SEM Studies on Immature Stages of the Drone Flies (Diptera, Syrphidae): *Eristalis similis* (Fallen, 1817) and *Eristalis tenax* (Linnaeus, 1758)

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ABSTRACT Adult drone flies (Syrphidae: *Eristalis* spp.) resemble male honeybees in appearance. Their immature stages are commonly known as rat-tailed maggots due to the presence of a very long anal segment and a telescopic breathing tube. The larvae are associated with decaying organic material in liquid or semi-liquid media, as in the case of other saprophagous eristalines. Biological and morphological data were obtained from both laboratory cultures and sampling in the field. Drone flies are important pollinators for wild flowers and crops. In fact, mass rearing protocols of *Eristalis* species are being developed to be used as efficient alternative pollinators. However, deeper knowledge of larval morphology and biology is required to improve artificial rearing. The production quality control of artificial rearing must manage the consistency and reliability of the production output avoiding, for example contamination with similar species. This article presents the first description of the larva and puparium of *E. similis*, including a comparative morphological study of preimaginal stages of the anthropophilic and ubiquitous European hoverfly species *E. tenax*. Scanning electron microscopy has been used for the first time to describe larvae and puparia of both species. Moreover, the preimaginal morphology of *E. similis* has been compared with all known descriptions of the genus *Eristalis*. The main diagnostic characters of the preimaginal stages of *E. similis* are the morphology of the anterior spiracles (shape of clear area and arrangement of facets) and pupal spiracles (length, shape, and arrangement of tubercles). *Microsc. Res. Tech. 76:853–861, 2013*.

INTRODUCTION

Many hoverflies that belong to the subfamily *Eristalinae* play an important ecological role, mainly due to their contribution in the pollination process. Drone flies (*Eristalis* spp) resemble honeybees (*Apis mellifera* Linnaeus, 1758) in both appearance (batesian mimics) and foraging behavior (Golding and Edmunds, 2000). The effectiveness of hoverflies as crop pollinators is becoming increasingly evident (Feldman, 2006; Jauker and Wolters, 2008; Kevan and Baker, 1983; Pontin et al., 2006; Rader et al., 2009). Drone flies play this important role in agricultural landscapes, or in natural habitats that are unsuitable for bee species (Jauker et al., 2009, 2012; Pérez Bañón et al., 2003a; 2007). Moreover, some *Eristalis* species [*E. tenax* (Linnaeus, 1758) and *E. cerealis* Fabricius, 1805] have been reared in captivity (including mass-rearing) in order to use them as pollinators under controlled conditions, such as in isolation cages or in greenhouses, to improve seed setting for diverse crops (Gladis, 1997; Jarlan et al., 1997; Kobayashi, 1972; Ohsawa and Namai, 1987, 1988; Okamoto et al., 2008; Takeda and Yanase, 1990).

In contrast with the flower-feeding habits of adults, rat-tailed maggots are associated with decaying organic material in liquid or semisolid media (Rotheray, 1993). *Eristalis* larvae could also be used as indicators of pollution, as they only occur where they can filter large quantities of bacteria from water (Gilbert, 1993). Furthermore, it has been demonstrated that the aquatic larvae of some drone flies can actually remove decaying organic substances that pollute the environment from water and release nutrients back into the system (Abou-El-Ela et al., 1978). These larvae could potentially be used to solve difficult problems associated with agricultural and horticultural processes, such as disposal of organic residues (Rotheray and Gilbert, 2011).

On the other hand, some species are frequent in environments with high organic and microbial contamination and can act as potential mechanical vectors of pathogens on pig and cattle farms (see Frankuski et al., 2011 and references therein). Moreover, *E. tenax* larvae have been found causing myiasis in dead animals or even humans and livestock (Aguilera et al., 1999;

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Catts and Mullen, 2002; González et al., 2009; Mumcuoglu et al., 2005; Salimi et al., 2010).

Preimaginal stages of some *Eristalis* species have been studied and described by different authors since the turn of the last century (e.g., Gabler, 1930, 1932; Klein-Krautheim, 1936; Metcalf, 1913; Vimmer, 1925; etc.). However, most of those descriptions are too general and, frequently without diagnostic characters that allow identification of a particular species. The most detailed and useful descriptions were provided by Hartley (1961), when he described and compared the morphology of larvae and puparia of six *Eristalis* species [*E. abusiva* Collin, 1931, *E. arbustorum* (Linnaeus, 1758), *E. intricaria* (Linnaeus, 1758), *E. nemorum* (Linnaeus, 1758), *E. pertinax* (Scopoli, 1763), and *E. tenax*], including the first identification key for their third larval stage. Dolezil (1972) described the preimaginal stages of *E. horticola* (De Geer, 1776) and *E. rupium* Fabricius, 1805, and included these two species in Hartley's key, improving it with new characters. Maibach et al. (1991) also described *E. rupium*, comparing it with Hartley's descriptions and including biological data of the species. Kuznetsov and Kuznetsova (1994) described and figured the preimaginal stages of three Northern European species: *E. anthophorina* (Fallen, 1817), *E. rossica* Stackelberg, 1958, and his *E. vitripennis* Strobl, 1893 (see Speight, 2011 for discussion about the taxonomical status of this species). Recently, Sasaki and Mikami (2007) described diagnostic characters of *E. cerealis* (Fabricius, 1805), *E. rossica*, and *E. tenax*. Unfortunately, about 75% of the larval stages of described species of the genus *Eristalis* are still unrecognized. Previous research using scanning electron microscopy (SEM) is very scarce, i.e.: descriptions of antennomaxillary organs of *E. tenax* (see Akent'eva, 2012), the chorion of the eggs of *E. cryptarum* (Fabricius, 1794) and *E. cerealis* (Kuznetsov, 1989; Sasaki and Mikami, 2007), first instar larvae of *E. cryptarum* (Kuznetsov, 1992), and the anterior spiracle of mature larvae of *E. cerealis*, *E. rossica*, and *E. tenax* (Sasaki and Mikami, 2007).

