a great variety of form and shape and have been important in taxonomy (Peters and Edmunds 1970, Müller-Liebenau 1973, Studemann et al. 1992). The function of the forceps in mating has been typically considered adhesive to allow the male to grasp the female abdomen. Fink and Andrikovics (1997) mentioned the presence of porous sensilla on the forceps of *Palingenia longicauda* (Olivier). McCafferty and Bloodgood (1989) observed, under SEM, numerous papilla-like filaments at the apex of the forceps in the genus *Tortopus* and suggested a sensory or stimulatory function. Although scales and papilla-like structures on the forceps of some mayflies have been hypothesized to perform sensory and mechanical activity, in particular to improve cohesion with the female cuticle (McCafferty and Bloodgood 1989, Gaino and Rebora 1995), their actual function remains unknown.

The current article describes, at ultrastructural level, the organization of the globular papillae in the forceps of the male *imago* of *Ecdyonurus venosus* (F.) and investigates their possible involvement in the mating process. Because Ephemeroptera are characterized by the preadult stage known as *subimago*, the same investigation has been also carried out on the forceps of this stage.

## Materials and Methods

Mature nymphs of *Ecdyonurus venosus* (F.) were collected in the Ierna Stream, close to Tavernelle, Perugia, Umbria Region, Italy, and in the Nera River, tributary of the Piediluco Lake, 13 km from Terni, Umbria Region, Italy, in autumn 2000. *Imago* and *subimago* specimens were secured by rearing larvae in the laboratory. The male *imago* and *subimago* were dissected under a stereomicroscope. The forceps 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 microscopy (SEM) observations, the specimens were dehydrated by using ethanol gradients, followed by critical-point drying in a CO2 Pabisch CPD apparatus (Pero, Milano, Italy). Specimens were mounted on stubs with silver conducting paint, sputter-coated with gold-palladium in a Balzers Union Evaporator (Fürstentum-Liechtenstein), and observed with a Philips EM 515 SEM (Eindhoven, Netherlands).

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.

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The thin sections were examined with Philips EM 400 and EM 208 electron microscopes.

## Results

The mating apparatus of *E. venosus* males includes broad penis lobes and a pair of forceps, each formed by four segments (Fig. 1a). In the *imago* the surface of each segment of the male forceps is uniformly covered with cuticular decorations (Fig. 1b) that assume a globular form along the inner side of the forceps (Fig. 1 c and d). The shape of these globular papillae varies with their location on the segments. On the first three segments of the forceps the globular papillae are loosely arranged and are convex distally (Fig. 1c). In contrast, those on the distal segment are clustered and exhibit a concave shape (Fig. 1 d and inset). The rest of the forceps is covered with triangulate scales, which tend to be pointed apically (Fig. 1 c and d), and with interspersed hairs set in a socket (Fig. 1c, inset of Fig. 1d).

In the *subimago* the forceps are reduced in length (Fig. 1e), and their surface is covered with cuticular folds, among which hairs set in a socket and microtrichia are present (Fig. 1f). Hairs and microtrichia are lacking on the inner side of the first three segments of the forceps where globular papillae are interposed among cuticular folds (Fig. 1 f and g). These papillae have a pointed apex (Fig. 1f) and are very sparse on the inner side of the distal segment, which is uniformly covered with microtrichia (Fig. 1g).

In the *imago*, TEM revealed that the globular papillae, whatever their shape and location on the segments, are innervated, indicating they are sensilla (Fig. 2 a, b, and d). Both concave papillae (Fig. 2 a-c) and convex papillae (Fig. 2d) have a similar ultrastructural organization.

The globular papillae (≈3–4 $\mu$m wide, 5 $\mu$m high) are delimited by a thin cuticle and have an internal lumen that is connected with the sensillum lymph cavity (Fig. 2 a and b). Serial sections show that the cavity is crossed by a cuticular septum (Fig. 2b), which partially divides the cavity of the globular papilla into two regions. Each papilla is innervated by a single neuron, with an unbranched dendrite having its outer portion (outer dendritic segment) wrapped by a dendritic sheath (Fig. 2b). The dendritic sheath shows an irregular pattern, consisting in a rough border (Fig. 2c). The neuron stops at the base of the papilla, where it forms a tubular body in the apical portion (Fig. 2d). The dendritic sheath of the tubular body is connected to the cuticle of the papilla by an electron-dense membrane (Fig. 2d).

