

## Surface ultrastructure of the immature stages of eristalines (Diptera: Syrphidae) from Egypt

Samy M Zalal<sup>1</sup> & Mona Ali M Mahmoud<sup>2</sup>\*

1 Department of Zoology, Faculty of Science, Suez Canal University, Ismailia & Taiba University at El-Ola, Saudi Arabia

2 Department of Zoology, Faculty of Science, Suez Canal University, Ismailia

### Abstract

The morphology of the larval and pupal stages of Egyptian eristalines (Diptera: Syrphidae) inhabiting a Gravel Bed Hydroponic system for sewage water treatment were studied using scanning electron microscopy. The egg and all three larval instars of *Eristalodes taeniops* were studied in detail, the egg and 3rd-instar *Lathyrphthalmus aeneus*, the 3rd-instar *Eristalodes megacephalus*, and the 3rd-instar of three other eristalines. The fine structure of the egg chorion can be used to differentiate species. The structure of the integument of the 1st instar is completely different from that of the 2nd and 3rd instars. The lateral, ventral and dorsal lips are coated with special large strong setae supported with many fine spines along their length. Such setae help the larvae to protect themselves from ingesting large particles, preventing mouth blockages. The posterior respiratory process of the 1st instar is not as highly developed as that of 3rd instar, and can be used to recognise different instars. The prothoracic spiracles of both 1st and 2nd are not developed.

**Keywords:** Scanning electron microscopy, morphology, pupa, larva

### Introduction

The Syrphidae (commonly known as hoverflies, a reflection of their mode of flight) comprise one of the largest and most sharply defined families of Diptera. The adults feed on nectar and pollen of flowers, especially of Compositae, and are good pollinators. The larvae exhibit a wide range of feeding habits: saprophagous, phytophagous and predacious, while a few are scavengers in the nests of social insects. Saprophagous larvae live in dung, wet decaying vegetation and wood, and tree sap. There are three larval instars, and the length of the life cycle varies greatly among species, taking less than two weeks in some, and possibly up to five years in others (Gilbert, 1993).

Species of *Eristalis* (subfamily Eristalinae, tribe Eristalini) all have filter-feeding saprophagous larvae, known as rat-tailed maggots from their extensible telescopic posterior respiratory process. This 'tail' enables the larva to descend deep into the water without losing contact with the atmosphere (Hynes, 1960). The normal habitats are evil-smelling ponds and other places where there is much decay, such as pools fouled by cattle dung. They often occur in large numbers along lake shores where the wind has piled up large masses of rotting water weeds. Descriptions of larvae and puparia of Eristalini have been published by a number of authors for non-Egyptian species (e.g. Beling 1888, Becher 1882, Metcalf 1913; Sack 1921, 1931, Kruger 1926, Dunavan 1929, Gäbler 1930, 1932; Weise 1938, Smart 1948, Dixon 1960, Hartley 1961, Maier 1982, Rotheray 1993, Perez-Bañon *et al.* 2003).

Four genera and four subgenera of Eristalinae are represented in Egypt (Mohamed 1975, Shaumar & Mohamed 1975, 1977) but their larval stages are poorly known or unstudied. This paper discusses the life cycle and morphology of different larval and pupal stages of Egyptian *Eristalis* spp obtained from a Gravel Bed Hydroponic system for sewage water treatment.

### Materials & Methods

The Gravel Bed Hydroponic system for sewage water treatment is based in two main sites, the Abu-Attwa station in Ismailia, and a station in the city of Tenth of Ramadan. The system

\* Author for correspondence

consists of a series of inclined gravel-filled channels, each lined with an impermeable membrane and planted with halophytes such as *Phragmites australis* (Dewedar *et al.* 1993). Typically a channel is 100 m long, and 2 m wide, filled with gravel to a depth of 300 mm, and with a bed slope of 1 in 100. After flowing through the 100 m long beds (the treatment phase), the effluent is fed into a crop phase consisting of a series of channels of similar construction to the reed beds, except they are 40 m long, 2 m wide and 150 mm deep, with a gradient of 1 in 50. Two beds were chosen for the collection of samples from Abu-Attwa: bed 4 (planted with *Phragmites australis*) and bed 5 (unplanted control). Both beds are filled with gravel and each fed with sewage water at a flow rate of 20 litres per min (21.6 m<sup>3</sup> per day). Larvae from Tenth of Ramadan were collected wherever they were found.

Larvae were collected twice per month for one year (1998), using a sieve of different mesh sizes: a fine sieve was particularly useful for collecting 1st instars. Larvae were examined with a binocular microscope for identification and for separating different instars and species. Live larvae were cleaned for examination either by putting them on moist filter paper in a Petri dish for two hours, or by using a soft brush (Rotheray, 1993). Larvae were fixed by placing them in boiling water for 5 min. Terminology follows Hartley (1961) and Rotheray (1993); taxonomy follows the suggestions of Perez-Bañón *et al.* (2003).

For taking scanning electron micrographs (SEM), larvae were fixed in 2% (w/v) aqueous osmium tetroxide for 12 h at 4 °C. Fixed material was then dehydrated through a graded ethanol series and then the alcohol substituted with absolute acetone; dehydrated specimens were critical-point dried in a Polaron E3000 apparatus (Biorad, Hemel Hempstead) using liquid CO<sub>2</sub> as a drying agent. Larvae were mounted onto aluminum stubs using double-sided adhesive tape and then coated with a 40-nm layer of gold using a Polaron E 5000 diode sputter-coating unit. All specimens were examined at 20 KV in a JEOL T20 scanning electron microscope.

## Results

### *Eristalodes taeniops* (Wied.)

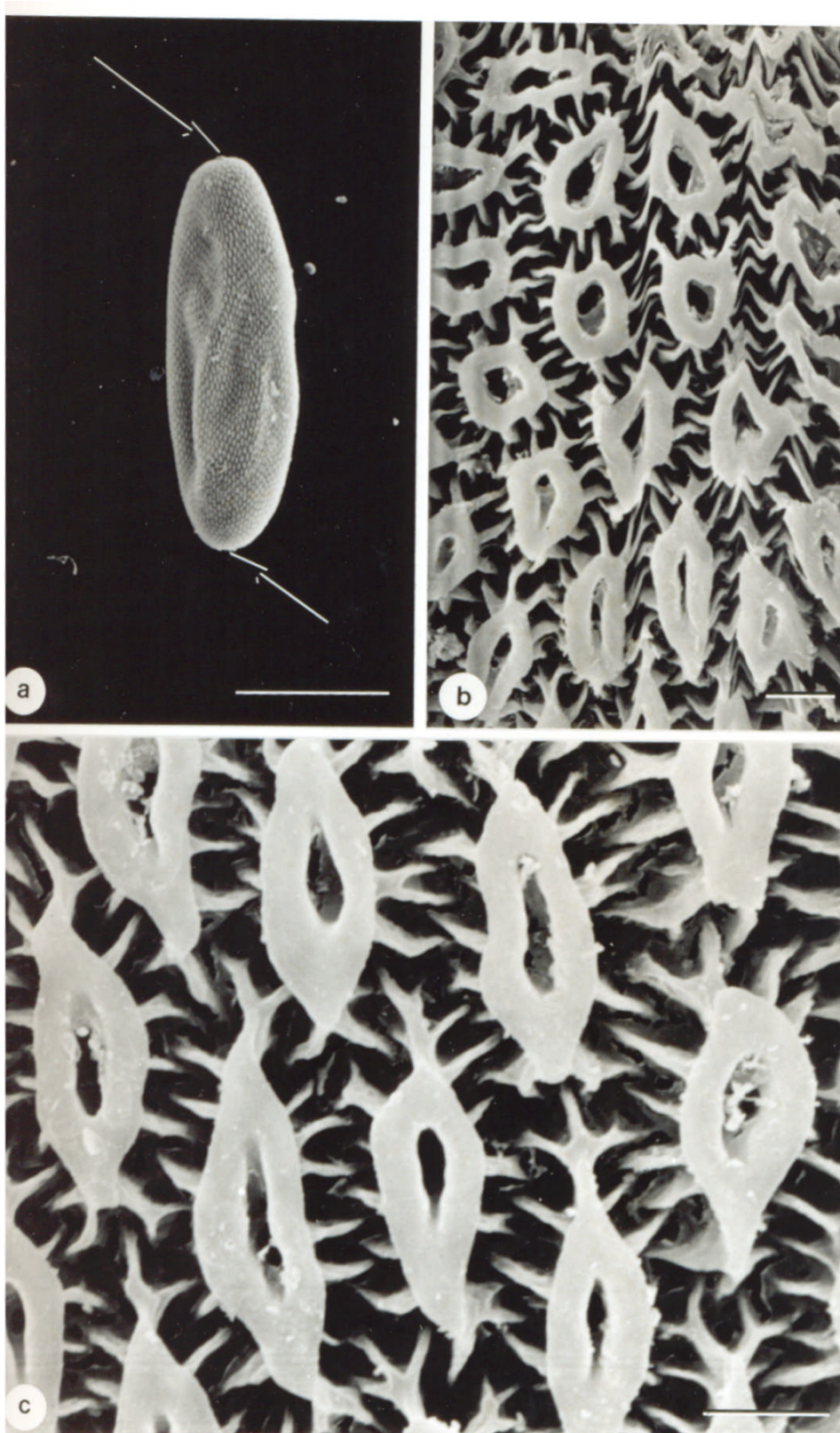
The duration of the egg stage of *Eristalodes taeniops* depends on water temperature and humidity, with increasing values accelerating development and hatching: it took six days to hatch at 15.5 °C. Eggs are difficult to collect directly in the field: the best way to obtain eggs is to catch mature females and encourage them to lay in a Petri dish provided with the sewage-water medium. Mature females are easy to recognize in the field and those which come frequently to the surface of the water are ready for egg-laying. The mean temperature of sewage water in the beds was 23 ± 8 °C. Dissected females had eggs arranged in rows of about three layers in the ovaries: mature females laid about 500 eggs, either singly or in small batches.

Each egg is white with an elongate cylindrical shape, and is 1.2 mm long and 0.4 mm wide (Fig 1a). The surface sculpturing (Fig 1 b,c) is diagnostic; the narrowing of the egg at both poles alters the shape of the sculpturing (compare Figs 1 b,c).

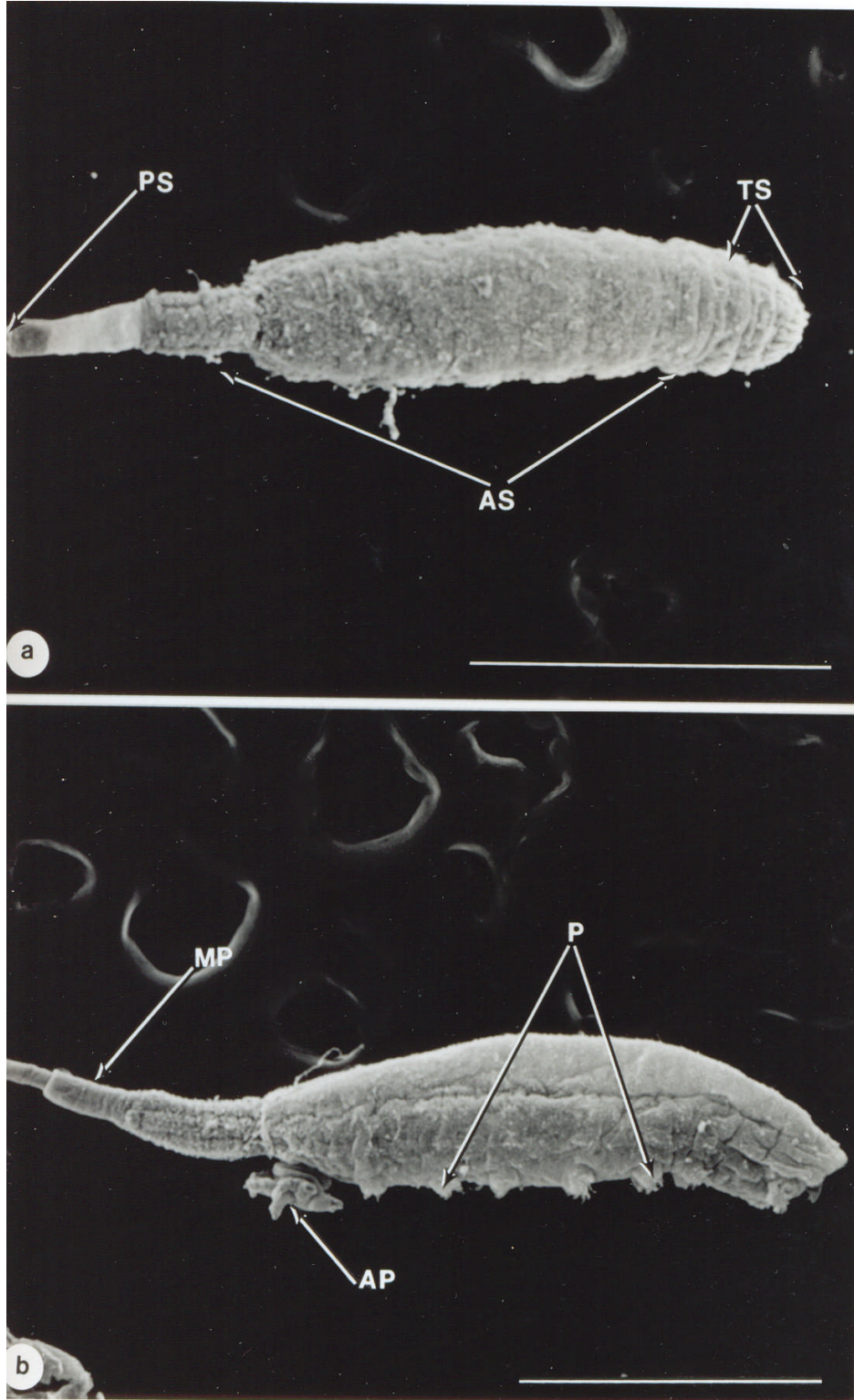
The 1st instar lasts about 10 days, and is white, semitransparent and approximately cylindrical: excluding the tail, the body is 1.1-2.0 mm long, and 0.4-0.5 mm wide. The long telescopic breathing tube can be extended several times the body length (Fig 2). The 1st instar can be distinguished from the other two instars by its characteristic type of sensillum distributed throughout the cuticle (Fig 3), consisting of a central long seta surrounded by numerous shorter ones. This is the only type of sensillum found throughout the whole body, including those on the lappets, the fleshy projections of the anal segment (Fig 3 d). The posterior respiratory process of the 1st instar at the tip of the breathing tube is surrounded by a four pairs of leaf-like setae on the periphery of the spiracular disc. These leaf-like structures comprise two short and two long pairs (Fig 4) with articulated bases: they are strongly

hydrophobic. There are six to eight straight slits on the spiracular disc, which has no spiracular scars (Fig 4 b).