We use an analysis of ultrastructure in our descriptions that permits detailed understanding of important diagnostic and comparative characters found useful in studies of other syrphids (Laska et al., 2006; Pérez-Bañón et al., 2003b; Rojo et al., 2006). In particular, these characters include the pseudocephalon and anterior and pupal spiracles.

We focus our research on two species of the genus *Eristalis*: *E. tenax* and *E. similis* (Fallen, 1817). The first is the most widely distributed syrphid species in the world, known from all regions except the Antarctic and found throughout Europe except in the far north. This anthropophilic and almost ubiquitous fly visits a wide range of flowers as an adult and its larvae are found in a large variety of organically rich aqueous and semi-aqueous rotting materials, including slurry, dung, etc. (Speight, 2011). The preimaginal stages of *E. tenax* have previously been described by other authors who used stereomicroscopy exclusively (Batelli, 1879; Buckton, 1895; Dixon, 1960; Gäbler, 1930, 1932; Hartley, 1961; Klein-Krautheim, 1936; Metcalf, 1913; Smart, 1948; Vimmer, 1925). According to Speight (2011), *E. similis* is a Palaearctic species distributed from the south of Finland to North Africa on the southern side of the Mediterranean basin, from Britain eastwards through central and southern Europe to the former Yugoslavia and on through Turkey and European Russia into Asia. Adults are frequent in mature deciduous forests and Mediterranean evergreen forests, but the biology and description of the preimaginal stages are still unknown.

The objectives of this study are: (1) to describe the morphology of the third larval stage and puparium of *E. similis*, using SEM; (2) to provide a comparative morphological study of the preimaginal stages of the two species, carried out using conventional stereomicroscopic descriptions and SEM photographs; (3) to present the preimaginal diagnostic characters of *E. similis* to distinguish it from all other known *Eristalis* species with immature stages described. Life histories and biological and rearing data of both species are also provided.

MATERIAL AND METHODS

The preimaginal stages of the two species examined in this study were collected as second and third larval stages in streams rich in organic matter in east Mediterranean localities (Lesbos Island, Greece). They were obtained from a culture derived from larvae collected on pig farms in the case of *E. tenax* and from wild gravid females in the case of *E. similis* in west Mediterranean localities (Alicante province).

Field collected larvae were introduced into plastic cages containing water from the same place where they had been collected. Rearing of immature stages was performed in a growth chamber at 16–22°C, 80 ± 5% RH, with a constant photo-regime of 15L:9D. Pupae were isolated in individual Petri dishes and inspected daily until the emergence of adults.

Larvae selected for preservation were third stage (L3). For permanent preservation, larvae were immersed in cold water to extend them, and then heated slowly for about 4 min to kill them. After this, they were preserved in 70% alcohol. Descriptions are based on preserved specimens with larval characters checked against living specimens in order to minimize errors due to preservation.

Larvae were studied using a cryo-scanning technique. This method has the great advantage that the material is frozen so quickly that vulnerable biological structures are well preserved. The larva was fixed on a holder with a layer of O.C.T. conductive glue and it was frozen rapidly with liquid nitrogen for 2 min. Afterwards the specimen holder was transferred to a system for cryo-SEM (Oxford CTI500) and was fixed on the cold stage of the freeze-etching unit, which was maintained under a vacuum and equilibrated from −150°C to −90°C. The specimen was freeze-etched under these conditions for about 2 min to eliminate contamination by frost and then a thin layer of gold was "sputtered" onto the material for five minutes. Finally, the sample was transferred to the cold stage of the SEM (S3000N Hitachi), which was kept at about −150°C, and secondary electron images were observed and recorded at an accelerating voltage of 5 kV.

Debris adhered to the puparium integument was removed by placing the specimens in an ultrasonic cleaner for a few minutes. The cleaned specimens were mounted on stubs and examined with a scanning

electron microscope (S3000N Hitachi) using variable-pressure (or low vacuum) mode. This technique allows a direct evaluation of the specimens without coating the samples with gold. Terminology used for larval and pupal descriptions follows Rotheray (1993) and Courtney et al. (2000). Dimensions of preserved specimens were measured using a stereomicroscope (Leica MZ9.5).

These studies were conducted in the technical research services at the University of Alicante.

RESULTS

Description of the Preimaginal Stages

Complete descriptions of the third larval stage and puparium of *E. similis* are presented. However, in the case of *E. tenax*, only characters undescribed until now or diagnostic morphological characters that distinguish it from *E. similis* have been included.

Eristalis similis (Fallen, 1817)

Third Larval Stage. Length not including posterior breathing tube (prp) 18–20 mm, maximum width 5–6 mm ($n = 10$).

Overall appearance: a long-tailed larva with internal mouth-hooks and a retractile anterior spiracle. Sub-cylindrical in cross-section with a flattened ventral surface, truncate anteriorly, and tapering posteriorly. Cuticle translucent when alive, cream to off-brown after fixation. Dorsal body surface coated in pubescence backwardly directed and slightly sclerotized on the terminal body segment. Setae on ventral surface are scarce except for the anal segment. Prolegs bearing crochets in two main rows, the first row bigger than the second.

