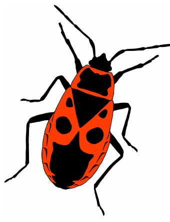

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ORIGINAL ARTICLE

Fine structure of the external sheaths of the ovipositor of *Aphidius ervi* (Hymenoptera: Braconidae)

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Abstract. The function and structure of the ovipositor in Hymenoptera have been studied intensively, although there is a lack of information on the external sheaths. We provide here a contribution on the structure of the external sheaths of the ovipositor of the parasitic wasp *Aphidius ervi*, in particular the secretory structure is described for the first time. These glands are made up of a large epithelial structure that consists a single layer of large secretory cells that occupy most of the lumen of the valve and belong to gland cell class 1. Based on the different features of the glands, a lubricating and/or host marking function is hypothesized and discussed.

INTRODUCTION

The insect ovipositor has developed from the primary segmental appendages of the eighth and ninth abdominal segments (Scudder, 1961a, 1964, 1971). In Hymenoptera, the ovipositor is unique among the endopterygotes in having a well-developed apparatus in which basal articulations are present (Kristensen, 1991; Vilhelmsen, 2000). The function of the ovipositor in parasitic Hymenoptera is to lay eggs on or inside different species of suitable hosts living in very different habitats (Vilhelmsen, 2000; Vilhelmsen et al., 2001). It has also a key role both in informing the parasitoid of the condition of the host and in marking the host with oviposition-deterring substances (Vinson, 1998; Ruschioni et al., 2015a).

The anatomy and mechanics of the ovipositor are presumably adapted to the diversity of hosts and host-associated substrates into or onto which parasitoids deposit their eggs and this might have contributed to the high diversity of species in the Hymenoptera (Field & Austin, 1994; Quicke et al., 1999). The basic functional elements of the ovipositor are, however, very similar (Snodgrass, 1935; Scudder 1961b, 1971; Smith, 1969; Copland & King, 1972; Richards, 1977; Austin & Browning, 1981). Ovipositors have three pairs of valvulae and two pairs of valvifers. The latter are situated basally and the second pair, with the ninth tergite, accommodates the main muscles that extend and retract the ovipositor proper (Oeser, 1961). The valvulae do not have a musculature (Quicke et al., 1999; Vilhelmsen, 2000). The first and second valvulae constitute the ovipositor proper and are joined together and connected in a way that they can be moved (Vilhelmsen, 2003). The third valvulae form the external sheath of the ovipositor (Vilhelmsen, 2003).

While the ovipositor proper of many parasitoid wasps has been studied in some detail (Quicke et al., 1995; Brown & Anderson, 1998; van Lenteren et al., 1998), the external sheaths have not received the same attention, and little data are available on their structural features. The ovipositor is generally very thin and therefore is easily damaged if it protrudes from the posterior end of the metasoma. It would thus appear that the external sheaths have evolved to protect the ovipositor when it is not in use (Scudder, 1971; Austin, 1983; Vilhelmsen, 2003). Although in the Aculeata the protective function of the third valvulae is taken over by the abdomen, the valvulae might still have a role in host detection and selection. Indeed, there are some sensilla on the valvulae that might fulfil such a role (Quicke et al., 1999; Larocca et al., 2007). Another theory proposes that

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the function of the external sheaths is to clean the ovipositor (Vilhelmsen, 2003).

In the Braconidae, the ovipositor generally protrudes from the metasoma, is strongly sclerotized and lodged within the external sheath (Snodgrass, 1933; Askew, 1971; Scudder, 1971). Larocca et al. (2007) report the ultrastructural details of the ovipositor of *Aphidius ervi* Haliday, but do not present any data on the external sheaths.

Here we describe the external sheaths of *A. ervi* and report for the first time an associated secretory structure. The morphological features and the possible functional significance of this gland are discussed.

MATERIAL AND METHODS

Insects

The *A. ervi* used in the present study was reared in the laboratory on pea aphid (*Acyrthosiphon pisum* Harris) maintained on potted broad bean (*Vicia faba* Linnaeus) plants. The aphid and parasitoid cultures were maintained in two separate environmental chambers at 20 ± 1°C and 75% ± 5% relative humidity, under 18L : 6D photoperiod.