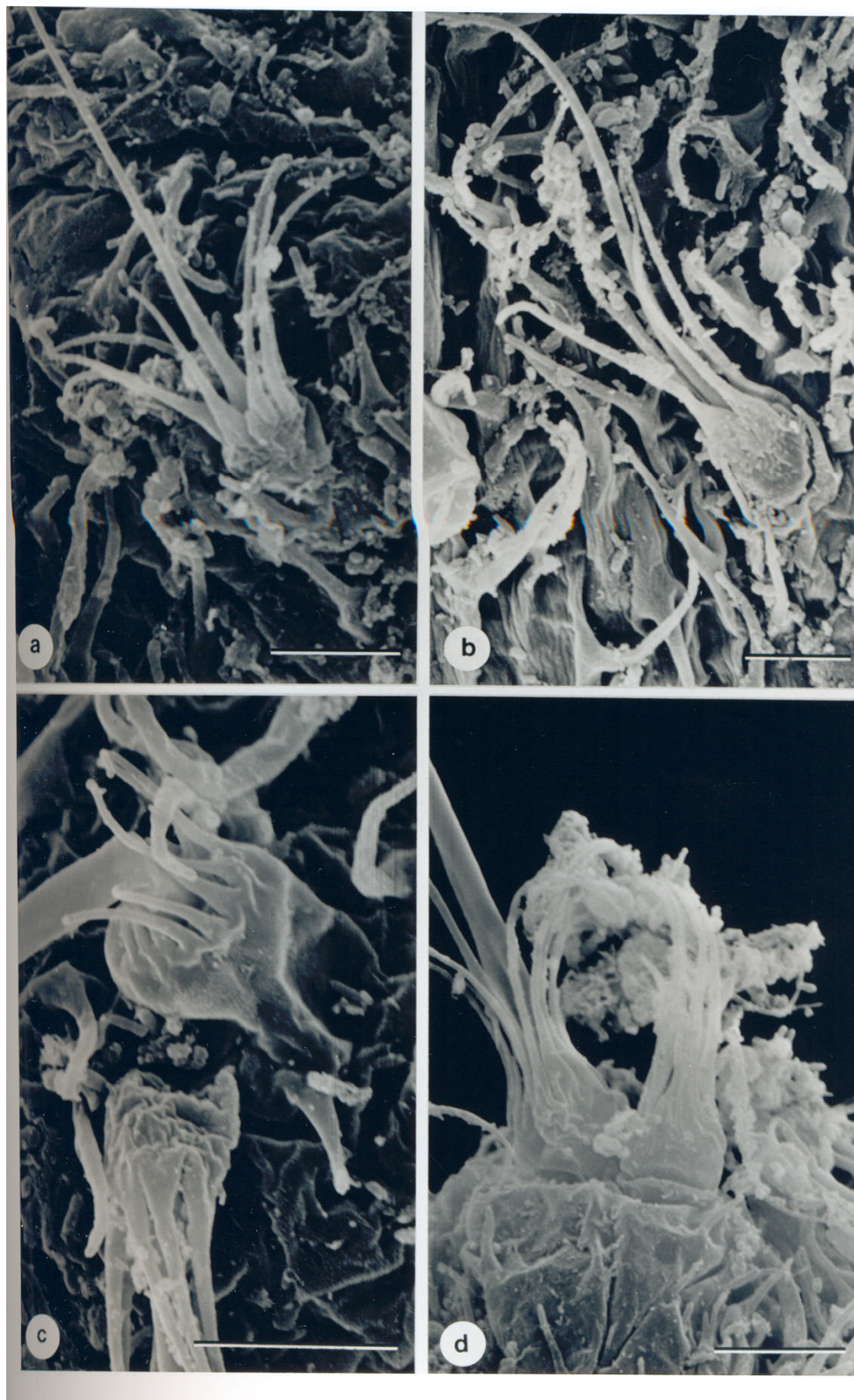
**Fig 1:** SEMs of the egg stage of *Eristalodes taeniops*: (a) Entire egg, arrows refer to the poles, bar = 500  $\mu\text{m}$ ; (b & c) higher magnification, showing surface texture at the pole (b) and mid-region (c), bar = 10  $\mu\text{m}$ . Note difference in shape as well as size of sculpture.



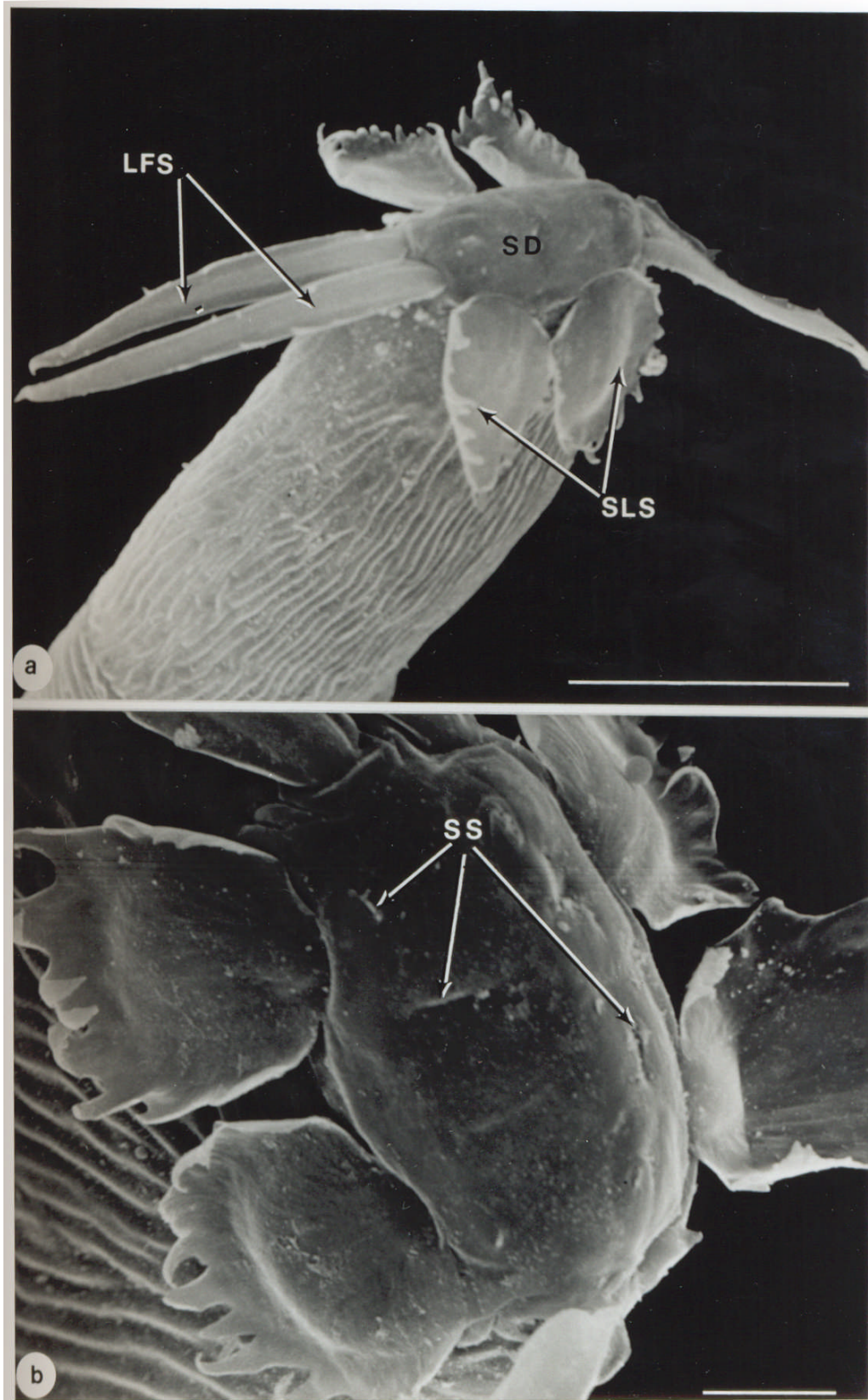
**Fig 2:** SEMs of 1st larvae instar of *Eristalodes taeniops*. (a) dorsal surface, showing three thoracic (TS) and eight abdominal segments (AS), the eighth extended to form a telescopic tube (the posterior respiratory process) bearing the posterior spiracles (PS); (b) lateral view, showing the prolegs (P), the anal papillae (AP) on the ventral side, and the central section (MP) of the telescopic tube. Bar = 1 mm



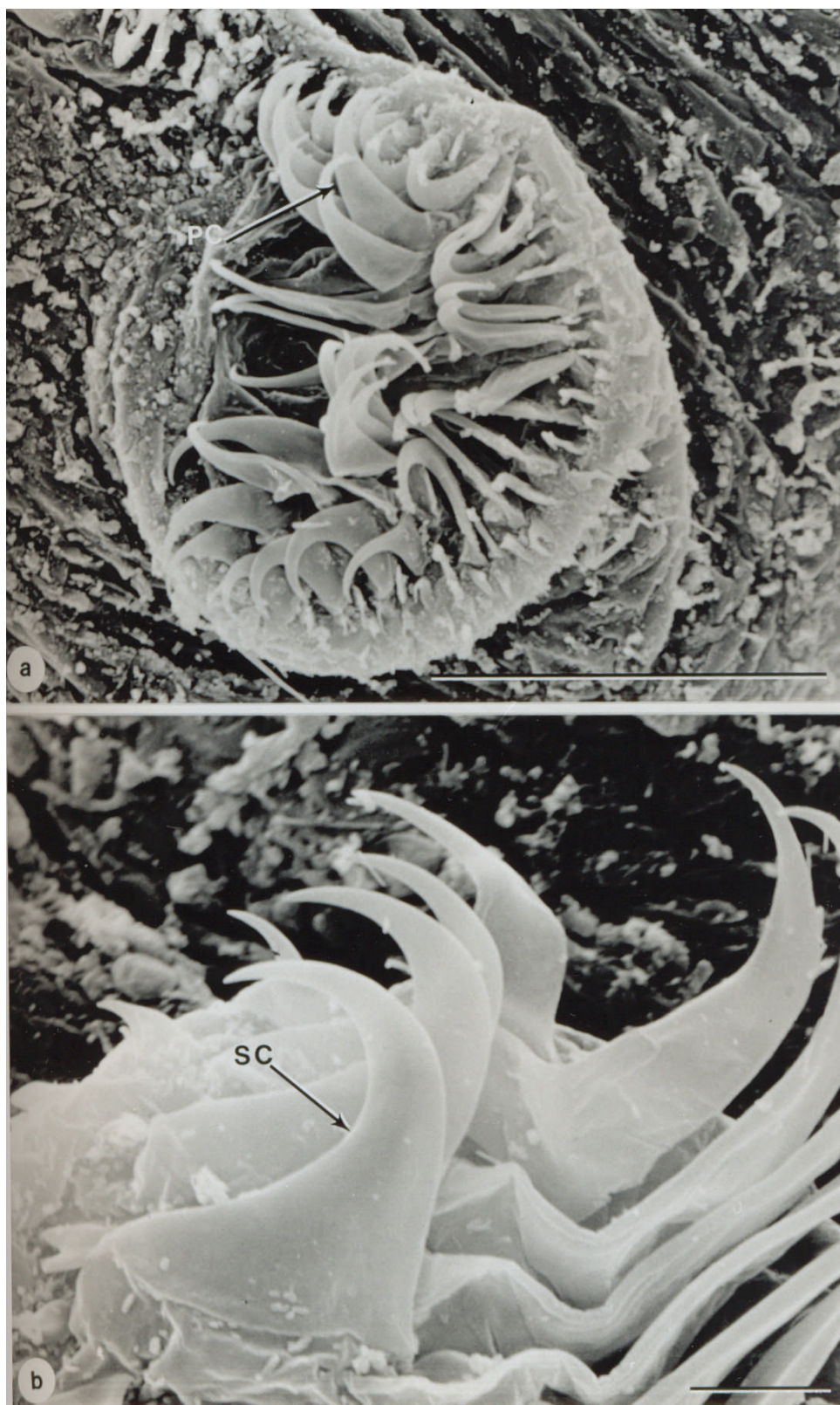
**Fig 3:** SEMs of four sensilla (a - d) on the integument of 1st-instar larva of *Eristalodes taeniops* from all over the body surface. Note that although there is a variation in the length of the setae, each sensillum comprises a long central seta surrounded by many shorter ones. All are sessile, i.e. not borne on papillae. Bar = 10  $\mu$ m