In longitudinal sections, TEM discloses that the hairs, which are set in a socket and located among the triangulate scales (Fig. 1 c and d), have a well-developed tubular body ending at the base of the lumen of the shaft (Fig. 2e). The cuticle of the hair is connected with the surrounding cuticle by the interposition of the joint membrane (Fig. 2e). Suspension fibers link the dendritic sheath of the tubular body to the cuticle of the socket (Fig. 2e).

In the *subimago*, longitudinal sections of the pointed globular papillae (Fig. 3a) (about 4 $\mu$m wide at its base, 6 $\mu$m high) have an internal lumen partially occluded by cuticle, which includes a more electron-dense material (Fig. 3a). At the insertion of the papilla with the surrounding cuticle, the electron-dense material protrudes to form a socket-like structure (Fig. 3a). Similar to that in the *imago*, the papillae are innervated by a single neuron whose unbranched dendrite forms a well-developed tubular body delimited by the dendritic sheath (Fig. 3 b and c). Suspension fibers connect the tubular body to the cuticle of the papilla (Fig. 3c). The fine structure of the hairs, which are set in a socket (Fig. 3d) and located among the cuticular folds, is similar to that described in the *imago.*

## Discussion

In the *imago*, the fine structure of the globular papillae projecting from the inner side of the forceps of *E. venosus* is consistent with their function as mechanoreceptors. Indeed, this function derives from the occurrence of a single dendrite ending in a tubular body, even if the papilla lacks a well-developed socket structure. Only an electron-dense membrane connects the tubular body to the basal cuticle of the papilla. The mechanical pressure acting on the forceps, due to the contact with the abdomen of the female during the mating flight, could be sensed by the cuticular surface of the globular papillae and transmitted to the tubular body. The clustering of the globular papillae along the most distal segment of the forcep is consistent with their function in sensing the female. In addition, the concave shape of the papillae may enhance adhesion to the female abdomen. In *Tortopus*, a representative of one of the four highly specialized mayfly lineages (Edmunds and McCafferty 1988), the loss of clasping ability in the forceps has been compensated by the parastyli, which hook to grooved excavations on the eight abdominal sternite of the female (McCafferty and Bloodgood 1989).

In the *subimago*, the presence of forceps with reduced length, the lack of fully developed external genitalia, and the lack of the flying agility do not allow the mating (Edmunds and McCafferty 1988). Although there are observations of mating by female *subimago* (Grandi 1960, Peters and Peters 1977), no such data have been reported for male *subimago.* The forceps of the *subimago* are shorter than those of the *imago* and covered with microtrichia, which probably act as a hydrophobic device to prevent drowning of the *subimago* as it emerges from the water (Edmunds and McCafferty 1988, Gupta et al. 1999). Among the microtrichia, sparse hairs, which are set in a socket, with the typical organization of mechanoreceptive bristles, are present. The bristles seem to be the most common type of mechanoreceptors and usually respond to direct touch (Keil 1997). These mechanoreceptive bristles also occur in the forceps of the *imago*, where they become more evident owing to the discarding of microtrichia during the *subimago* molt, which diminishes air friction.