Head (pseudocephalon). Mandibles and mandibular lobes internal, mandibles supporting expanded mandibular lobes [mouthparts adapted for filter-feeding (*sensu* Roberts, 1970)]. Antennomaxillary organs well developed, located between mouth and dorsal surface of prothorax (Figs. 1A and 1B). These organs consist of two pairs of cylindrical-shaped structures tipped with different types of sensilla (Fig. 1C). Antenna easily identified by the presence of antennal sensory cone. Antennal segment at the base of the antennal cone and on the maxillary palp with several satellite sensilla (Fig. 1C). Antennomaxillary organs with mechano- and chemoreceptors bearing fleshy basal papilla with three sections. Basal section of the papilla supporting antennomaxillary organs divided medially almost to the base (Fig. 1B). Dorsal lip (a projection between the mouth and the antennomaxillary organs) broad, lacking a medial groove and covered with a conspicuous tuft of long setae (Figs. 1A and 1D). One pair of sensilla located above the mouth and below the tuft of setae (Fig. 1D). Ventral lip well developed with one pair of sensilla (Figs. 1A, 1E, and 1F).

Thorax. Lateral lips well developed, rounded, inner upper margin and the base coated in long, fine, and densely aggregated setae, outer upper margin coated with spicules (Figs. 1A and 2A). Dorsal surface of prothorax with 6–8 longitudinal grooves. Anterior fold with a broad band of backwardly directed, slightly hooked, and sclerotized spicules, which become progressively shorter posteriorly. Dorsal surface of prothorax with a pair of anterior spiracles about three times longer than broad, light brown in color, sclerotized, with pointed slightly recurved tips, and completely retractile within inverted integumental pockets (Fig. 2B). Spiracular openings situated on a clear area weakly sclerotized of the ventral surface, extending along the distal two-thirds of spiracle length. Clear area about three times longer than broad, with the fold in the middle of its length. Lower part of clear area widened, encircling less than three-fourths the perimeter of the spiracle. Number of facets around 17, arranged in one row at the edge of the clear area (Fig. 2B). Lateral margins of the mesothorax with two patches of sclerotized spicules arranged as follows: a group between 10 and 15 spicules immediately anterior to the 4th pair of sensilla and another group with 25–30 spicules located in front of the 5th pair of sensilla. Mesothorax bearing well developed prolegs with more than 60 crochets arranged in multiple rows (Fig. 2C).

Abdomen. Primordia of pupal spiracles on the dorsal surface of first abdominal segment. Six pairs of ventral prolegs on segments 1–6. Prolegs well developed, in frontal view, having a circular-shape with two main rows of apically brown crochets, which are broader at the apex than the base in ventro-lateral view. About 6–7 primary crochets long and slender, with distal third sclerotized, and larger than the posterior ones (Fig. 2D). Arrangement of crochets varies from abdominal segments 1–6, with a few crochets facing sideways out from the body in segment 1 (Fig. 2E), to most facing sideways out from the body by segment 6 (Fig. 2F). Rows of crochets of paired prolegs on abdominal segments 1–6 separated by a distance greater than their individual length (Figs. 2E and 2F). Sensilla 4 aligned horizontally with sensilla 5 and 6 on segment 7. Dorsal surface of anal segment covered with long dense setae. Anal segment extended, with three pairs of weakly developed lappets. Second and third pairs of lappets are together at the end of the anal segment, with the first pair about halfway along its length. Posterior breathing tube (prp) shiny, sclerotized, brown in color, with three pairs of spiracular openings arranged around fused central scars (Fig. 3D). Four pairs of long interspiracular setae.

Chaetotaxy. Prothorax with 12 pairs of sensilla; mesothorax and metathorax with 9 pairs; abdominal segments 1–7 with 11 pairs; anal segment with 3 pairs of sensilla (sensilla 9, 10 and 11) and three pairs of lappets.

Puparium. Anterior end truncate, tapered posteriorly, flattened ventrally and subcylindrical in cross-section. Cream to dark brown in color. Pupal spiracles projecting from middle of upper part of operculum, separated by a distance of more than one-third of spiracle length. These processes are sub-cylindrical structures about 2–2.5 mm in length (length width ratio of spiracle 10-9:1) dorsal surface facing toward the lateral margins of the puparium and with the distal one-fourth slightly curved and flattened (Figs. 3A and 3B). About 80% of the dorsal and lateral surfaces covered with irregularly spaced, oval-shaped tubercle each bearing 6–10 oval spiracular openings (Figs. 3B and 3C). Entire surface smooth, including the space between tubercles.

Material examined: 15 larvae obtained from a culture derived from wild gravid females collected in Agost (Alicante), Spain, VI. 2009 E. Gras & C. Pérez-Bañón. 20 puparia (15 m, 5 f), Thermi (Lesbos), Greece 8. IV. 2001 C. Pérez-Bañón & S. Rojo.