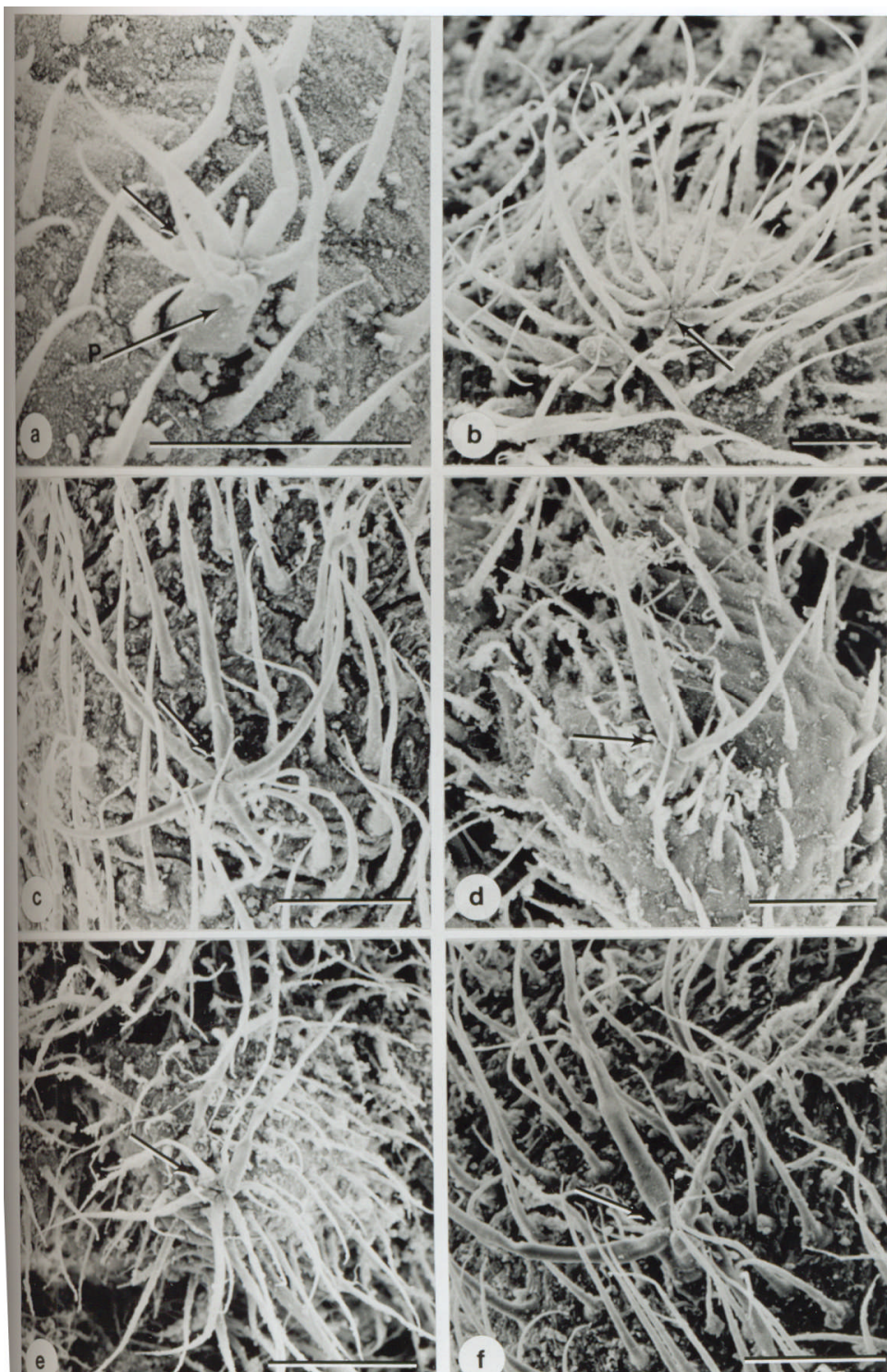
**Fig 4:** SEMs of the respiratory process of the 1st-instar larva of *Eristalodes taeniops*: (a) posterior respiratory spiracles at the tip, with spiracular disc (SD) and four pairs of hydrophobic setae at the outer margin, two short leaf-like setae (SLS) and two outer long feather-like setae (LFS), bar = 50  $\mu\text{m}$ ; (b) higher magnification showing the spiracular disc with eight spiracular slits (SS) on its surface, bar = 10  $\mu\text{m}$



**Fig 5:** SEMs of prolegs of 2nd- instar *Eristalodes taeniops*: (a) prolegs with crochets of different lengths, the longest being the primary crochets (PC), bar = 100  $\mu\text{m}$ ; (b) the secondary crochets (SC) are shorter and stouter than the primary crochets (PC), bar = 10  $\mu\text{m}$ .

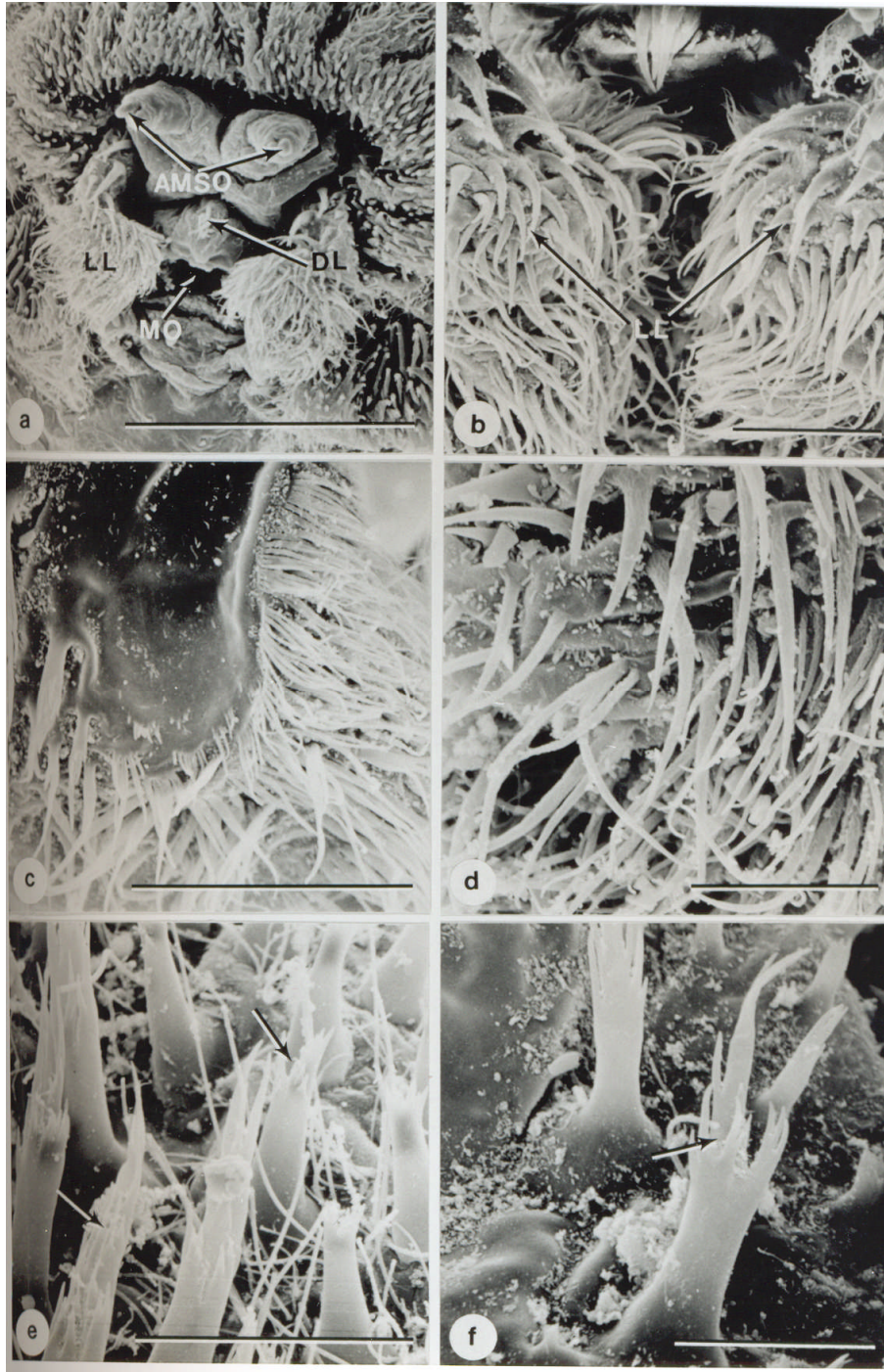


**Fig 6:** SEMs of the segmental sensilla of the 3rd-instar larva of *Eristalodes taeniops*: (a) a dorsal sensillum (arrow); note it is borne on a papilla (P); bar = 100  $\mu$ m; (b-f): different forms of lateral and posterior sensilla (arrowed) with varying numbers of long and short setae; bars (b, d, f) = 100  $\mu$ m, (e) = 250  $\mu$ m

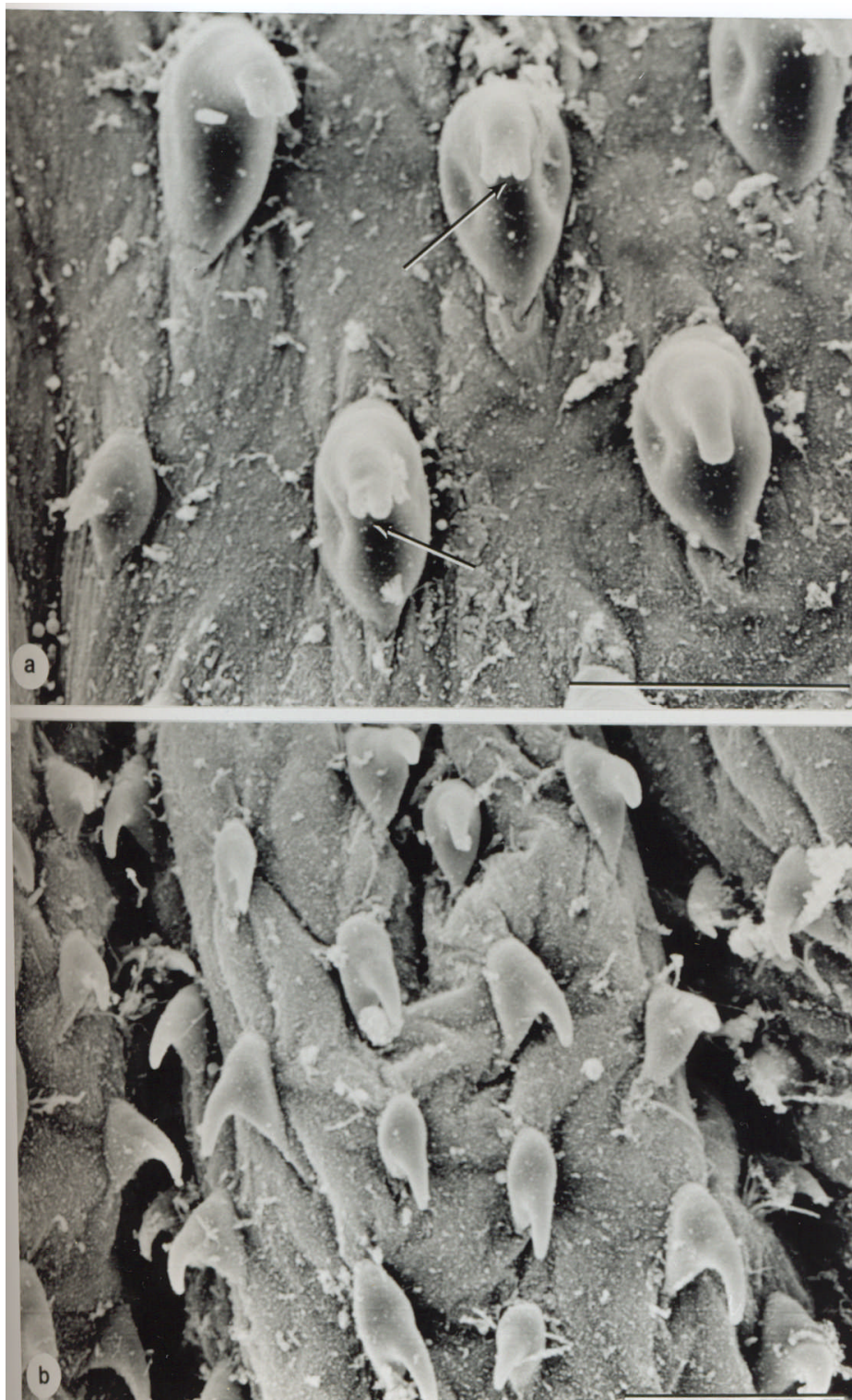




**Fig 7:** SEMs of mouthparts of 3rd-instar larva of *Eristalodes taeniops*: (a) ventral view showing mouth opening (MO), lateral lips (LL), dorsal lips (DL) and antenna-maxillary sense organ (AMSO); bar = 500  $\mu$ m; (b) lateral lips (LL); bar = 200  $\mu$ m; (c) inner side of lateral lips; bar = 100  $\mu$ m; (d) higher magnification of lateral lips (LL); note the long thin setae surrounding them; bar = 50  $\mu$ m; (e) a group of stout, highly sclerotised setae which terminate in fine spines (arrowed), situated on each side of the mouth opening; bar = 100  $\mu$ m; (f) fine spines (arrow) on the sclerotized setae; bar = 100  $\mu$ m.