Scanning electron microscopy

Ten *A. ervi* females were anaesthetized by exposure to cold temperature (–18°C) for 60 s and then dipped in 60% alcohol. The ovipositor was removed from the abdomen and dehydrated using a graded series of ethanol concentrations, from 60% to 99%, 15 min in each; the last step was repeated twice. After dehydration, the 99% ethanol was replaced with pure hexamethyldisilazane (Sigma), and the specimens were allowed to dry under a hood, under room conditions. Ten ovipositors were mounted on aluminium stubs and gold-sputtered (SCD 040 unit; Balzers Union, Vaduz, Liechtenstein). Observations were carried out using a scanning electron microscope FE-SEM SUPRA 40 (Carl Zeiss NTS GmbH, Oberkochen, Germany) at 10 kV and with a window diameter of 9 mm to 10 mm, and analyzed using SMART-SEM software (V05.04: 08.v.2009; Zeiss, Oberkochen, Germany).

Transmission electron microscopy

Ten females of *A. ervi* were anaesthetized by exposure to a temperature of –18°C for 60 s, and then immediately immersed in a solution of 1% glutaraldehyde and 2.5% paraformaldehyde in 0.1 M cacodylate buffer and 5% sucrose, pH 7.2–7.3. The entire ovipositor was detached from its base, transversally cut in the middle to facilitate the penetration of the fixative, and left at 4°C for 2 h. The specimens were kept at 4°C overnight in 0.1 M cacodylate buffer and 5% sucrose, pH 7.2–7.3, then post-fixed in 1% OsO_4 for 1 h at 4°C and rinsed in the same buffer. Dehydration in a graded series of ethanol from 60% to 99% was followed by embedding in Epon-Araldite, with propylene oxide as the bridging solvent. Thin sections were cut using a diamond knife (Drukker, Cuijk, The Netherlands) on an ultramicrotome (Nova; LKB, Bromma, Sweden) and then mounted on formvar-coated 50-mesh grids. The sections on the grids were stained with uranyl acetate (20 min, room temperature) and lead citrate (5 min, room temperature). Finally, the sections were examined under a transmission electron microscope (EM 208; Philips, FEI Company, Eindhoven, The Netherlands). Digital pictures (8 bit greyscale