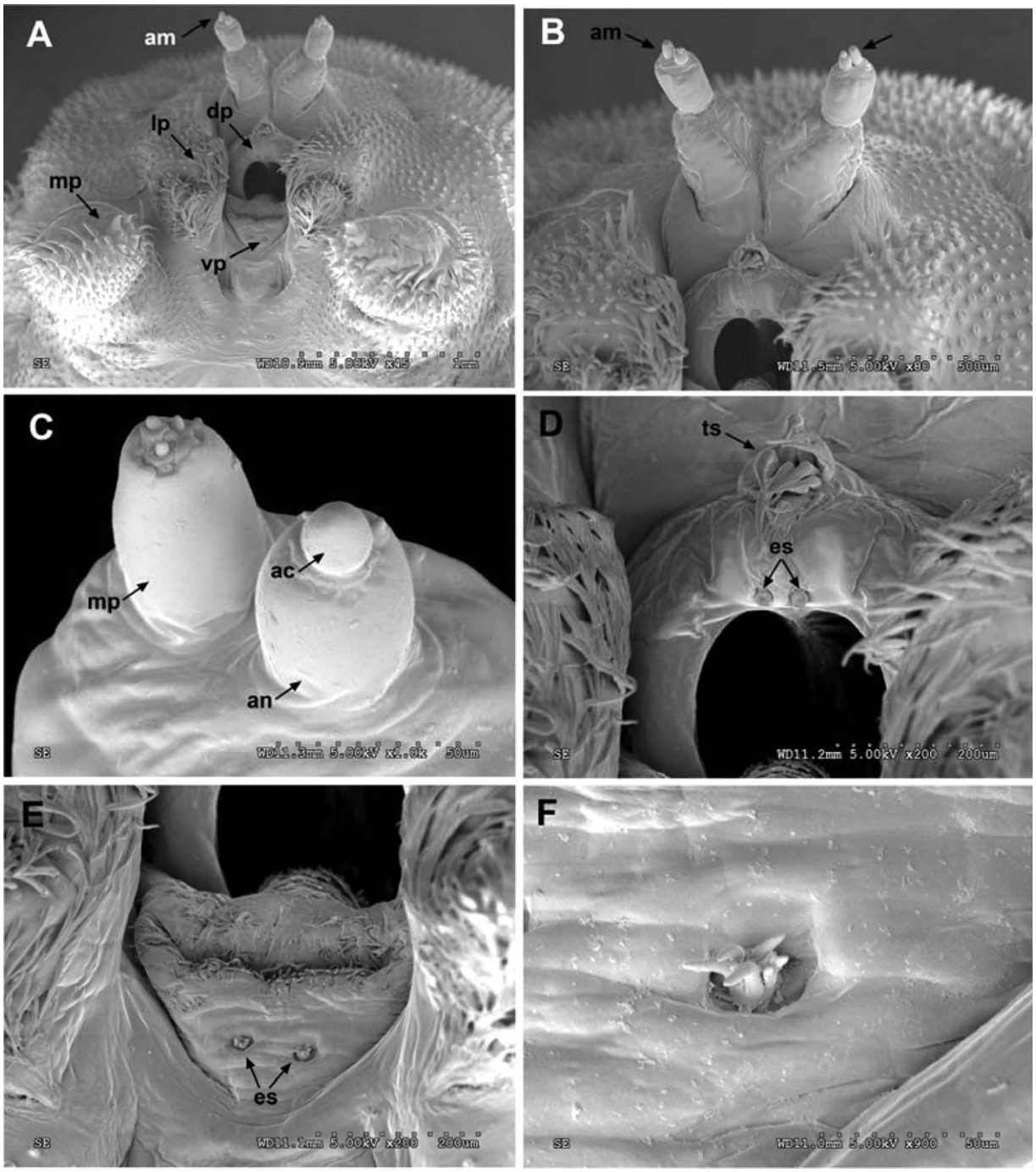

Fig. 1. Scanning electron micrographs of third larval stage of *Eristalis similis*. **A**: Head and thoracic segments (pro- and mesothorax), ventral view; (**B**) cephalic region with antennomaxillary organs; (**C**) details of antennomaxillary organs; (**D**) Position of the extra pair of sensilla on the dorsal lip; (**E**) Position of the extra pair of sensilla on the ventral lip; (**F**) details of the extra sensilla of ventral lip. Abbreviations: (am) antennomaxillary organs; (dp) dorsal lip; (lp) lateral lip; (vp) ventral lip; (mp) mesothoracic proleg; (an) antenna; (mp) maxillary palp; (ac) antennal cone; (ts) tuft of long setae; (es) extra pair of sensilla.

Diagnostic Characters of *E. tenax* (Linnaeus, 1758).

Third Larval Stage. Length, overall appearance of the body, chaetotaxy, and head as *E. similis*.

Thorax. *Anterior spiracles:* Spiracles are short, about two times longer than broad, stout, sclerotized, with rounded and slightly recurved tips, dark brown in color, completely retractile within inverted integumental pockets (Figs. 4A and 4B). Spiracular openings located on a clear area of the ventral surface, extending along the distal three-fourths of the spiracle length. Clear area about three times longer than

