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Cuticular and sensory structures on the copulatory apparatus of *Rhithrogena semicolorata* (Ephemeroptera: Heptageniidae)

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The male copulatory apparatus of *Rhithrogena semicolorata* shows paired penis lobes, armed with teeth laterally projected and equipped with ventral titillators. As revealed by observations under scanning and transmission electron microscopy (SEM, TEM), the titillators are unarticulated appendages that lack sensory structures. The presence of two depressions, located inside the female genital apparatus, is coherent with a possible function of the titillators in stabilising the couple at mating. Sensory structures are located ventrally in the distal portion of each penis lobe and consist of two kinds of sensilla: campaniform sensilla and short trichoid sensilla. Both sensilla show an organisation consistent with a mechanosensory function, particularly related to pressure stimulations. The presence of mechanoreceptors in the penis lobes is coherent with the need of the male to receive mechanosensory information about the female position during mating.

Keywords: mayflies; mechanoreceptors; penis lobes; sensilla; ultrastructure; titillators

Introduction

Insect genitalia show a variety of forms whose function is still a matter of debate (Arnqvist 1997, 1998). In Ephemeroptera, the general morphology of male genitalia has been extensively investigated for taxonomic and phylogenetic purposes (see review in Kluge 2004) but functional studies are limited to few species (Brinck 1957; Takemon 2000). In particular, the sensory role of the coupling apparatus in insects has been poorly investigated (Acebes et al. 2003). Some studies have been carried out on Diptera (Acebes et al. 2003), Coleoptera (Ramaswamy et al. 1980; Kim and Yamasaki 1996; Kim et al. 1999; Kim and Teiji 2004; Düngelhoef and Schmitt 2006), Orthoptera (Snell and Killian 2000; Kumashiro and Sakai 2001) and Odonata (Uhia and Cordero Rivera 2005). As far as mayflies are concerned, the occurrence of sensory structures has been hypothesised only for the forceps of some species (McCafferty and Bloodgood 1989; Gaino and Rebora 1995; Fink and Andrikovics 1997) and then demonstrated in *Ecdyonurus venosus* (Gaino and Rebora 2002). So far, the possible presence of sensilla on the penis lobes has not been verified.

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In the genus *Rhithrogena*, titillators on the penis lobes have been described in most species. These titillators have been interpreted either as movable or immobile appendages (see review in Kluge 2004).

The aim of the present study is to describe the fine structure (SEM, TEM) of the penis lobes of *Rhithrogena semicolorata* Curtis, 1834 to ascertain the presence of sensory structures and to interpret the actual function of the titillators. The morphology of the inner female vestibulum is also investigated with regard to the shape of the penis lobes, and to understand how cuticular differentiations can facilitate mating.

Materials and methods

Adult males and females of *R. semicolorata* were obtained in the laboratory from mature larvae collected in Nera River (Terni, Umbria Region, Central Italy) in spring 2007. The specimens were kept in water provided with supplementary oxygen, stones and detritus from the collecting site, at 25 ± 2°C, LD 12:12 h light conditions.

Penis and female genitalia were dissected from anaesthetised imagines and fixed for 12 hours in 2.5% glutaraldehyde in cacodylate buffer, pH 7.2.

For scanning electron microscopy (SEM) analysis, the fixed material was repeatedly rinsed in the same buffer, then dehydrated by using ethanol gradients, followed by critical-point drying in a CPD 030 critical-point dryer (Bal-Tec Union Ltd., Balzers, Liechtenstein). Specimens were mounted on stubs with silver conducting paint, sputter-coated with gold-palladium in a K550X sputterer (Emitech, Ashford, England), and observed with a XL30 SEM (Philips, Eindhoven, The Netherlands), at an accelerating voltage of 18 kV.