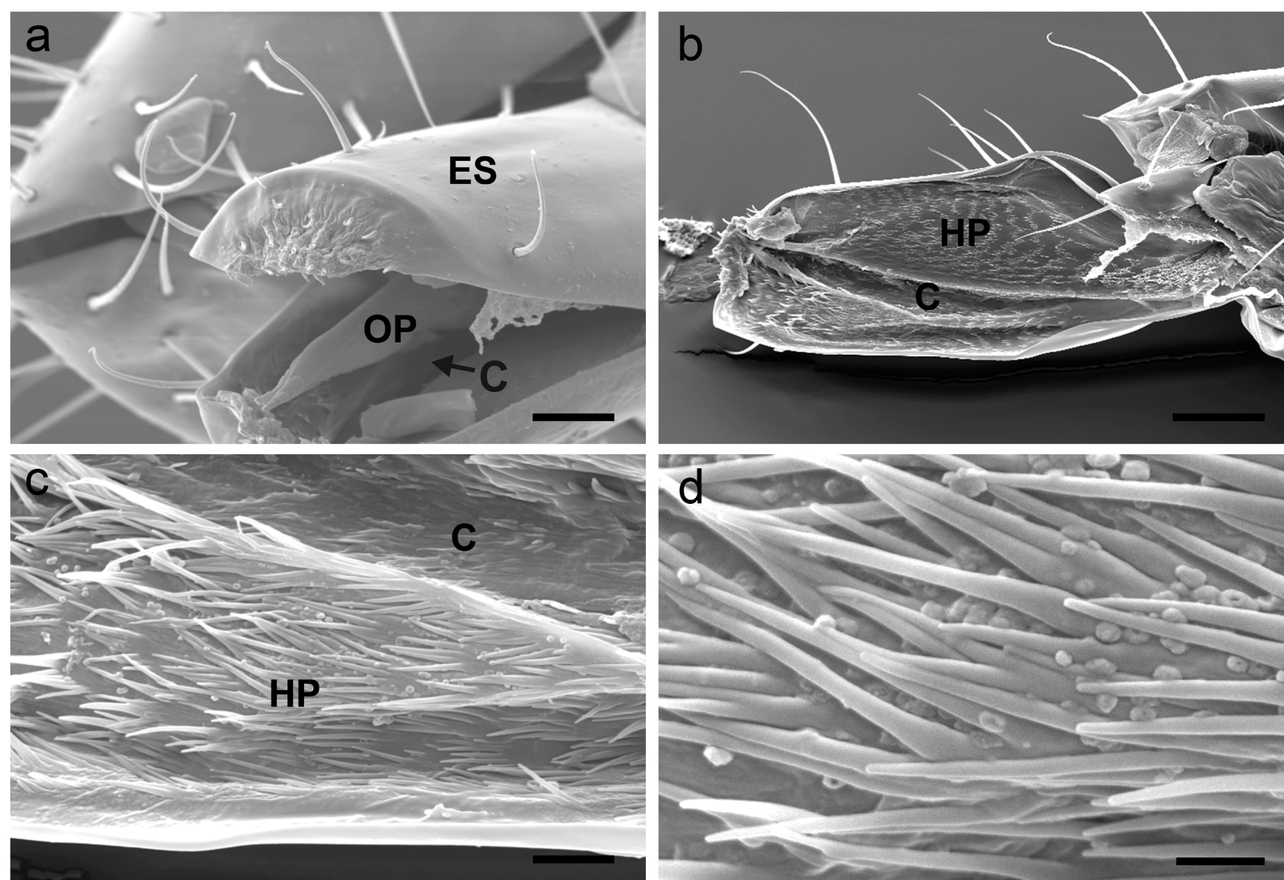

Fig. 1. Scanning electron micrographs. a – *Aphidius ervi* ovipositor (OP) enclosed by the external sheaths (ES). The ovipositor canal is visible (C). b – internal view of the external sheath, showing the ovipositor canal (C) and the hair-like projections (HP). c – details of the structure of cuticle characterized by hair-like projections. d – hair-like projections. Scale bars: a, b: 25 µm; c: 2.5 µm; d: 1.5 µm.