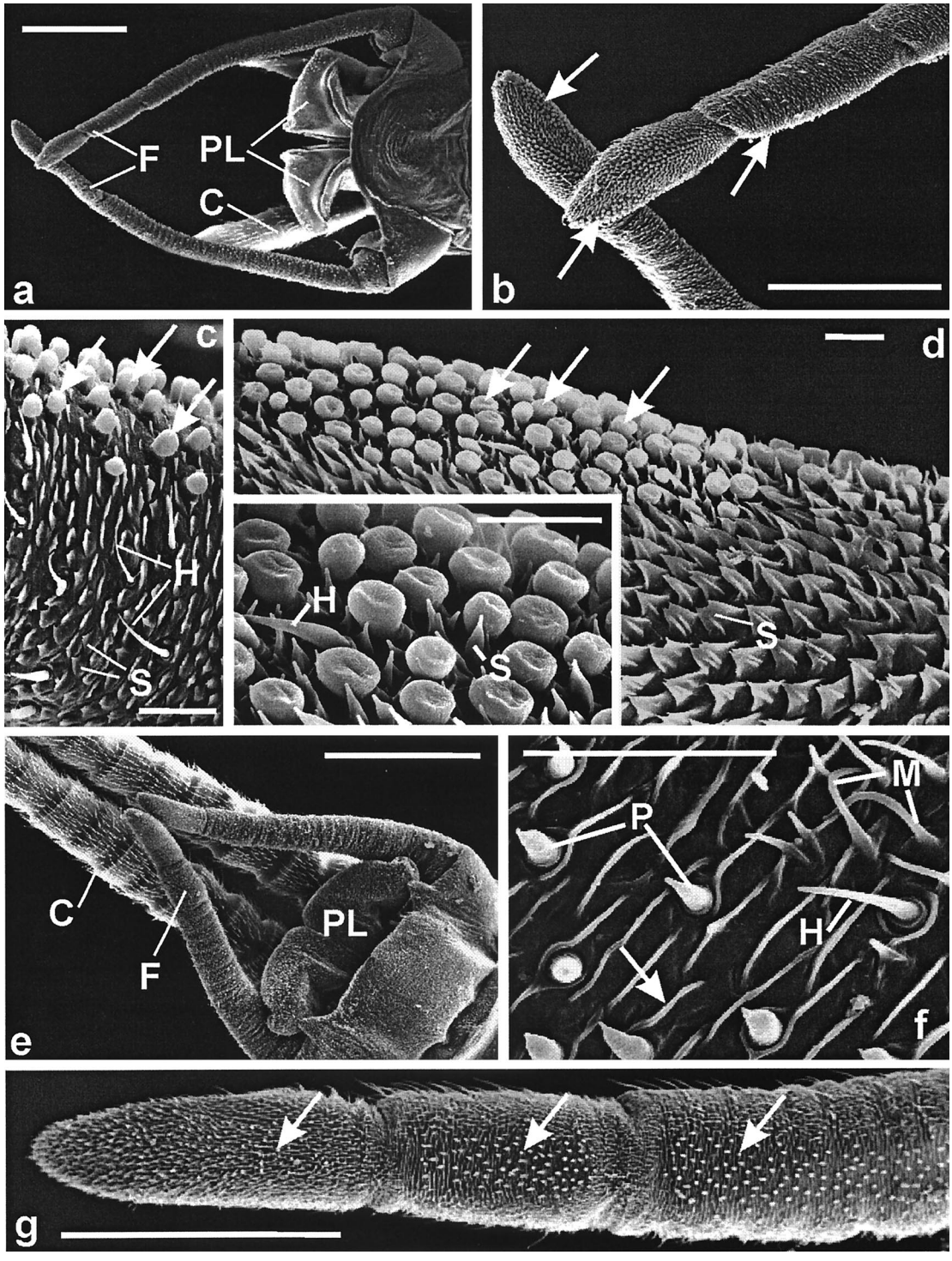


**Fig. 1.** SEM view of the forceps of the *imago* (a-d and inset) and of the *subimago* (e-g) of *Ecdyonurus venosus.* (a) Ventral view of the terminal portion of the mating apparatus showing forceps (F) and penis lobes (PL). C, cerci. Bar = 500 $\mu$m. (b) Distal segments of the forceps. Note the globular papillae on their inner side (arrows). Bar = 200 $\mu$m. (c) Apically convex papillae (arrows) on the first segment of the forceps. H, hairs; S, scales. Bar = 20 $\mu$m. (d) Concave papillae (arrows), at higher magnification in the inset (bar = 10 $\mu$m), clustered on the distal segment of the forceps. H, hairs; S, triangulate scales. Bar = 10 $\mu$m. (e) Ventral view of the mating apparatus showing forceps (F) and penis lobes (PL). C, cerci. Bar = 500 $\mu$m. (f) Slightly pointed globular papillae (P) detailed on the inner side of the forceps with cuticular folds (arrow). Note the presence of hairs (H) and microtrichia (M). Bar = 20 $\mu$m. (g) Inner side of one of the forceps showing the arrangement of the slightly pointed globular papillae (arrows). Note their reduction in number on the last segment. Bar = 200 $\mu$m.