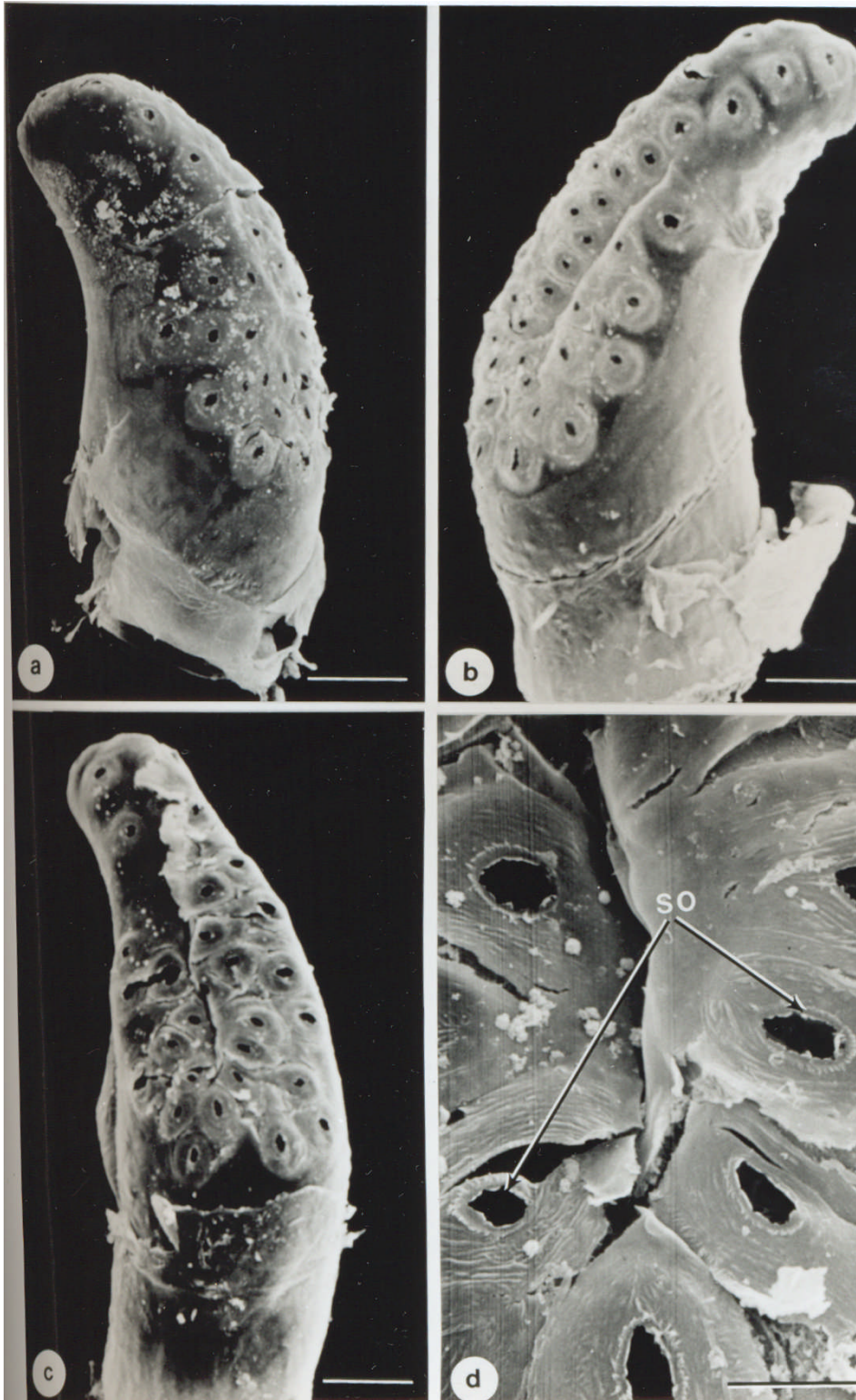
**Fig 8:** SEMs of front margin of the prothorax of 3rd-instar larva of *Eristalodes taeniops*: (a) a group of short, stout recurved spinules; note the tips (arrowed) bearing 2 or 3 digits; (b) a group of short, hooked non-digitate setae. Bar = 50  $\mu$ m



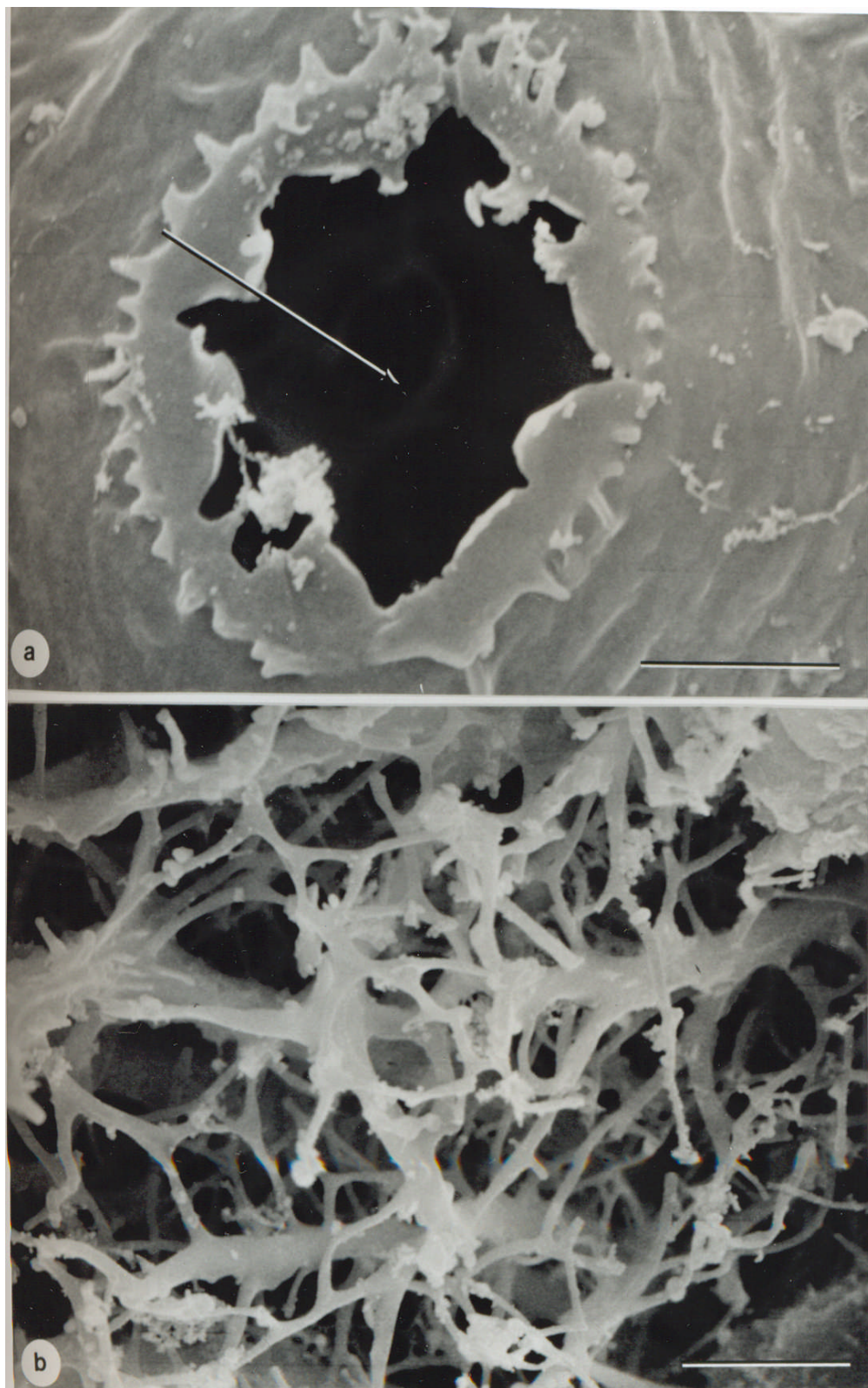
**Fig 9:** SEMs of the prothoracic spiracle of 3rd-instar larva of *Eristalodes taeniops*: note the anterior spiracle (AS) protected by a sheath (arrowed). Bar = 100  $\mu$ m.



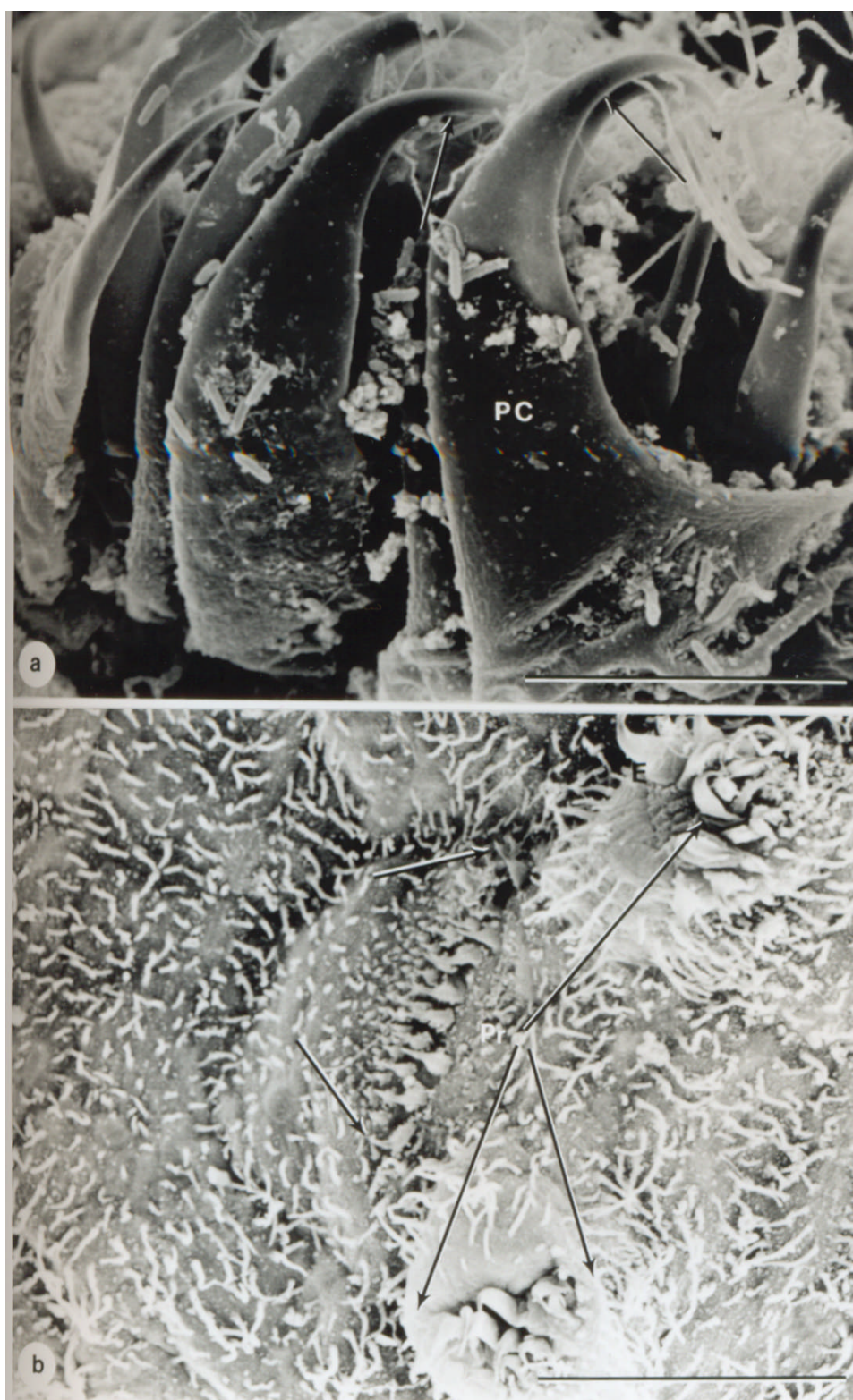
**Fig 10:** SEMs of the structure of the prothoracic spiracles of 3rd-instar larva of *Eristalodes taeniops*: (a, b) lateral views; bar = 100  $\mu\text{m}$ ; (c) ventral view; bar = 100  $\mu\text{m}$ ; (d) higher magnification, showing the arrangement of the spiracular openings (SO); bar = 25  $\mu\text{m}$ .



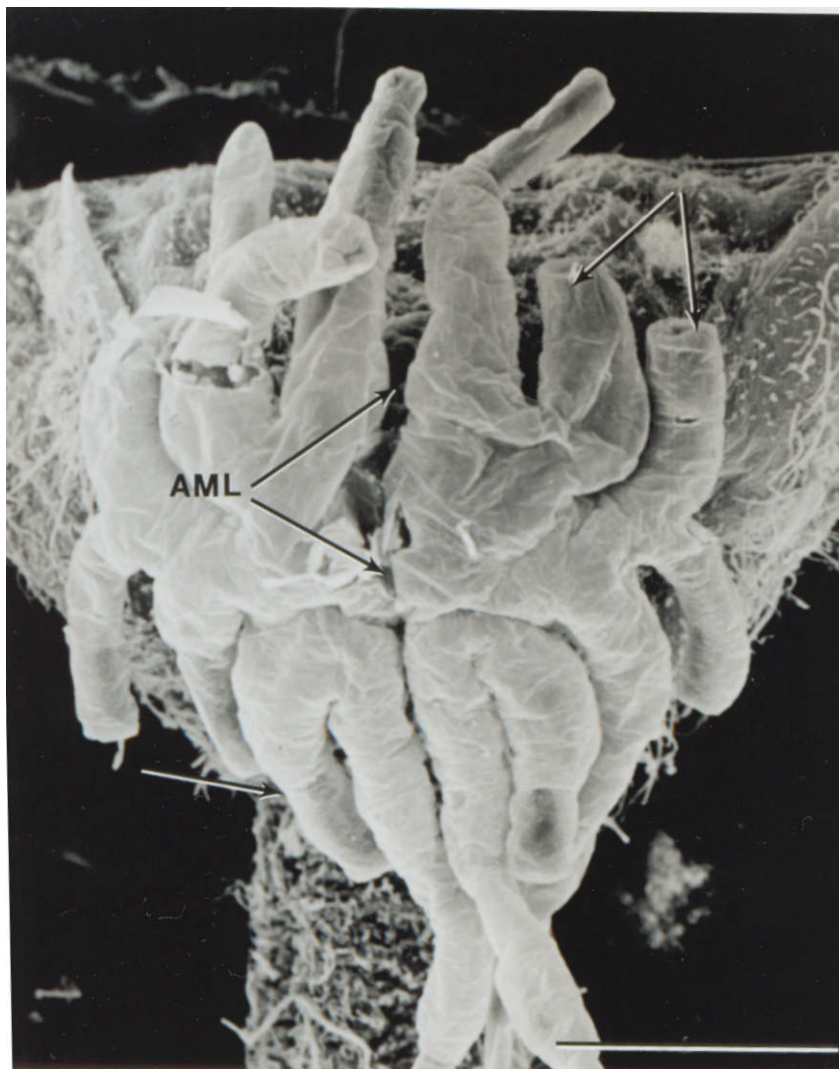
**Fig 11:** SEMs of the opening of the prothoracic spiracle of the 3rd-instar larva of *Eristalodes taeniops*: (a) the spiracular opening (arrowed) surrounded by a crenate thickening of the cuticle; bar = 5  $\mu\text{m}$ ; (b) network lining the interior of the spiracle; bar = 10  $\mu\text{m}$ .