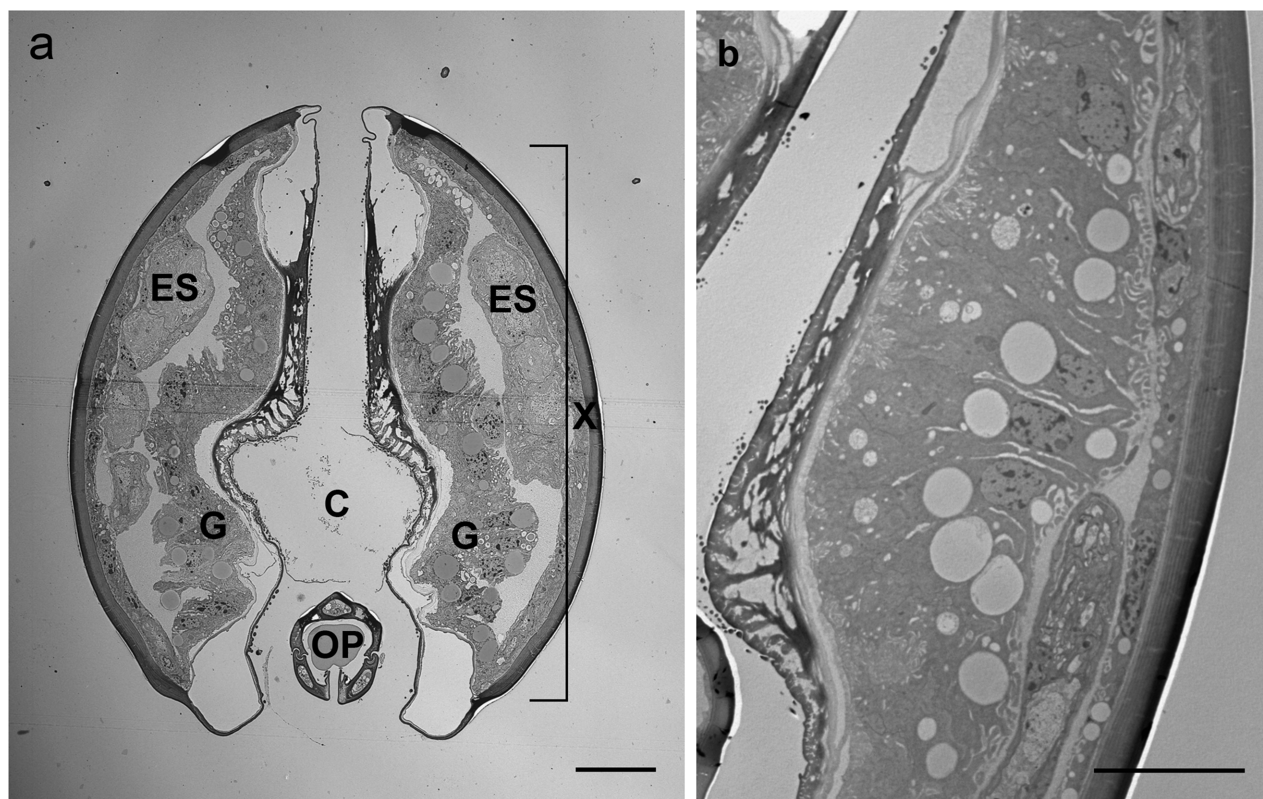

Fig. 2. Transmission electron micrographs of cross-sections of: a – external sheaths (ES) delimiting the canal (C) where the ovipositor (OP) is located, showing the gland (G) and its extension (x = 150 μm). In this preparation the ovipositor is located just below the ovipositor canal. b – detail of the glandular epithelium. Scale bars: a: 10 μm; b: 20 μm.

TIFF files) were obtained using a high resolution digital camera (MegaViewIII; SIS and Olympus, Tokyo, Japan) connected to the transmission electron microscope.

RESULTS

The third valvulae (forming the external sheath) of *A. ervi* are seven times broader than the ovipositor proper, and have a smooth cuticle on their external surface (Fig. 1a). Scanning electron microscopy of the inner surface shows numerous hair-like projections, interspersed with small sub-spherical particles, with their apex orientated towards the distal end of the valvula (Fig. 1b–d). A longitudinal internal canal is delimited by the medial surfaces of both valvulae (Fig. 1b) and is where the ovipositor is held at rest. Distally, the external sheaths are truncated and have a wrinkled cuticle (Fig. 1a). The cross-section of the external sheaths shows a half-elliptical shape (Fig. 2a); at rest the internal surfaces delimit two cylindrical hollow spaces, the upper of which is the ovipositor canal (Fig. 2a). The cuticle lining the external surface of the third valvula is smoother and thicker when compared with the inner surface, on which there are many typical multiple cuticular invaginations (Fig. 2a, b). Lining the lumen of the external sheath is an extensive epithelium (Fig. 2a, b) the cells of which are in close contact with the internal cuticular wall. The epithelium is made up of a single layer of large secretory cells that occupy most of the valvula lumen (Fig. 2a, b). At the bases of the cells there are deep infoldings and large electron-lucid vesicles (Figs 2b, 3b), which are also present in the cytoplasm of the cells along with stacks of rough endoplasmic reticulum (Fig. 3b) and numerous slender mitochondria. In the apical region of the cells the microvilli are connected with the innermost cuticular layer, which is electron dense and has many filaments. The cuticle is thicker in the medial part of the valvula, and has a complicated pattern of digitiform projections and infoldings (Fig. 3a, c). In cross-section, this part shows a sponge-like structure, which is perforated by numerous tiny pores (Fig. 3a, d). The release sites of these glands are positioned very close to the longitudinal internal upper canal.

DISCUSSION

This study shows that in *A. ervi*, the interior of each external sheath is characterized by the presence of numerous hair-like projections (microtrichia) that are interspersed with small sub-spherical particles, with their apex orientated towards the distal end of the valve. In other Braconidae, Vilhelmsen (2003) reports microtrichia of different densities and shapes on the inner wall of the external sheaths. According to Vilhelmsen (2003), this structure appears to clean the ovipositor proper between ovipositions. This function is fundamental, since it is important for female parasitoids to keep the ovipositor sensilla clean in order to maintain their functionality.

The present study reports for the first time, in a parasitic wasp, the presence of a gland in the external sheaths. Usually the size of the third valvulae is just sufficient to enclose the ovipositor proper, in *A. ervi* the area enclosed