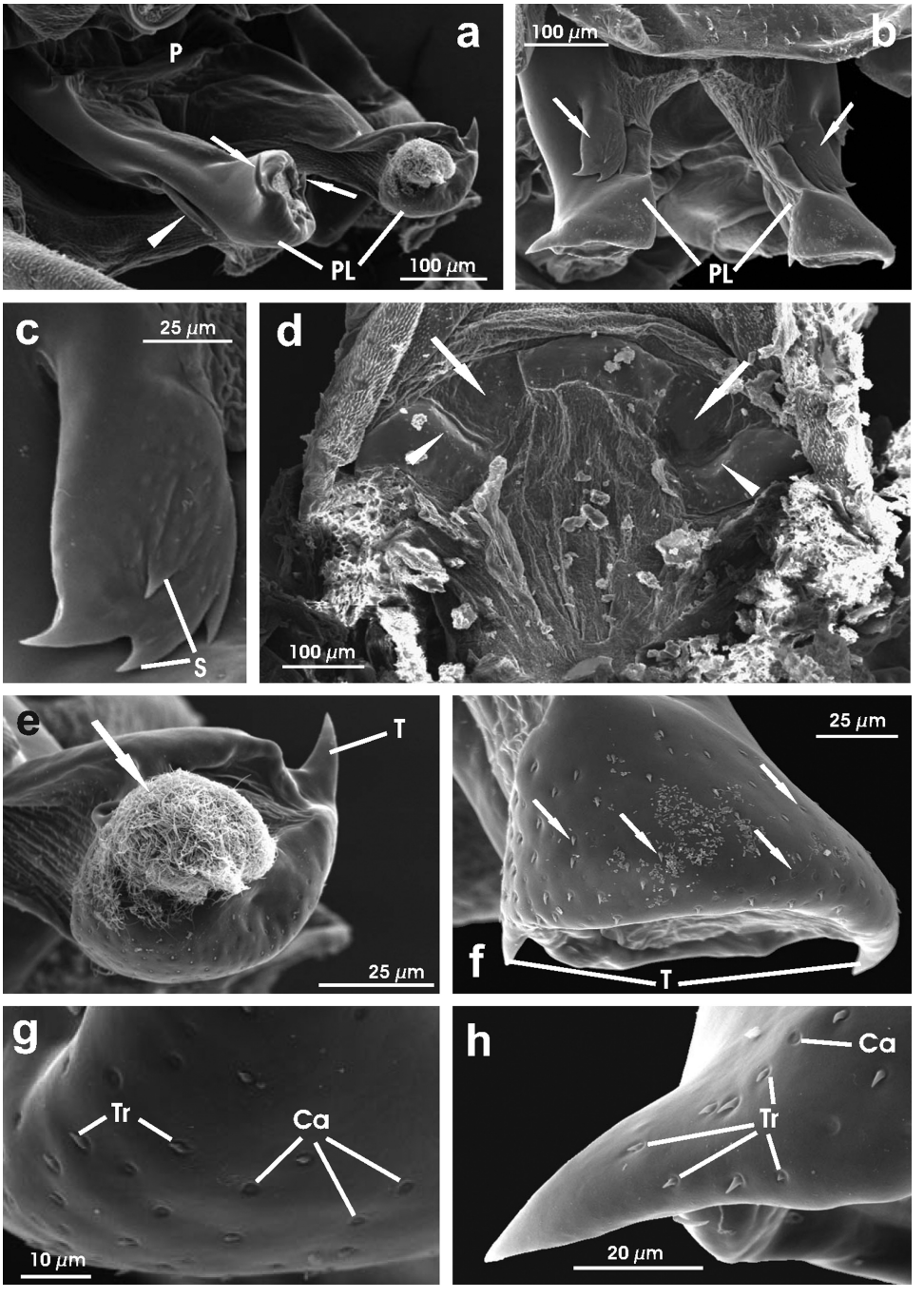
For transmission electron microscopy (TEM), the fixed genitalia were repeatedly rinsed in cacodylate buffer and post-fixed for 1 hour at 4°C in 1% osmium tetroxide in cacodylate buffer. Then the material was repeatedly washed in the same buffer, dehydrated by using ethanol gradients, and finally embedded in an Epon-Araldite mixture resin. Ultrathin sections, cut on a EM UC6 ultracut (Leica Microsystem GmbH, Wien, Austria), collected on formvar-coated copper grids, stained with uranyl acetate and lead citrate, were examined with a EM 208 TEM (Philips, Eindhoven, The Netherlands).