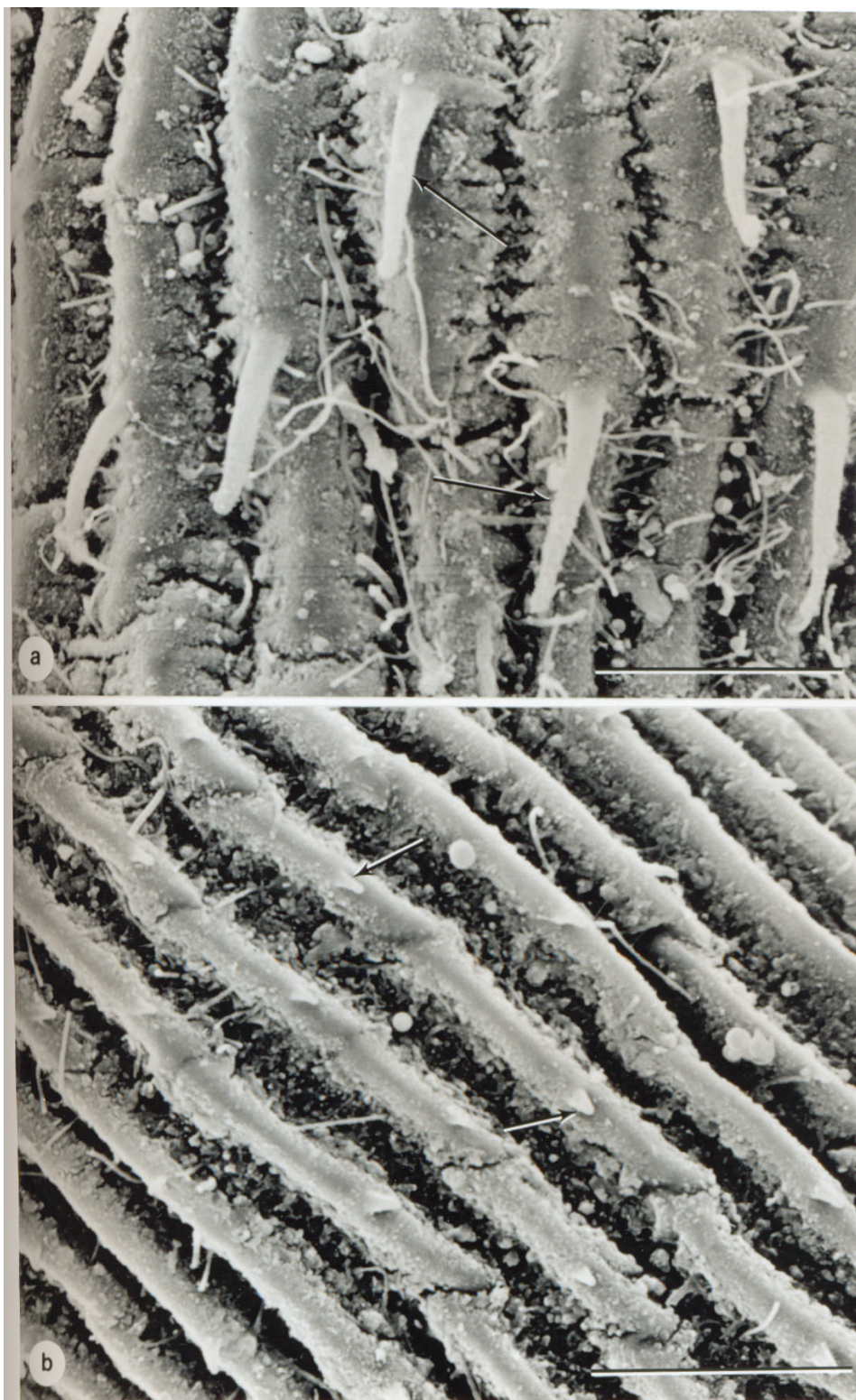
**Fig 12:** SEMs of crochets on the ventral surface of 3rd-instar larva of *Eristalodes taeniops*: (a) primary crochets (PC) on the prolegs, showing highly sclerotized curved tips (arrowed); bar = 100  $\mu$ m; (b) row (arrowed) of eight anteriorly directed, crochet-like spines just anterior to the sixth abdominal prolegs (Pr); bar = 500  $\mu$ m.



**Fig 13:** SEM of the anal papillae of 3rd-instar larva of *Eristalodes taeniops*: the tubes (arrowed) comprising the anal papillae are arranged on either side of the anterior mid-line (AML). Bar = 200  $\mu$ m.

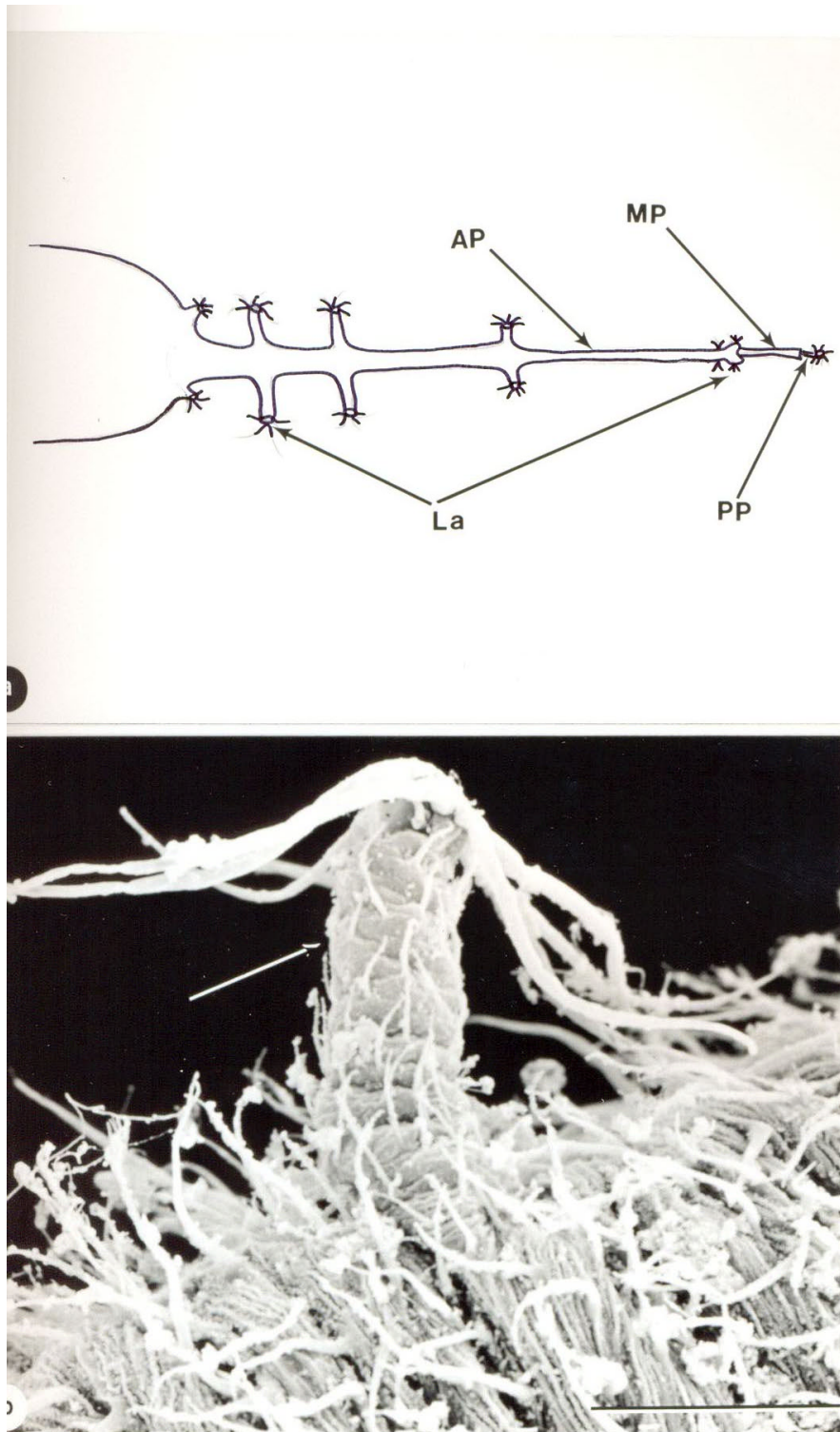


**Fig 14:** SEMs of the central section of the breathing tube of 3rd-instar larva of *Eristalodes taeniops*: (a) proximal region, with long setae (arrowed); (b) distal region, with short setae (arrowed). Note the longitudinal striations. Bars = 50  $\mu$ m

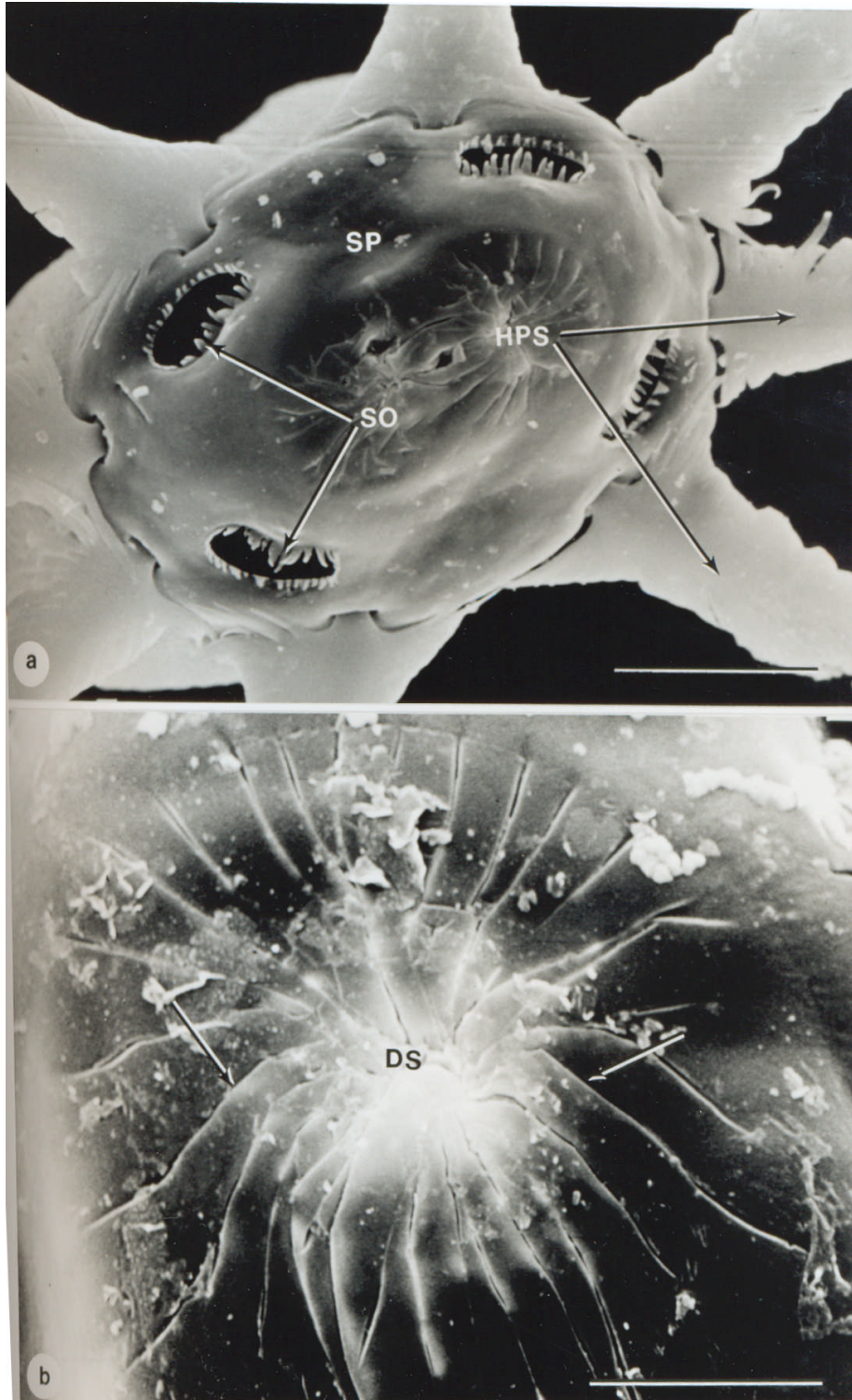




**Fig 15:** 3rd-instar larva of *Eristalodes taeniops*: (a) Diagram of the last abdominal segment (ventral surface) with fleshy projections, the lappets (La), bearing sensillae; AP anterior part of the segment, MP middle part, and PP posterior part; (b) SEM of anal segment; lappet (arrowed) with sensillae on one side of the anal segment; bar = 200  $\mu$ m



**Fig 16:** SEMs of posterior respiratory process of 3rd-instar larva of *Eristalodes taeniops*: (a) the posterior spiracles; note the four spiracular openings (SO) at the edge of the spiracular plate (SP), and the two dorsal scars in the centre; at the periphery of the spiracular plate are four pairs of hydrophobic plumose setae (HPS); note the way each seta is attached to the spiracular plate; bar = 50  $\mu$ m; (b) dorsal scar (DS) with pattern on its surface (arrowed); bar = 20  $\mu$ m