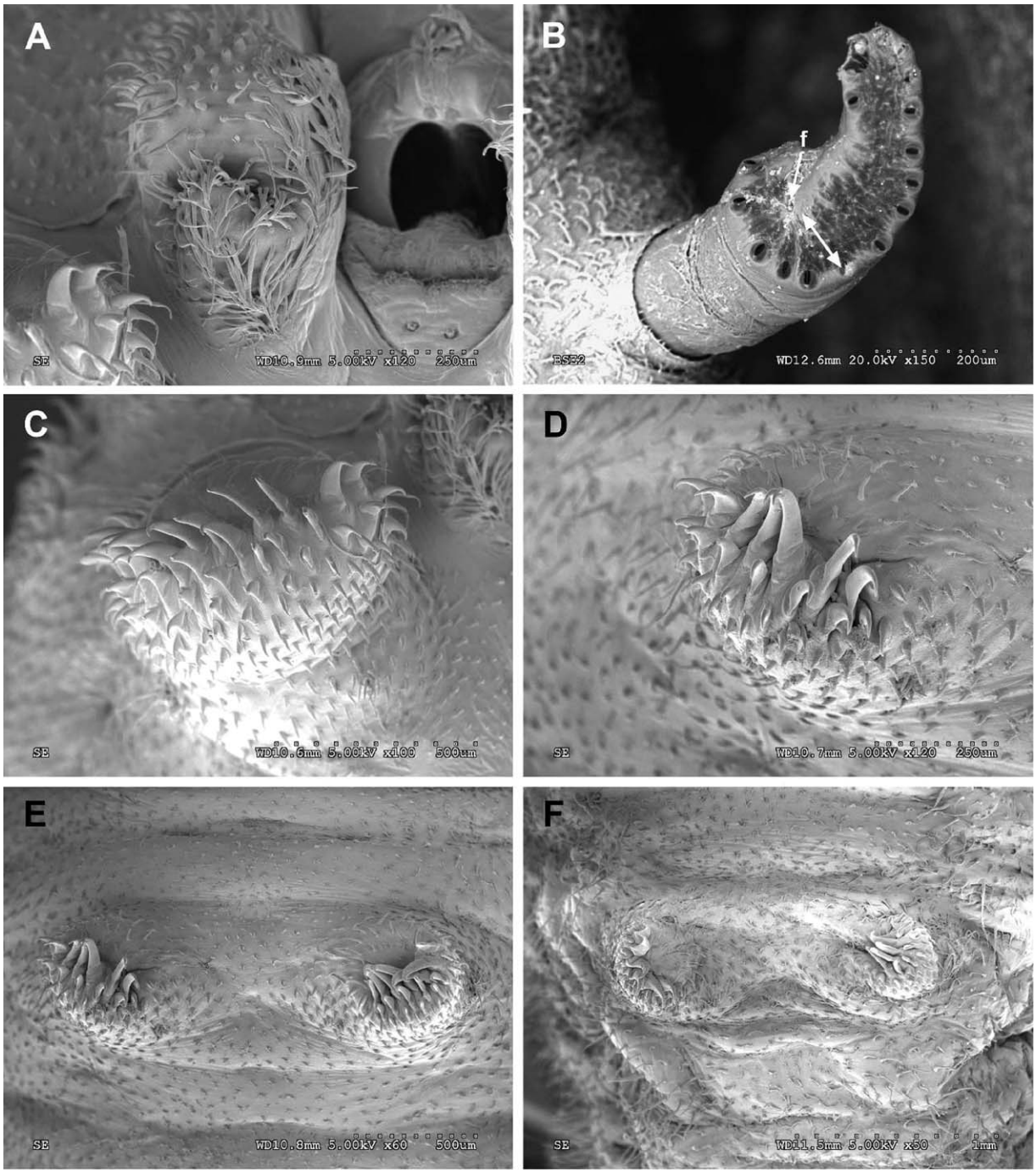

Fig. 2. Scanning electron micrographs of a third larval stage of *Eristalis similis*. **A**: Detail of lateral lips; (**B**) anterior spiracle, antero-dorsal view; (**C**) details of mesothoracic prolegs; (**D**) abdominal proleg; (**E**) first pair of abdominal prolegs; (**F**) Prolegs of the sixth abdominal segment. Abbreviations: (f) fold of anterior spiracles.

broad, with the fold slightly above the middle of its length. Lower part of clear area not widened, all facets could be seen from one position. Facets (17–21) arranged in one row around the edge of the unsclerotized area (Fig. 4B).

Abdomen. *Prolegs:* Well developed, in ventro-lateral view, as small cones with an oval base broader at the apex than the base with two main rows of apically brown crochets, broader at the apex than the base in ventro-lateral view. About 6–7 primary crochets as long as wide, sclerotized at distal third, larger than the posterior ones (Fig. 4C). Crochets on abdominal segments 1–5, arranged in semi-circular rows with all crochets facing backwards. Last pair of prolegs with most

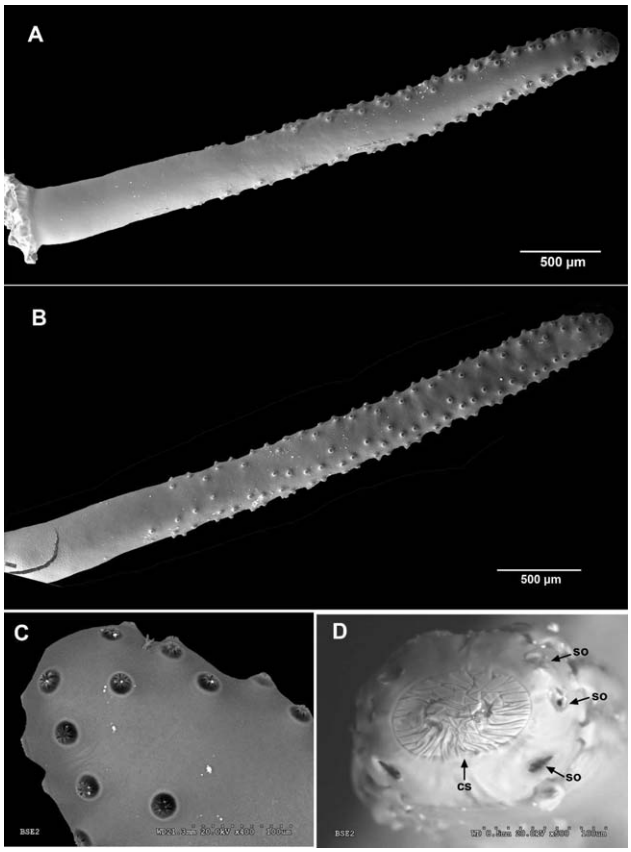

Fig. 3. Scanning electron micrographs of a puparium of *Eristalis similis*. **A**: Pupal spiracle, ventral view; (**B**) pupal spiracle, dorsal view; (**C**) spiracular openings of pupal spiracles; (**D**) posterior breathing tubes, apical view. Abbreviations: (cs) central scars; (so) spiracular openings.

of the large primary crochets facing toward the lateral margins of the body (Fig. 4D). Rows of crochets of paired prolegs on abdominal segments 1–6 separated by a distance less than their individual length (Figs. 4C and 4D).