The terminology used for the description of the ultrastructural organisation of sensilla is after Keil (1997).

Figure 1. Penis lobes (a–c, e–h) and vestibulum (d) of the imago of Rhithrogena semicolorata under SEM. (a) Dorsolateral view of the penis lobes (PL) armed with two lateral teeth (arrows). Note the plate (P) formed by the proximal portion of the lobes, and the titillator (arrowhead). (b) Ventral view of the penis lobes (PL) showing unarticulated titillators (arrows) in their proximal portion. (c) Detail of a titillator characterised by four spines (S) externally projected. Note the lack of sensory structures on the surface. (d) Dorsal wall of the female vestibulum, visible after removing the genital plate. Note paired depressions (arrows), delimited by a rim mainly uplifted along their external board (arrowheads). (e) Dorsal view of the distal portion of a single penis lobe showing the fairly round board. Only a single lateral tooth (T) is visible while the other is covered with the sperm (arrow). (f) Ventral view of the distal portion of a single penis lobe. Note the two lateral teeth (T) and numerous sensilla (arrows) on the surface of the lobe. (g–h) Details showing campaniform (Ca) and trichoid (Tr) sensilla on the dorsal surface of a penis lobe (g) and a tooth (h).

Results

The male copulatory apparatus of *R. semicolorata* consists of a pair of penis lobes armed with two lateral teeth (Figure 1a, b). The basal portions of the lobes are jointed, forming a plate dorsally (Figure 1a). The ventral surface of each lobe



proximally bears a single titillator, which is about 80 μm long and 50 μm wide (Figure 1b). The titillators are unarticulated appendages, lacking sensory structures, armed with four spines externally projected (Figure 1c).

The dorsal surface of the female vestibulum shows two paired depressions (Figure 1d), about 80 μm long, the size of which gradually decreases from the apical (70 μm large) to the basal portion (55 μm large). The depressions are delimited by a rim mainly uplifted along their external board.

Each penis lobe is slightly enlarged towards the terminal region, which has a fairly round shape with two lateral teeth (Figure 1e, f). Some images show the presence of sperm on the dorsal side of the lobes (Figure 1a, e).

Along the ventral surface, the terminal portion of each lobe bears numerous cuticular structures (Figure 1f): small circular depressions, surrounded by a ring of raised cuticle about 2.5 μm in diameter (Figure 1g, h), and short bristles, about 4 μm long, set in a socket and almost adherent to the surface (Figure 1g, h).

Thin sections, observed under TEM, reveal that the circular depressions are campaniform sensilla and the short bristles are trichoid sensilla, both innervated by one unbranched neuron (Figure 2a, b). The neuron is enveloped by the accessory cells (Figure 2b).

Campaniform sensilla are visible as neurons under the cuticle surface (Figure 2a, c). The dendrite ends at the base of the cuticle and forms a tubular body in the apical portion (Figure 2a, c). Above the dendrite, the cuticle is in contact with the dendrite sheath of the tubular body by the socket septum (Figure 2c).

Trichoid sensilla have a short, smooth bristle, without pores, attached to the socket by an electron dense joint membrane (Figure 2d). The socket septum is visible beneath the bristle (Figure 2d). Deeper sections show a well developed tubular body at the base of the bristle (Figure 2e). The socket septum links the dendrite sheath of the tubular body with the socket (Figure 2e).

Discussion

It is well known that mayfly copulation takes place in flight: the male catches the wing base of the female with its forelegs and curves the abdomen upwards, inserting the penis lobes into the vestibulum and thus allowing insemination (Brinck 1957; Grandi 1973).