**Fig 17:** SEMs of the pupal spiracles of *Eristalodes taeniops*: (a) dorsal surface (DS), showing numerous openings (facets - F) covering the distal two-thirds; bar = 500  $\mu$ m; (b) lateral view with arrow indicating ventral surface; proximal (Pr) and distal (Di) regions; bar = 500  $\mu$ m; (c) texture of ventral surface showing regular depressions each with a seta (S); bar = 5  $\mu$ m; (d) spiracular openings (SO) borne on slightly raised regions of the cuticle; bar = 5  $\mu$ m

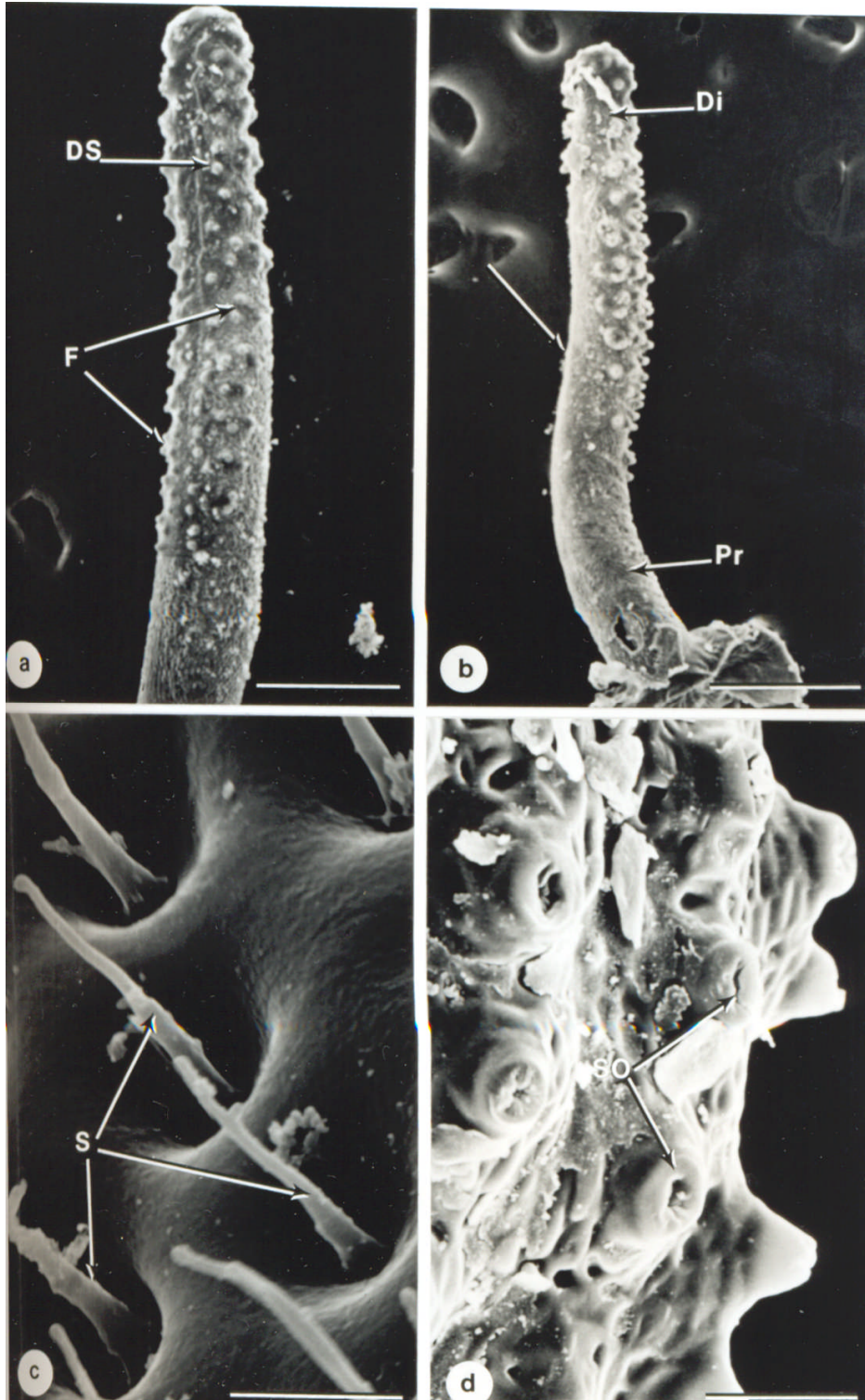
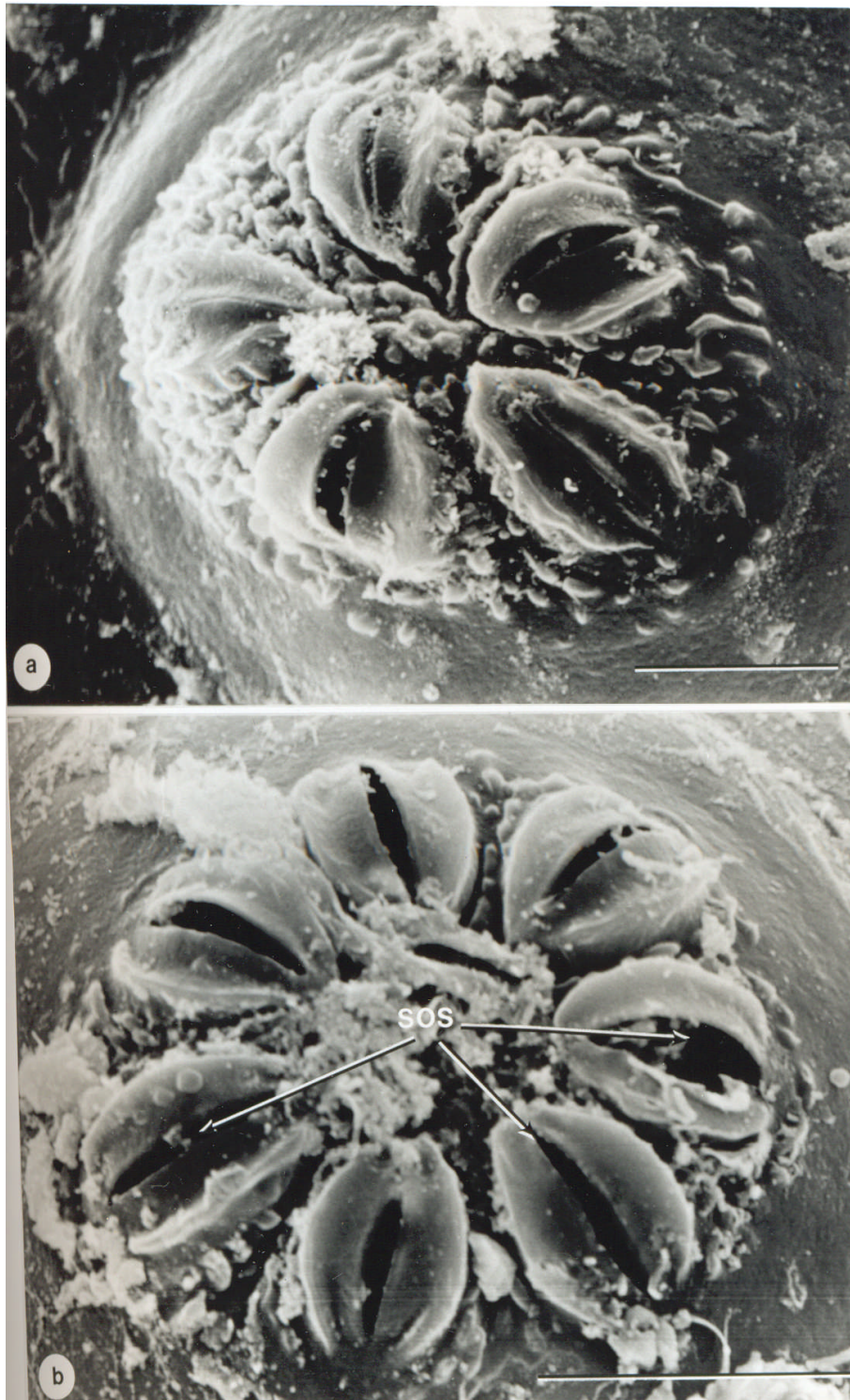


Fig 18: SEM of pupal spiracular openings in *Eristalodes taeniops*: (a) one with five slits; bar = 5  $\mu\text{m}$ ; (b) one with eight slits; bar = 10  $\mu\text{m}$ .



2nd instars are similar in structure to the 3rd except they are smaller and lack the anterior respiratory spiracles found only in the 3rd instar. This is an important feature to distinguish between 2nd and 3rd instars morphologically. Fig 5 a,b shows the primary and secondary crochets on the prolegs of the 2nd instar.

3rd-instar larvae are cylindrical, about 19-20 mm long and 4 mm wide. The cuticle is generally unpigmented and transparent, although in some larvae it becomes whitish and opaque. It is covered with setae which are longer on the lateral and posterior parts of the body. There is a segmental arrangement of sensilla, and each consists of one or more setae. The number of setae per sensillum is not constant (Fig 6 a,f) among individuals within species. A typical abdominal segment has nine to ten sensilla. The sensilla tend to be borne on papillae (Fig 6 a), but are sometimes sessile (Fig 6 b).

The reduced head is represented only by the pair of bilobed antenna-maxillae above the mouth and the connecting cuticle. There is a fleshy lobe just above the mouth and below the antenna-maxillae, known as the dorsal lip (Fig 7 a). On the ventral prothorax there is a lobe-like fleshy structure on either side of the mouth forming a pair of lateral lips (Fig 7 b). These lateral lips are coated in specialised setae of two kinds, one made up of long, thin smooth hairs on the outer surface (Fig 7 d), while the other consists of thick, highly sclerotized hairs with very fine teeth in different layers along their length (Fig 7 e, f).

The anterior surface of the thorax is folded inwards to meet the cuticle of the head, and bears two rows of large, stout, recurved spinules. The tip of each spinule is digitate, with two or three fine teeth. Next to these are groups of slightly shorter, non-digitate spinules (Fig 8).

The 3rd instar has two sets of respiratory organs: the anterior and posterior spiracles. The anterior spiracles appear as a pair of brown structures on either side of the prothorax, dorso-lateral near the posterior edge. They are recessed into specialized pockets (Fig 9), and can be retracted by a series of small protractor muscles. They are elongate (0.5-0.6 mm) and have numerous (32) facets (Fig 10), each facet having a single opening slit (Fig 11 a). The interior is lined with a fine network (Fig 11 b).

There is a pair of prolegs on the mesothorax and on each of the first six abdominal segments. The prolegs bear claw-like spines or crochets, which differ slightly from one species to another. They are arranged in several rows, the primary crochets being the largest and longest (Fig 12 a). The secondary crochets of the subsequent rows are stouter and shorter, while subsequent rows have distinctly smaller crochets. Crochets usually point backwards and slightly outwards. Anterior to the sixth abdominal prolegs, there is a row of anteriorly directed, small, crochet-like spines, about 8-9 in number (Fig 12 b).

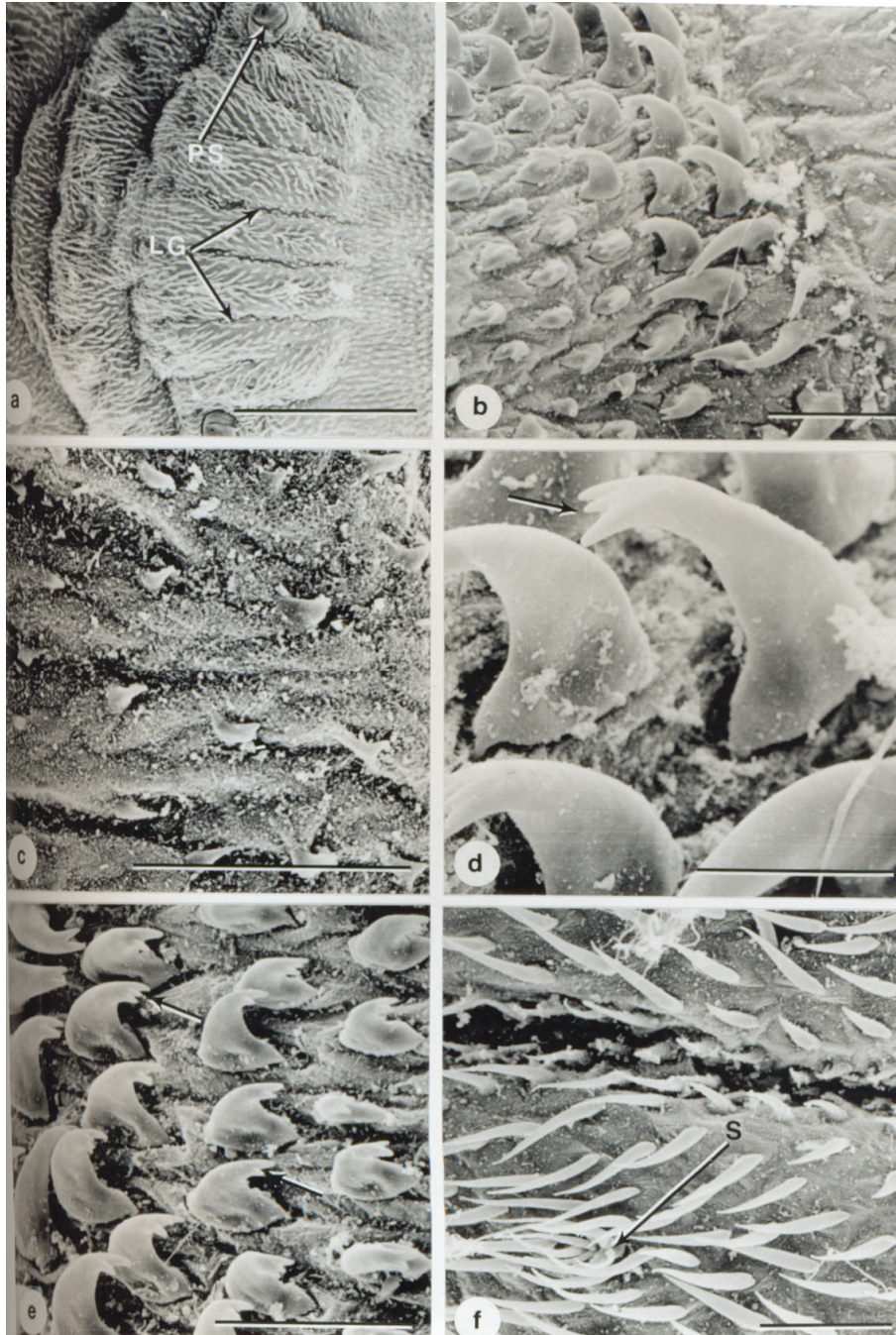
On the ventral surface of the last segment of the body, there are a number of reversible tubes which make up the anal papillae: they can be protruded through the anal fold. In their retracted position, the tubes lie in the body cavity alongside the hind-gut and open into the perianal cavity. They are retracted by a series of muscles and protruded by blood pressure. Each tube has a trachea running along its length, and the papillae are bilaterally symmetrical, and are described with a set of numbers representing the number of tubes with a common tracheal supply, starting from the anterior mid-line: *Eristalodes taeniops* has the formula 2:3:2 on each side (Fig 13).