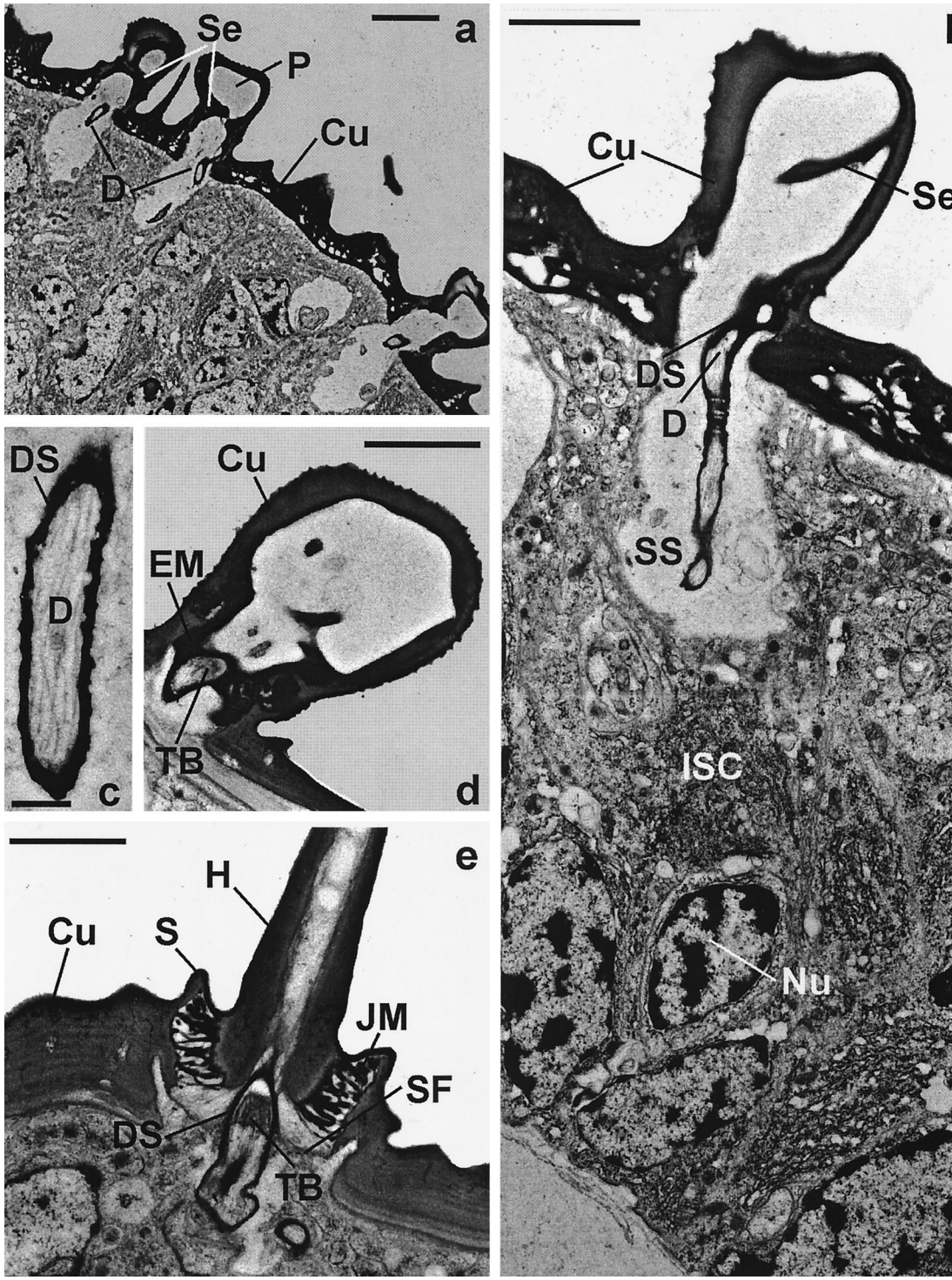


**Fig. 2.** TEM view of globular papillae (a-d) and hairs (e) located on the forceps of the *imago* of *Ecdyonurus venosus* in longitudinal section. (a) Globular papillae (P) on the inner side of the last segments of the forceps showing their innervation. Cu, cuticle; D, dendrite; SE, septum. Bar = 3. 5 $\mu$m. (b) A globular papilla showing the dendrite (D) in its dendritic sheath (DS). The cavity of the papilla is in contact with the sensillar sinus (SS) and is partially crossed by a septum (SE). Cu, cuticle; N, nucleus of the inner sheath cell (ISC). Bar = 2. 5 $\mu$m. (c) Detail of a dendrite (D) in its dendritic sheath (DS), which shows a rough border. Bar = 0. 6 $\mu$m. (d) Globular papilla showing the tubular body (TB) located in the apical portion of the dendrite. The tubular body is wrapped in its dendritic sheath, which is connected to the cuticle (Cu) of the papilla by an electron-dense membrane (EM). Bar = 2. 5 $\mu$m. (e) Basal portion of a hair (H) set in a socket (S) showing a well-developed tubular body (TB) wrapped in its dendritic sheath (DS). Cu, cuticle; JM, joint membrane; SF, suspension fibers. Bar = 2 $\mu$m.