Though titillators are fairly common in Heptageniidae, their function is still questionable (see review in Kluge 2004). In *R. semicolorata* titillators are unarticulated appendages, lacking sensory structures on their surface. The depressions identified on the dorsal wall of the female vestibulum suggest that during copula the titillators can be inserted into these depressions to anchor the male to the female genitalia. In fact, the length of titillators and depressions is fairly similar, while the width of the depressions slightly exceeds that of the titillator but decreases towards the proximal end. The presence of four large spines projecting externally at the apex of the titillators could enhance the anchorage to the female. This system could perform a mechanical action for stabilising the partners during copulation. In this regard, as reported by Arnqvist (1998), the diversification of accessory parts in male genitalia of insects could represent a modality to enhance fertilisation and therefore the reproductive success. Mechanical systems of stabilisation of the genitalia contact during mating have been previously described in the genus *Tortopus*, where the male parastyli have a primary function in coupling

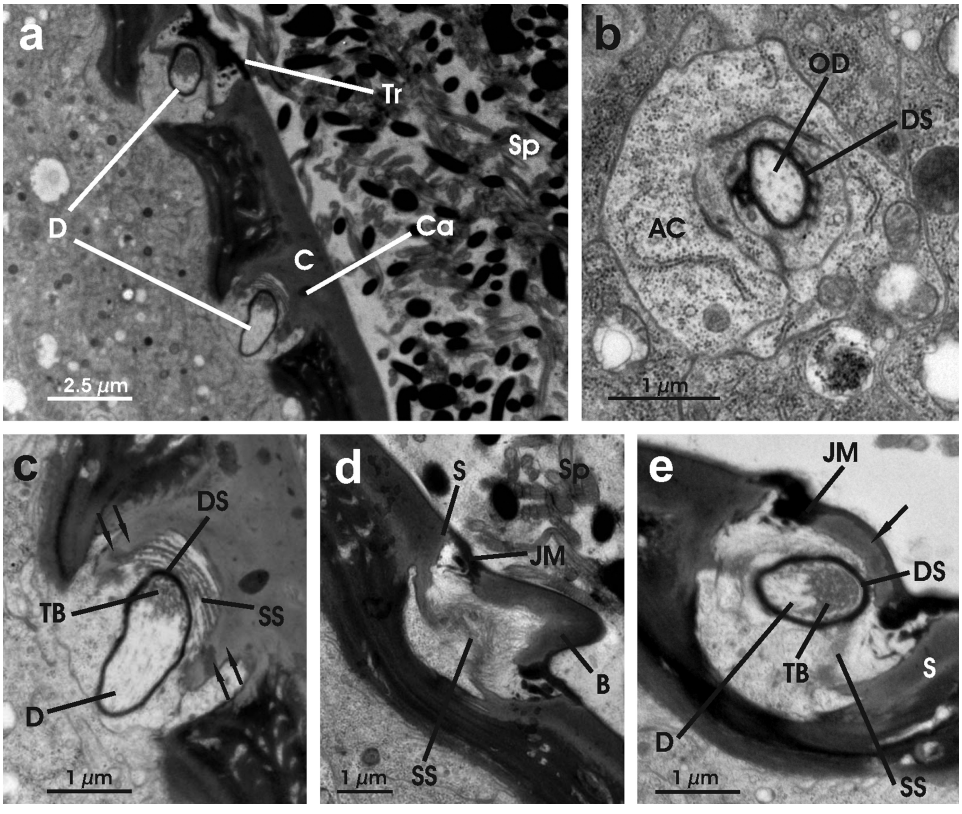


Figure 2. Thin sections of the penis lobes of the imago of *Rhithrogena semicolorata* under TEM. (a) Cross section of the distal portion of the lobe. Campaniform (Ca) and trichoid (Tr) sensilla are innervated by one dendrite (D); C, cuticle; Sp, sperm. (b) Cross section of the outer dendritic segment (OD) enveloped by the accessory cells (AC). At this level, campaniform and trichoid sensilla are not distinguishable; DS, dendrite sheath. (c) Longitudinal section of a campaniform sensillum. Note the tubular body (TB) at the apex of the dendrite (D). The dendrite sheath (DS) of the tubular body is in contact with the cuticle (arrows) by the socket septum (SS). (d) Longitudinal section of a trichoid sensillum. Note the short smooth bristle (B), connected to the socket (S) through an electron dense joint membrane (JM). Note the suspension fibers of the socket septum (SS); Sp, sperm. (e) trichoid sensillum in a deeper section showing the dendrite (D) forming the tubular body (TB) at its apex. Socket septum (SS) links the dendrite sheath (DS) of the tubular body to the socket (S). Note the joint membrane (JM) linking the base of the bristle (arrow) to the socket.

and holding the female and male together during copulation (McCafferty and Bloodgood 1989).

The copulation requires both partners to be able to detect their mutual position. The genital sensilla are most likely to perform such a function (Acebes et al. 2003).