The anal segment extends to form a telescopic tube consisting of three parts: the anterior and posterior parts have circular striations, while the middle part is longitudinally striated (Fig 14). There is a fleshy projection of the anal segment which bears four long pairs of sensilla (Fig 15). At the tip the posterior spiracles comprise a spiracular plate bearing two circular pits (the dorsal scars) at the centre, surrounded by four rounded spiracular openings near the margin (Fig 16 a). There are four pairs of hydrophobic setae on the periphery of the disc, and each dorsal scar has a reticulate pattern on its surface (Fig 16 b).

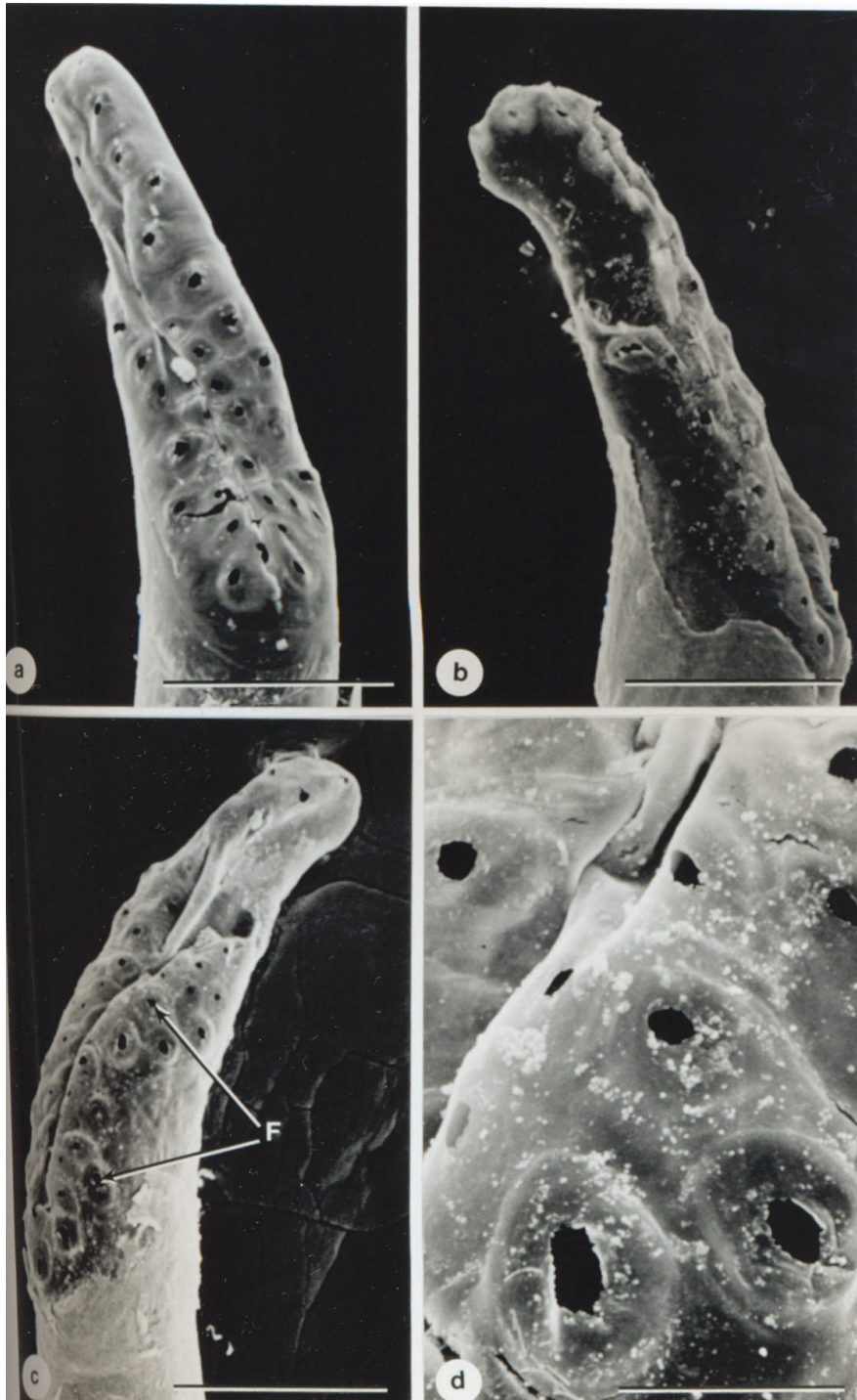
The pupa is formed within the cuticle of the 3rd larval instar, which is modified to form a puparium. The pupal stage of this species lasts about  $13 \pm 3$  days before eclosion. The pupa is dark in colour, 11-13 mm long and 3-9 mm wide. The pupal spiracles push out through the puparium, through circular patches on the dorsum of the first abdominal segment. Each is about 2.0-2.4 mm long (Fig 17 a, b) and bears a number of spiracular openings, each of which

consists of a number of spiracular slits born on a short base (Fig 17 d): these slits increase in number from the proximal (3) to the distal part (8) of the pupal spiracle (Fig 18).

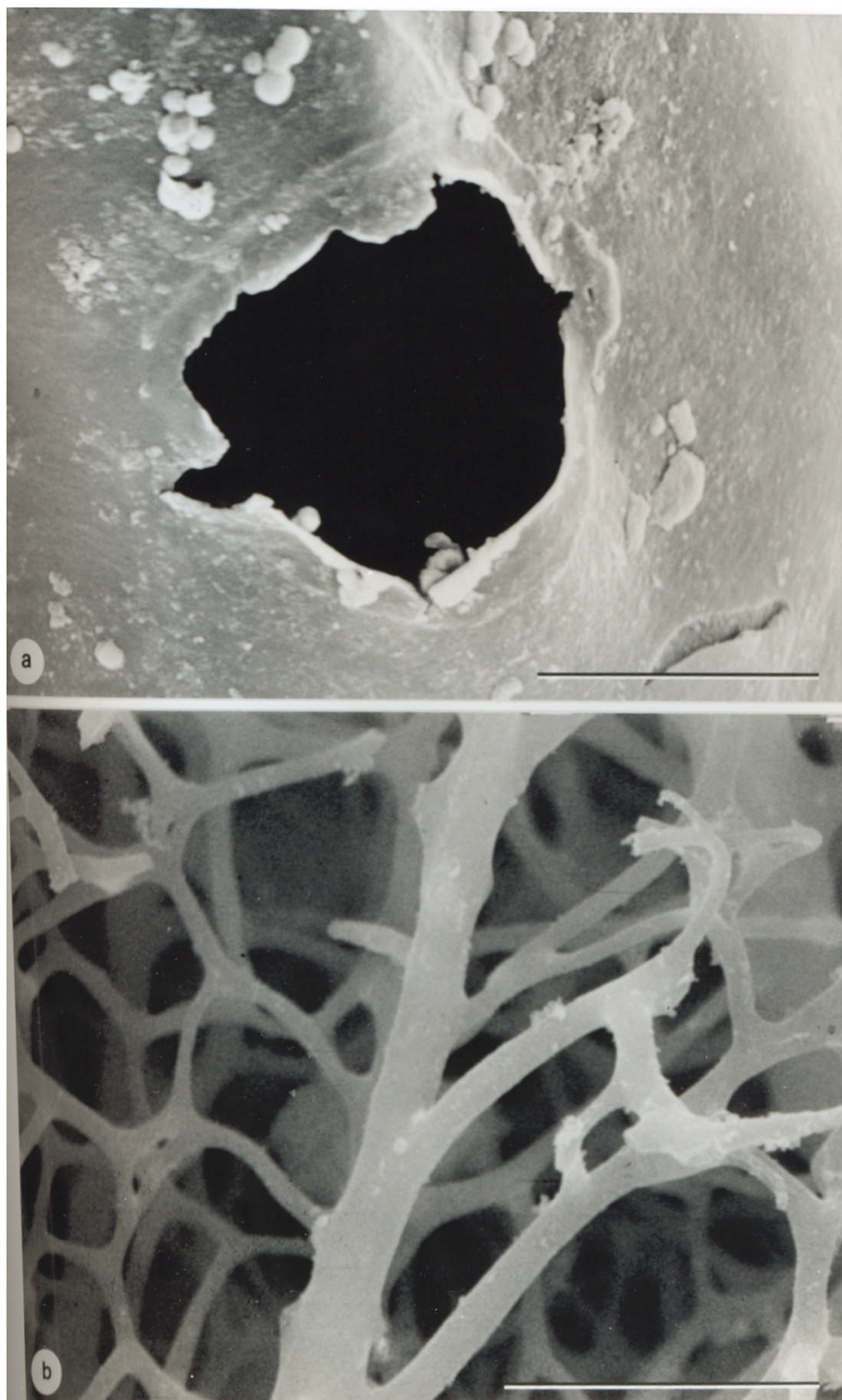
**Fig 19:** SEMs of the prothorax of the 3rd larval instar of *Eristalodes megacephalus*: (a) dorsal surface of prothorax, with the two prothoracic spiracles (PS) separated by 8-9 longitudinal grooves (LG); bar = 1 mm; (b) front margin of the prothorax showing groups of morphologically different setae; bar = 100  $\mu$ m; (c) short, immature, anteriorly directed setae; bar = 100  $\mu$ m; (d) two rows of 2-3 digitate (arrowed) setae, recurved, and highly sclerotized; bar = 50  $\mu$ m; (e) stout, backwardly directed setae with digitate tips (arrowed); bar = 100  $\mu$ m; (f) long hair-like setae covering the longitudinal grooves of the prothorax and body; note the sensilla (S); bar = 100  $\mu$ m



**Fig 20:** SEMs of prothoracic spiracles of 3rd larval instar of *Eristalodes megacephalus*: (a) ventral surface of spiracle; (b, c) lateral surface, showing the spiracular opening and the facets (F); bar = 250  $\mu$ m; (d) higher magnification of (a); bar = 50  $\mu$ m

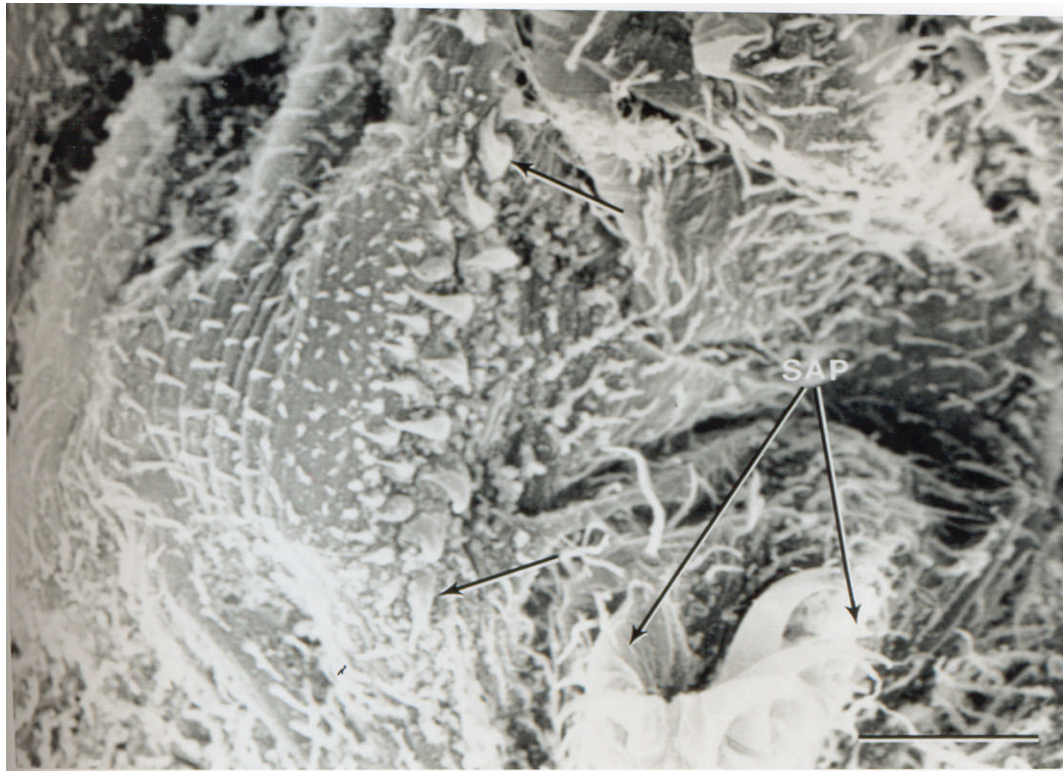


**Fig 21:** SEMs of the prothoracic spiracles of 3rd-instar larvae of *Eristalodes megacephalus*: (a) spiracular opening; (b) network lining interior of the spiracle. Bar = 10  $\mu$ m.