Puparium. *Pupal spiracles:* Subcylindrical 1–1.5 mm in length (length: width ratio of spiracle 6-4:1), projecting forward and then bending sharply downward, slightly tapering, and flattened apically (Figs. 5A and 5B). Light brown in color. About 60% of the dorsal and lateral surfaces covered with irregularly spaced and oval-shaped tubercles, each bearing 6–10 oval spiracular openings (Figs. 5B and 5C). Tubercles not reaching the ventral surface. Entire surface smooth, including space between tubercles.

Material examined: 25 larvae obtained from a culture derived from puparia collected at a pig farm in Villena (Alicante), Spain, IV. 2008 P. Hurtado & C. Pérez-Bañón. 25 puparia (15 m, 10 f), from Thermi (Lesbos), Greece 8. IV. 2001 C. Pérez-Bañón & S. Rojo.

Life History

We found larvae of *E. similis* and *E. tenax* together with *Eristalinus* spp. in natural streams rich in organic matter in the East Mediterranean localities. The origin of this organic matter was waste from olive oil or cheese factories. Oviposition of both species took place at the edges of the stream under and between the stones situated near the water. Eggs were ovoposited in clusters of up to a hundred eggs if several females used the same place. Pupation took place between the vegetation and stones slightly above the water level.

In the laboratory, *E. similis* was reared from wild gravid females. The adults collected in the field were individualized, kept in cages ($40 \times 40 \times 40$ cm^3) and supplied food *ad libitum* with water, sugar, and pollen (25°C, 60% RH and 12L:12D photoperiod). Soaked cereals, mainly barley, were provided as an oviposition medium following Gladis (1997). The eggs hatched after 24 h. The larvae were reared with a liquid medium consisting of water and decaying barley. The larvae completed their development in two weeks. The length of the pupal period varied from 8 to 11 days.

Larvae of *E. tenax* in the East and West Mediterranean localities are also associated with slurry manure from pig farms. Adults of *E. tenax* could be found throughout the year on pig farms. Larvae and puparia of this species were found together with *Eristalinus* spp from early spring to autumn (March to October), with two peaks of activity. The first peak of activity was at the end of spring and beginning of summer (June); and the second at the end of summer (September). The length of the pupal period varied from 8 to 10 days ($n = 25$) under laboratory conditions (25°C, 60% RH and 12 L:12 D photoperiod).

DISCUSSION

The larvae of the two species studied were found submerged in water bodies that receive excess nutrients linked to human activities (eutrophic water). As is typical of most Eristalinae, larvae show an elongated anal segment and a telescopic breathing tube. This allows these larvae to descend to depths many times their body length, usually into the mud or accumulated debris at the bottom, where they can feed and breathe and at the same time remain hidden. This character is shared with other aquatic saprophagous genera (see Rotheray, 1993 for a comparison).

According to Rotheray and Gilbert (1999), European species of *Eristalis* can be distinguished from other long-tailed syrphid larvae by the following characters: prolegs with crochets in three rows with spicules gradually becoming smaller below; abdominal segments 2–6 with lateral sensillum 4 above 5 and 6; last pair of prolegs with curved tips of most of the primary crochets facing out to the lateral margins of the larva; without transverse row of spicules just in front of the last pair of prolegs. Our results demonstrate that the larvae of *E. similis* also share these features and can be easily distinguished from the genus *Eristalinus* Rondani, 1845, which frequently has a similar type of habitat (Pérez-Bañón et al., 2003b).

As occurs with the imagoes, the larvae of *E. similis* and *E. tenax* are very similar, but easily recognized by the length and shape of anterior spiracles. Spiracles are short, up to two times longer than broad, stout, sclerotized, with rounded and slightly recurved tips in *E. tenax* (Fig. 4B) but are up to three times taller than broad in *E. similis* (Fig. 2B). Other characters that separate these species are the shape of the primary