Electron microscopy observations revealed that the penis lobes of *R. semicolorata* bear numerous sensilla on their ventral surface: (i) campaniform sensilla, visible as small circular depressions surrounded by a ring of raised cuticle; (ii) trichoid sensilla, visible as short bristles set in a socket and almost adherent to the penis surface. In section, both sensilla show a well developed tubular body, connected with a modified cuticle by the suspension fibres of the socket septum. This organisation is consistent with a mechanosensory function (McIver 1985; Keil 1997). During mating, these

mechanoreceptors, which are exclusively located in the ventral-distal portion of the penis lobes, make contact with the dorsal wall of the female vestibulum: the campaniform sensilla perceive pressure stimulations, while the short bristles of the trichoid sensilla, almost adherent to the penis surface, could be mainly stimulated by the sliding contact.

The role of mechanoreceptors in affecting the mating position and mating success has been hypothesised for the papillae on the forceps of *Ecdyonurus venosus* (Ephemeroptera: Heptageniidae) (Gaino and Rebora 2002) and were demonstrated for the bristles located on the claspers and lateral plates of *Drosophila melanogaster* (Acebes et al. 2003). The presence of campaniform sensilla on the surface of the endophallus in *Lilioceris lilii* (Coleoptera: Criocerinae) has been interpreted as a device to sense the correct position inside the female bursa to provide the best opportunity to perform direct sperm transfer (Düngelhoef and Schmitt 2006). In damselflies the male probably uses chemoreceptors on its penis to detect the female mating status (virgin or previously-mated) and, consequently, to regulate the duration of copulation and sperm removal (Uhia and Cordero Rivera 2005). In some coleopterans the presence of hairs and campaniform sensilla on the genitalia median lobes is interpreted as a reproductive barrier to prevent accidental mating among closely-related congeneric species (Epila-Otara and Triplehorn 1990).

In conclusion, ultrastructural investigations on mayfly genitalia, beyond their relevance for taxonomic and phylogenetic research, can provide information about the coupling mechanisms leading to successful reproduction in these insects.

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References

Acebes, A., Cobb, M., and Ferveur, J.F. (2003), 'Species-specific effects of single sensillum ablation on mating position in *Drosophila*', *Journal of Experimental Biology*, 206, 3095–3100.

Arnqvist, G. (1997), 'The evolution of animal genitalia: distinguishing between hypotheses by single species studies', *Biological Journal of the Linnean Society*, 60, 365–379.

Arnqvist, G. (1998), 'Comparative evidence for the evolution of genitalia by sexual selection', *Nature*, 393, 784–786.

Brinck, P. (1957), 'Reproductive system and mating in Ephemeroptera', *Opuscola entomologica*, 22, 1–37.

Düngelhoef, S., and Schmitt, M. (2006), 'Functional morphology of copulation of Chrysomelidae-Criocerinae and Bruchidae (Insecta: Coleoptera)', *Bonner Zoologische Beiträge*, 54, 201–208.