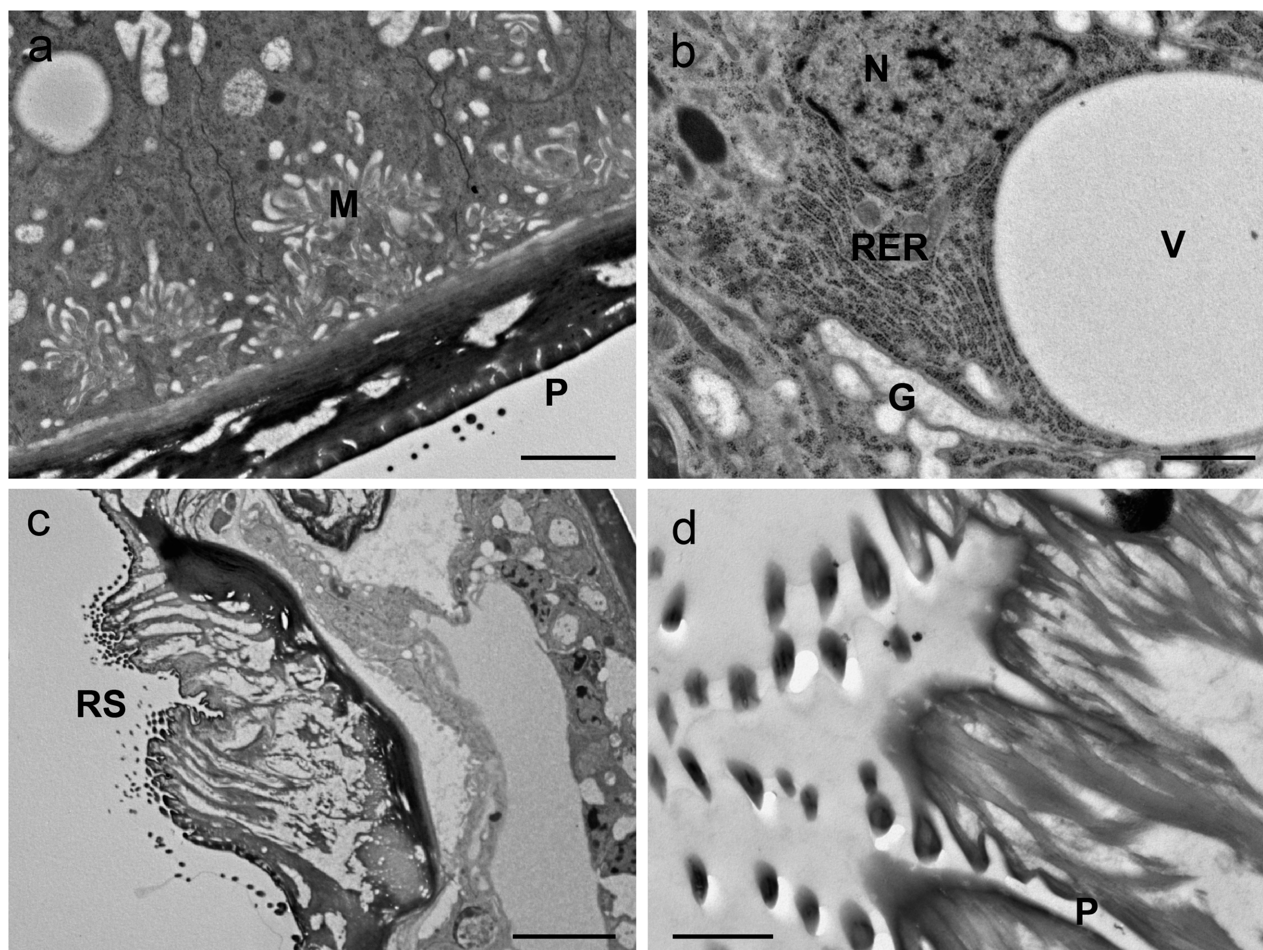

Fig. 3. Transmission electron micrographs of cross-sections of the external sheaths at the gland level. a – detail of the apical part of the secretory cells, showing abundant microvilli (M) just below the cuticle. b – close-up of a secretory cell, showing rough endoplasmic reticulum (RER), nucleus (N), Golgi apparatus (GO) and large electron-lucid secretory vesicles (V). c – cross-section at the level of the release site (RS). d – detail of the release site, showing the canal pores (P). Scale bars: a: 2 µm; b, d: 1 µm; c: 5 µm.

is much greater than that needed to enclose the ovipositor proper, most likely to make room for the glands. The glandular epithelium is made up of a single layer of secretory cells that are directly in contact with the cuticle. According to the classification of insect epidermal glands proposed by Quennedey (1998), the secretory cells associated with the external sheaths in *A. ervi* belong to class 1, as they are in direct contact with the external cuticle and lack specialized transporting cells or cuticular ducts.