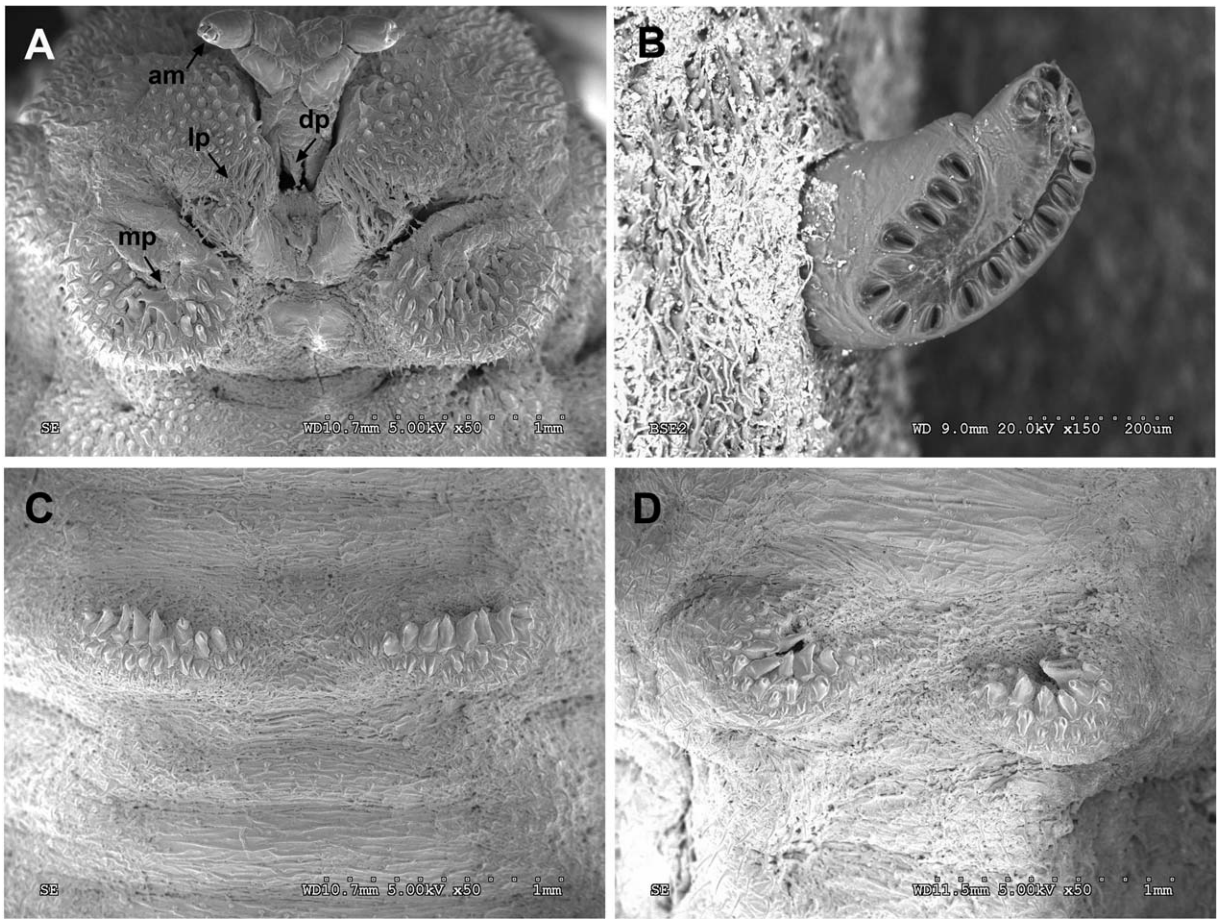

Fig. 4. Scanning electron micrographs of third larval stage of *Eristalis tenax*. **A**: Head and thoracic segments (pro- and mesothorax), ventral view; (**B**) anterior spiracle, antero-dorsal view; (**C**): first pair of abdominal prolegs; (**D**) Prolegs of the sixth abdominal segment. Abbreviations: (am) antennomaxillary organs; (dp) dorsal lip; (lp) lateral lip; (mp) mesothoracic proleg.

abdominal crochets, which are about as long as they are wide in *E. tenax* and nearly twice as long as wide in *E. similis*. Furthermore, the shape of prolegs and the arrangement of crochets can also be used to distinguish both species. The outer primary crochets on abdominal segments 1–5 are arranged with the tips facing out to the lateral margins of the larva in *E. similis* (Fig. 2E), and facing backwards in *E. tenax* (Fig. 4C). The change in orientation of crochets from pointing mainly backwards at the front end of the body to pointing laterally at the anal end is not related to locomotion so much as to facilitating larvae to grip the bottom substrate when breathing (Rotheray and Gilbert, 1999).

As would be expected, the larva of *E. similis* also shows similarities with other congeneric species. However, close examination revealed slight but obvious differences among them. The morphology of the anterior spiracle (shape of clear area and arrangement of facets) was used as a diagnostic character in Eristalinae by Hartley (1961) and Dolezil (1972). This feature is a useful character to distinguish *E. similis* from the group of species with the clear area not widened in the lower part (Fig. 4B) (*E. tenax*, *E. anthophorina*, *E. rossica*, *E. nemorum*, *E. intricaria*, *E. horticola*, and *E. rupium*). *E. similis* presents the lower part of the clear area widened, encircling less than three-fourths of the spiracle perimeter as occurs in *E. arbustorum* and *E. abusiva* (see Fig. 5D). The species *E. pertinax* also presents the lower part of the clear area widened, but in this case clearly encircling three-fourths of the spiracle perimeter. The differences between the last three species are more difficult to detect. In fact, Dolezil (1971) did not include the larva of *E. abusiva* in his key because he was unable to distinguish it from *E. arbustorum*. The larvae of *E. similis* can be separated from these two species by the shape of the clear area, abruptly narrower above the fold, not gradually tapering to the apex as occurs in *E. arbustorum* (Fig. 5D). This character is visible using light stereomicroscopy, but the shape and the arrangement of facets can be difficult to make out. Ultrastructural analysis overcomes this difficulty and easily reveals the shape of the clear area and the arrangement of facets.