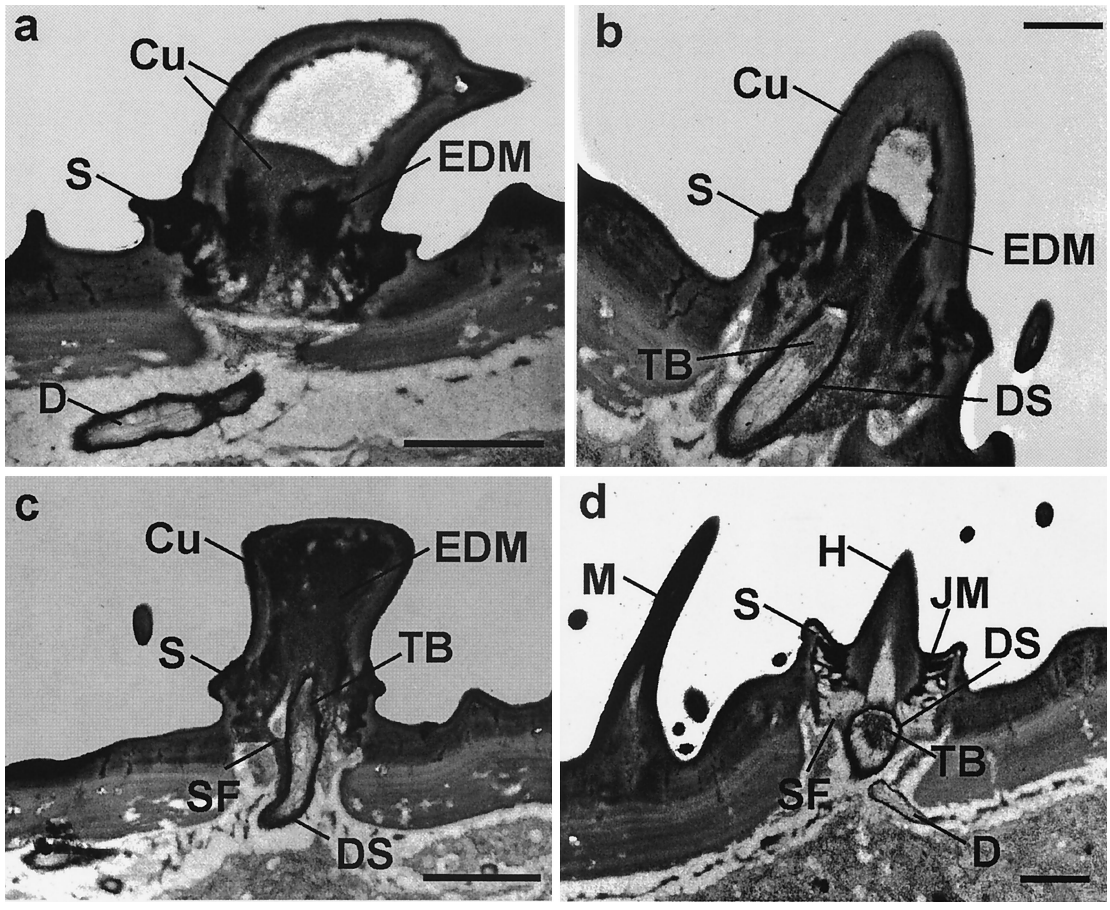


**Fig. 3.** TEM view of slightly pointed papillae (a-c), hairs and microtrichia (d) located on the forceps of the *subimago* of *Ecdyonurus venosus* in different longitudinal section. (a-c) Cuticle (Cu) and electron-dense material (EDM) partially occlude the lumen of the papillae. Note the socket-like structure (S), the dendrite (D) in its dendritic sheath (DS), the tubular body (TB) and the suspension fibers (SF). (a) Bar = 1. 5 $\mu$m, (b) Bar = 1 $\mu$m, (c) Bar = 2. 5 $\mu$m. (d) One of the microtrichia (M) in proximity of a hair (H) set in a socket (S) and characterized by the joint membrane (JM), the suspension fibers (SF), the dendrite (D) ending in a tubular body (TB) wrapped in its dendritic sheath (DS). Bar = 1. 2 $\mu$m.

In exopterygotes, additional sensilla are often formed during a molt (Carline et al. 1984, Chapman and Greenwood 1986), and this increase is also coupled with growth (Zacharuk and Shields 1991). In the molt from *subimago* to *imago*, the globular papillae, located on the inner side of the forceps, show a remarkable change in their outline and in their distribution. Whereas in the *imago* the mechanosensory role of the globular papillae is related to mating, such a function is difficult to hypothesize for the *subimago*, a short-lived stage that is unable to mate and usually rests close to the water waiting for its final imaginal molt (Gupta and Gupta 1996). The occurrence of a well developed tubular body in these globular papillae supports a mechanosensory function.

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