Epila-Otara, J.S., and Triplehorn, C.A. (1990), 'Mechanoreceptors on the male genitalia of Platypodidae (Coleoptera): a Pergamous isolation mechanism?', *Journal of African Zoology*, 104, 345–350.

Fink, T.J., and Andrikovics, S. (1997), 'The presumed role of wing sensory structures in the unique mating behavior of the endangered European mayflies *Palingenia longicauda* (Olivier) and *Palingenia fuliginosa* (Georgi) (Insecta, Ephemeroptera)', in *Ephemeroptera and Plecoptera: biology-ecology-systematics*, eds. P. Landolt and M. Sartori, Fribourg, Switzerland: M.T.L., pp. 326–331.

Gaino, E., and Rebora, M. (1995), 'Comparative study of the mating apparatus of three species of Leptophlebidae (Ephemeroptera)', *Aquatic Insects*, 17, 95–104.

Gaino, E., and Rebora, M. (2002), 'Sensory receptors on the forceps of the male imago and subimago of *Ecdyonurus venosus* (Ephemeroptera: Heptageniidae)', *Annals of the Entomological Society of America*, 95, 244–249.

Grandi, M. (1973), 'I comportamenti di volo nelle diverse fasi della vita aerea degli Efemerotteri', *Bollettino dell'Istituto di Entomologia della Università di Bologna*, 31, 109–127.

Keil, T. (1997), 'Functional morphology of insect mechanoreceptors', *Microscopy Research and Techniques*, 39, 506–531.

Kim, J.L., Park, S.O., and Yamasaki, T. (1999), 'Sensilla on the aedeagi of the genus Damaster (Insecta, Coleoptera, Carabidae)', *Korean Journal of Biological Sciences*, 3, 23–28.

Kim, J.L., and Teiji, S. (2004), 'Sensilla on the external genitalia of the carabid beetle, *Carabus (Ohomopterus) dehaanii dehaanii* Chaudoir (Coleoptera, Carabidae)', *Entomological Research*, 34, 151–155.

Kim, J.L., and Yamasaki, T. (1996), 'Sensilla of *Carabus (Isiocarabus) fiduciaries saishutoicus* Csiki (Coleoptera: Carabidae)', *International Journal of Insect Morphology & Embriology*, 25, 153–172.

Kluge, N. (2004), *The phylogenetic system of Ephemeroptera*, Dordrecht: Kluwer Academic Publishers.

Kumashiro, M., and Sakai, M. (2001), 'Reproductive behaviour in the male cricket *Gryllus bimaculatus* DeGeer I. Structure and function of the genitalia', *The Journal of Experimental Biology*, 204, 1123–1137.

McCafferty, W.P., and Bloodgood, D.W. (1989), 'The female and male coupling apparatus in *Tortopus* Mayflies', *Aquatic Insects*, 11, 141–146.

McIver, S.B. (1985), 'Mechanoreception', in *Comprensive insect physiology, biochemistry and pharmacology, vol. 6: Nervous System: Sensory*, eds. G.A. Kerkut and L.I. Gilbert, New York: Pergamon Press, pp. 71–131.

Ramaswamy, S.B., Gupta, A.P., and Fowler, H.G. (1980), 'External ultrastructure and function of the speculum copulates (SC) of the german cockroach, *Blattella germanica* L. (Dictyoptera: Blattellidae)', *Journal of Experimental Zoology*, 214(3), 287–292.

Snell, L.C., and Killian, K.A. (2000), 'The role of cercal sensory feedback during spermatophore transfer in the cricket *Acheta domesticus'*, *Journal of Insect Physiology*, 46, 1017–1032.

Takemon, Y. (2000), 'Reproductive behaviour and morphology of *Paraleptophlebia spinosa* (Ephemeroptera: Leptophlebidae): implications of variation in copula duration', *Limnology*, 1, 47–56.

Uhia, E., and Cordero Rivera, A. (2005), 'Male damselflies detect female mating status: importance for postcopulatory sexual selection', *Animal Behaviour*, 69, 797–804.