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Transmission and scanning electron microscopic observations on antennal apical pegs in the wasp species Pimplinae (Insecta: Hymenoptera)

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ABSTRACT

Pimplinae are parasitoids belonging to the family Ichneumonidae that attack and develops inside hidden host; female wasps evolved a peculiar host recognition strategy, that involves the use of self-produced vibrations which are transmitted through the antennae on the substrate and perceived back as an echo using the leg subgenual organ. In this study we investigated, using both scanning and transmission electron microscopy, the antennal tips of a few Pimplinae. In all the investigated species, the antennal tips present peculiar apical pegs with different shape and number, often defining a flattened sole devoid of other antennal structures, such as sensilla. These pegs are present in both sexes with different number and development, are inserted on the antennal wall through an inflexible socket and present a cuticular shaft with cuticle of different thickness. We never found the presence of sensory neurons or glandular epithelium associated with these pegs. Because of their peculiar morphological features, we hypothesize for the antennal apical pegs a role in the context of host searching behavior (in the case of the female through the vibrational sounding strategy), as well as during mating behavior.

1. Introduction

Parasitoids are highly specialized insect mainly belonging to the Hymenoptera, which depend for their development on the presence of a suitable host. Such hosts can be other insect species or other arthropods, i.e. spiders. Once mated, a parasitoid female needs to localize on the environment the suitable host species, recognize them as belonging to the right pool of hosts, and accept them in order to allocate its progeny. Given the high level of specialization, insect parasitoids have often evolved very specific sensory structures, in most cases located on the antennae, that are used to locate and exploit exposed or hidden hosts (Bin et al., 1989; Isidoro et al., 1996, 2001; Romani et al., 2002, 2010; Ruschioni et al., 2012; Polidori and Nieves-Aldrey, 2014; Riolo et al., 2016). Among others, Pimplinae (Hymenoptera: Ichneumonidae) are endoparasitoids which attack and develop on hidden hosts, manly Lepidopterans (Henaut and Guerdoux, 1982; Wäckers et al., 1998). Differently from parasitoids that exploit exposed host, parasitoids of hidden hosts have to face the problem of locate their invisible prey. In some cases, hidden hosts are larvae actively feeding within plant tissues. Therefore, parasitoids exploit vibratory stimuli produced by the host itself, which have a high level of detectability and reliability for the parasitoid (Meyhöfer et al., 1994, 1997a, 1997b; Casas et al., 1998). Scenario changes in the case of hidden hosts that are no active since they do not feed, move or release chemical substances, as it is the case of pupae. Some pupal parasitoids belonging to the Pimplinae have evolved a finely tuned strategy to locate a potential hidden host. In fact, they rely on the self-production of vibrations which are transmitted through the substrate and detected as an echo. This peculiar behavioral adaptation was defined as “vibrational sounding” and, functionally speaking, involves the antennae as vibratory transmitters, and the legs as receivers (Wäckers et al., 1998; Otten et al., 2001, 2002). Morpho-functional studies carried out on the legs showed the presence of a well-developed subgenual organ located inside the tibiae, most likely involved in detection of vibratory stimuli coming from the substrate (Otten et al., 2002). During host searching behavior, Pimplinae perform a continuous antennal tapping on the substrate. Borden et al. (1973) showed the presence of apical pegs in both sexes of *Itoplectis conquisitor* (Say), for which they hypothesized a sensory function despite the lack of transmission electron microscopy analysis. Antennal apical pegs were also described in *Pimpla instigator* F., referring to them as “vesicles filled with hemolymph” involved in the transmission of “shock waves which originate within the body of the insect” (Henaut and Guerdoux, 1982; Henaut, 1990). The presence of specialized antennal tips modified into hammering structures suitable