A similar structure is described by Tagawa (1977) on the inner surface of the second valvifer of the braconid *Apanteles glomeratus* L., and is known to secrete a pheromone (Obara & Kitano, 1974; Tagawa, 1977). Several studies report that Hymenoptera, including parasitic wasps, secrete female sex pheromones (Whiting, 1932; Boush & Baerwald, 1967; Cole, 1970; Vinson, 1972; Cormier et al., 1998). However, only in a few cases is the location of these pheromone-producing glands known. In *Campoletis sonorensis* (Cameron) (Ichneumonidae), the sex pheromone appears to be produced over the whole body (Vinson, 1972), in *Cardiochiles nigriceps* Viereck (Braconidae), Dufour's gland is the source of the sex pheromone (Vinson, 1978), while in *Apanteles melanoscelus* Ratzeburg (Braconidae), the gland is located on the ninth tergite. Tagawa (1983) investigated seven other braconid species, and reports the female sex-pheromone-producing glands being located on the ninth tergite and/or second valvifer.

In the present study, we observed that in *A. ervi* the release sites of the gland located in the external sheaths are on the inner surface of the valvulae, which partially hides them. These release sites are positioned very close to the longitudinal internal canal, where the ovipositor is held when at rest. This position does not appear suitable for the release of a volatile sex pheromone and is not in accordance with that usually described in the literature for sex-pheromone-producing glands (Percy & Weatherst, 1971; Levinson & Levinson, 1995; Raina et al., 2000; Billen, 2009; Riolo et al., 2014), although this needs to be confirmed.

Host marking involves the release of chemical and/or physical signals (marks) on and/or inside a host, and is common among parasitoid insects (Hofsvang, 1990; van Alphen & Visser, 1990). The evidence that insects host-mark with pheromones and that they are perceived by contact chemoreceptive sensilla is well documented (Rabb & Bradley, 1970; Holler et al., 1991; Wang & Huang, 1991;

Ruschioni et al., 2015b). Parasitized hosts that remain in a habitat can be re-encountered, and host-marking pheromones that indicate that the host has already been parasitized regulate the host choice for oviposition of females (Gardner et al., 1984). A female that perceives the host-marking pheromone can reject that host and invest time in searching for other hosts. Host-marking pheromones also act as epideictic messages that trigger the dispersal of parasitoids from sites that are already occupied when the population density is near optimal (Vinson, 1985; Roitberg & Prokopy, 1987; Roitberg & Mangel, 1988). Parasitoid females may use internal or external marks, or both, to indicate which hosts are already parasitized (Salt, 1937; van Lenteren, 1976, 1981; Hofsvang, 1990). In most cases, the sources of marking pheromones are associated with the female reproductive tract (Vinson, 1985; Holler et al., 1993; Marris et al., 1996; Quicke, 1997; Rosi et al., 2001). In addition, in some cases the secretion of accessory gland acts both as a lubricant, to facilitate the passage of eggs (Hosken & Ward, 1999; Sturm & Pohlhammer, 2000; Dallai et al., 2008) and a host-marking pheromone (Quicke, 1997; Rosi et al., 2001). Our observations show two canals enclosed by the third valvulae, only the upper of which is occupied by the ovipositor proper. The glandular epithelium and the release site of the glands are located on the inner surface of the upper canal, which may serve as a reservoir for the secretions from these glands. One function of the substances secreted by these glands can be an external host-marking pheromone; they could be smeared onto the outer surface of the ovipositor proper or applied directly during egg laying.

The valves of the ovipositor proper are held together by a complicated interlocking mechanism that allows them to slide back and forth on one another (Snodgrass, 1933; Smith 1968; King & Copland, 1969; Smith, 1970; Askew, 1971; Quicke et al., 1995; van Lenteren, 1998). Austin (1983) describes the mechanics of the ovipositor system in detail, which are characterized by the extension and retraction of the ovipositor dependent on the rotation of the valvifers; here, the external sheaths should provide support and guidance for the ovipositor proper. This movement provides the functional basis for piercing the host and for moving the eggs along the shaft of the ovipositor (Austin & Browning, 1981). The substances produced by the glands in the external sheaths could also serve as a lubricant during the extension and retraction of the ovipositor proper, especially as oviposition by *A. ervi* is very quick (< 0.5 s) (Volkl & Mackauer, 2000). However, further studies are needed to determine the chemical nature of the substances secreted by the gland and their function(s).

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