The main diagnostic characters of the *Eristalis* puparium are length, shape, and arrangement of tubercles on pupal spiracles. The most distinctive pupal spiracles occur in *E. tenax*, projecting forward

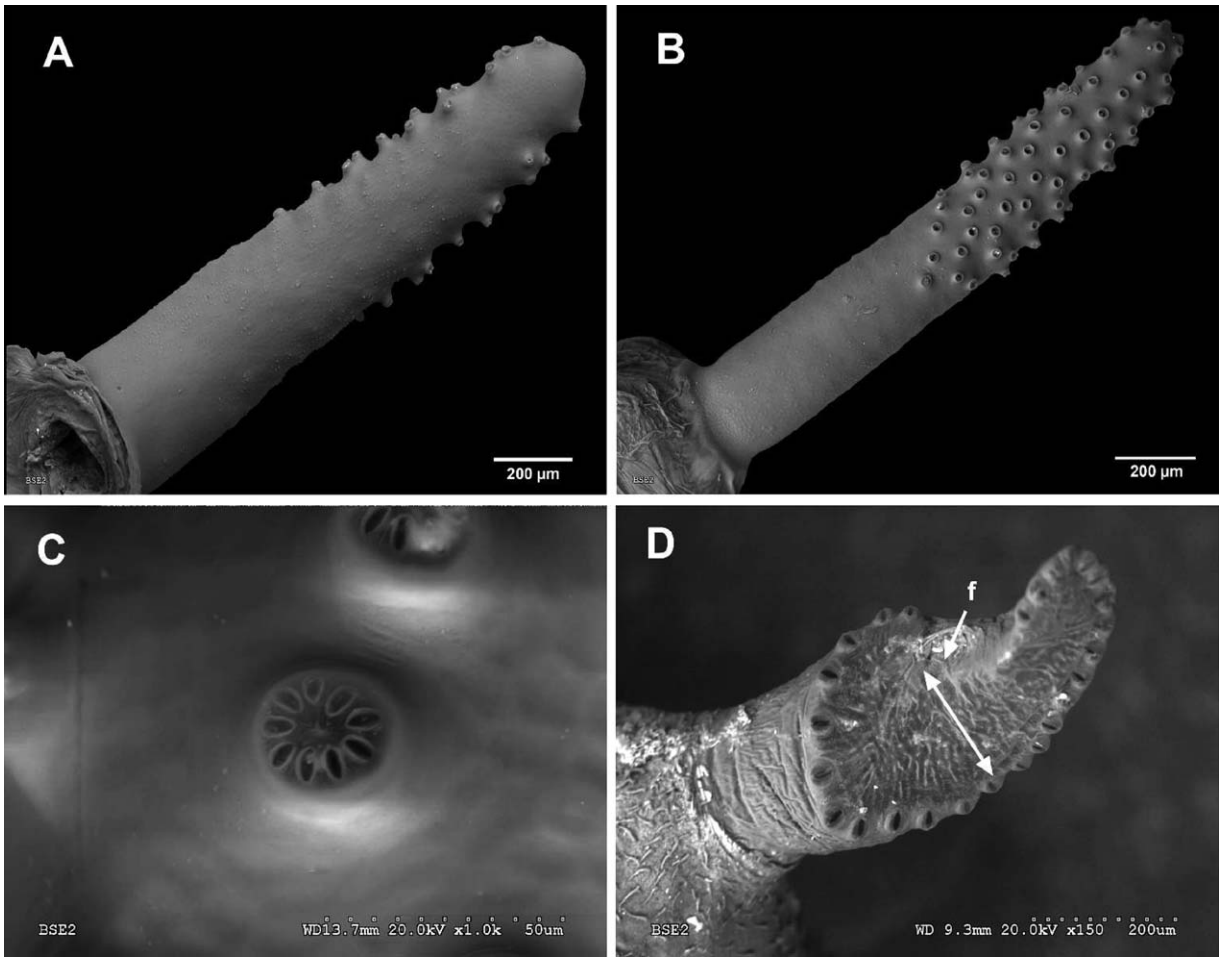

Fig. 5. **A–C**: Scanning electron micrographs of a puparium of *Eristalis tenax*. A: pupal spiracle, ventral view; (**B**) pupal spiracle, dorsal view; (C) spiracular openings of pupal spiracles. **D**: Scanning electron micrographs of anterior spiracles of *Eristalis arbustorum*, antero-dorsal view. Abbreviations: (f) fold of anterior spiracles.

and then bending sharply downward. According to this character, *E. similis* seems to be more closely related to *E. pertinax*, also with very long pupal spiracles (3.5 mm), with about 80% of their length covered by tubercles.

Detailed ultrastructural analysis of cephalic sensorial organs in *E. tenax* larvae showed several satellite sensilla positioned on the antennae (Akent'eva, 2012), apparently innervated with several receptor cells in the cephalic region, suggesting the presence of both mechanoreceptors and chemoreceptors. Both types of sensory receptors are also present in *E. similis* and have been found in predatory hoverflies based on SEM studies (see Ngamo et al., 2002; Rojo et al., 2006). On the other hand, results from the present study show the presence in *Eristalis* of two pairs of sensilla on the dorsal and ventral lips on the head (Figs. 1D–1F). These sensilla correspond to the maxillary sensilla and the labial sense organ, respectively described by Hartley (1961). In fact, the sensilla of the dorsal and ventral lip are homologous to the ventral organs and labial organs that appear in other cyclorrhapha such as *Calliphora* Robineau-Desvoidy (Courtney et al. 2000; Hükesfeld et al., 2010). In predatory hoverfly larvae (Syrphinae), we found one or two pairs of sensilla located above the mouth and below the antennomaxillary organs (see Laska et al., 2006; Rojo et al., 2006) that could be homologous to the maxillary sensilla.

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