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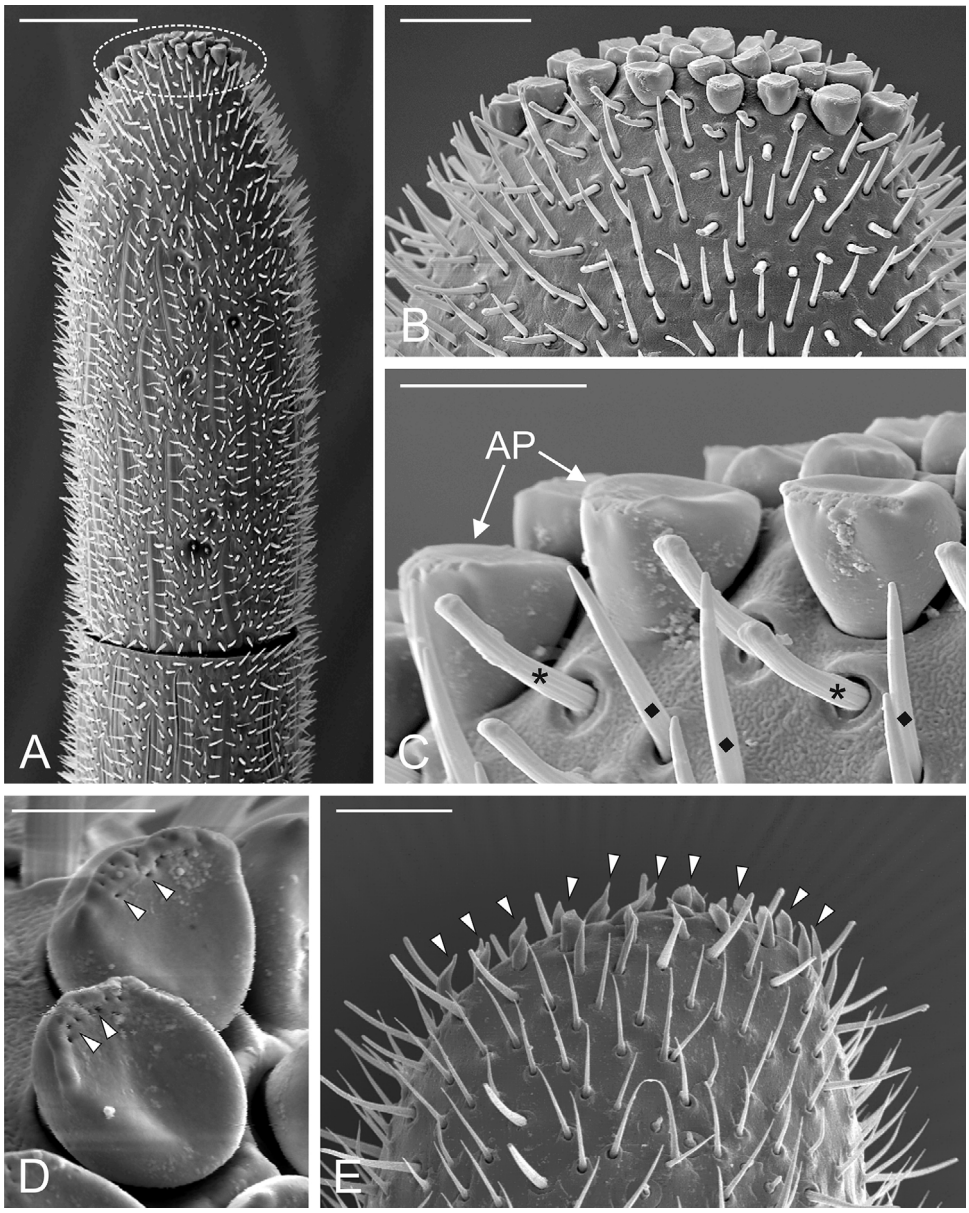

Fig. 1. SEM images of *Pimla turionellae* antenna A) female apical antennomere showing the area where the apical pegs are located. B) Detail of the previous image. C) Close-up view of the apical pegs (AP). Their typical drum-like shape is clearly visible, as well as the presence of the sensilla trichoidea 1 (*) and sensilla trichoidea 2 (◆). D) apical view of the antennal apical pegs sowing the smooth, flattened area and the pores (arrowheads) close to the outer margin. E) View of the antennal tip of *P. turionellae* male: antennal apical pegs are still present (white arrowheads) but with a reduced number and development. Scale bar: A: 50 μm; B, E: 20 μm; C, D: 10 μm.

to knock the substrate was reported for the Cryptinae and Pimplinae (for the latter only in the tribe Pimplini, not in the larger group Ephialtini) (Broad and Quicke, 2000; Quicke et al., 2003; Laurenne et al., 2009). Laurenne et al. (2009) and Laurenne and Quicke (2010), hypothesized for the antennal apical pegs a possible transition from sensilla to specialized solid structures resembling hammers, though no structural data were provided to support this hypothesis. In this paper, we report for the first time a fine structural study of the antennal apical pegs of some Pimplinae species, showing that antennal pegs are cuticular specialized structures lacking any sensory or secretory function.

2. Materials and methods

INSECTS. *Pimpla turionellae* L. and *Xanthopimpla stemmator* (Thunberg) were laboratory reared using *Galleria mellonella* L. (Lepidotera: Pyralidae) pupae. Parasitized pupae were maintained at 25 ± 1 °C. Upon emergence, adult parasitoid were transferred to Plexiglas cages (25 × 25 × 25 cm) at the following conditions: 15 ± 1 °C, 16:8 l:D and 80% RH. Adult individuals were fed with water and honey. Adults of *Pimpla luctuosa* Smith and *Pimpla nipponica* Uchida were kindly provided by Dr. Felix L. Wäckers (Biobest Sustainable Crop Management, Westerlo, Belgium).

2.1. Scanning electron microscopy (SEM)

Ten individuals of each sex of *P. turionellae* and *X. stemmator* were used for the observations. As regards *P. luctuosa* and *P. nipponica*, we investigated only 5 female individuals each, since males were unavailable. Insects were anaesthetized using CO_2 and kept at low temperature (−18 °C) for 60 s, then they were dip in alcohol 60%. Individuals were dissected removing the antennal tip from the rest of the antenna. Specimens were dehydrated in a series of graded ethanol, from 60% to 99%, 15 min each. After dehydration, 99% ethanol was substituted with pure HMDS (Hexamethyldisilazane, Sigma®) and the

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