**Fig 22:** SEM of ventral surface of 3rd-instar larva of *Eristalodes megacephalus*: row (arrowed) of 9 anteriorly directed crochet-like spines just in front of sixth abdominal prolegs (SAP). Bar = 25  $\mu$ m

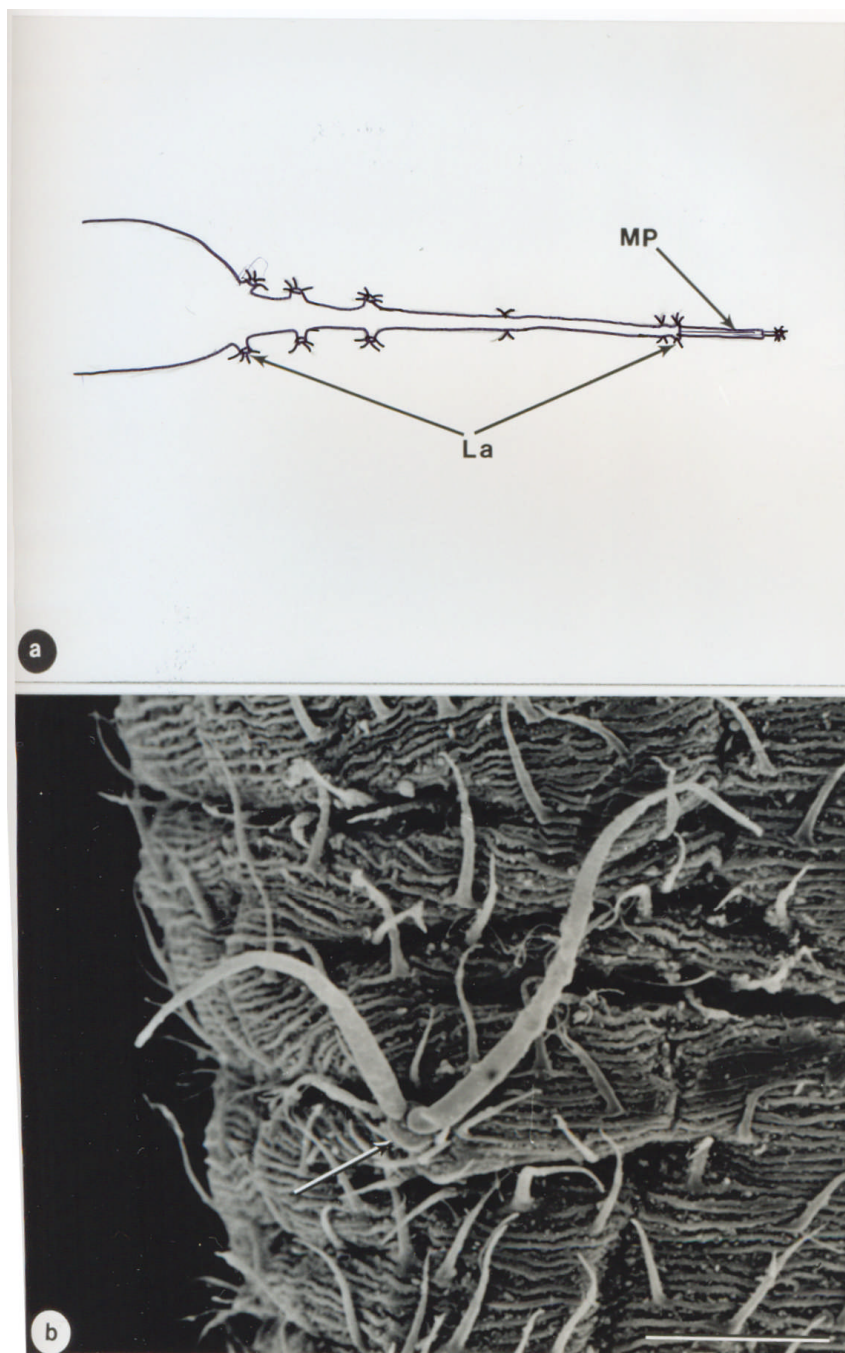


***Eristalodes megacephalus* (Rossi)**

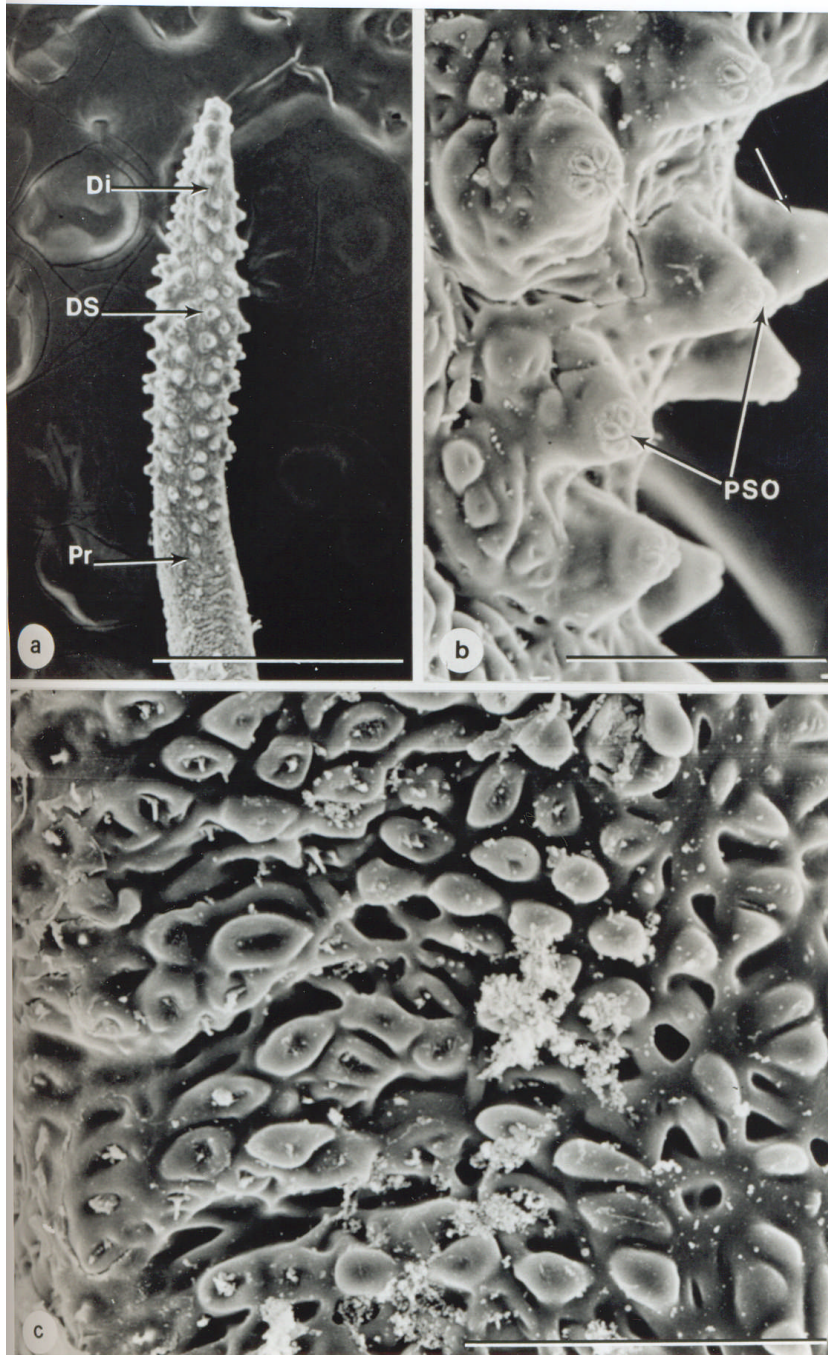
The life cycle of this species is similar to *E. taeniops*; adults are found in Abu-Attwa but are more dominant in the Tenth of Ramadan site. 1st and 2nd larval instars are very similar to those of other species and it was very difficult to separate them. The 3rd instar is about 12-14 mm long and 3-4 mm wide. The cuticle is transparent, unpigmented and covered with setae of different sizes and shape, as in *E. taeniops* (see Fig 6). The front margin of the prothorax bears groups of hooked setae (Fig 19): first there is a group of short, anteriorly directed setae (Fig 19c), followed by two rows of long setae with digitate tips (Fig 19 d), and behind them there is a group of stouter, backwardly directed short setae (Fig 19 e). Very thin long setae cover the longitudinal grooves on the prothorax (Fig 19 f).

The prothoracic spiracles are about 0.7-0.75 mm in length (Fig 20), and contain about 35 facets. The spiracular opening (Fig 21) has an irregular margin. The posterior spiracles have the same structure as in *E. taeniops* (see Fig 16). The 8-9 crochets on the prolegs are thin and elongated. There are about 8-9 crochet-like spines just anterior to the sixth abdominal prolegs (Fig 22). The fleshy projections of the anal segment are shorter than in *E. taeniops* (Fig 23). The puparium of *E. megacephalus* is brown, and about 10-12 mm long and 3.5-4.0 mm wide. The pupal spiracles are dark, slightly thinner and longer than in *E. taeniops*, and about 0.5-0.6 mm long. The openings of the pupal spiracles comprise a number of spiracular slits (4-8) born on the long base (Fig 24). The pupal stage lasts for  $5 \pm 1$  days at room temperature.

**Fig 23:** Anal segment of 3rd-instar larva of *Eristalodes megacephalus*: (a) diagram of the ventral 8th abdominal segment; note the lappets (La); MP middle section of telescopic breathing tube; (b) SEM of lappet (arrowed) with sensilla. Bar = 100  $\mu$ m



**Fig 24:** SEMs of pupal spiracles of *Eristalodes megacephalus*: (a) dorsal surface (DS), showing the spiracular opening on the distal (Di) two-thirds; Pr = proximal region; bar = 250  $\mu\text{m}$ ; (b) pupal spiracular openings (PSO), each raised on a slightly long shaft (arrowed); bar = 100  $\mu\text{m}$ ; (c) texture of the ventral surface of the spiracle; bar = 100  $\mu\text{m}$ .

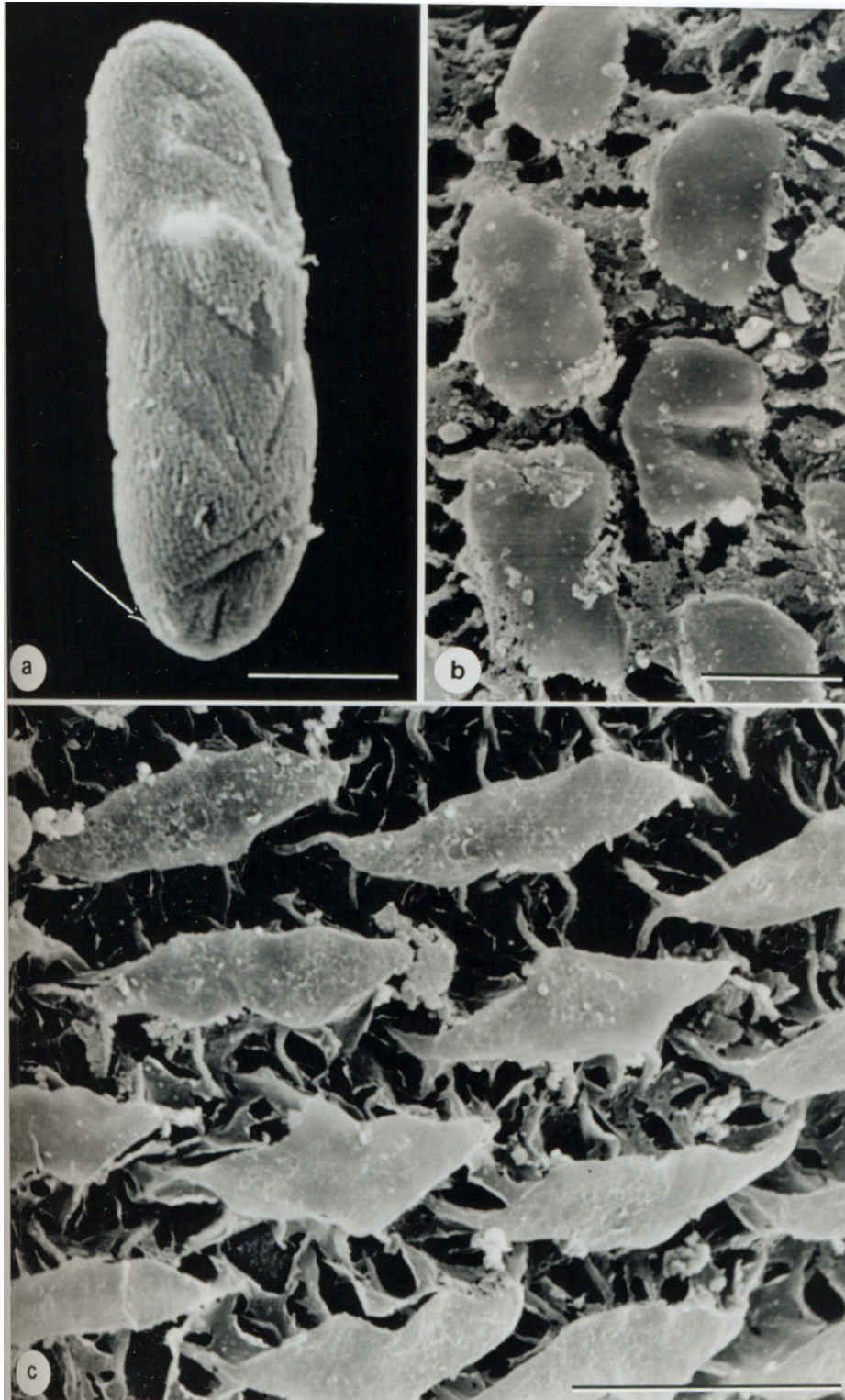


***Lathyrophthalmus aeneus* (Scopoli)**

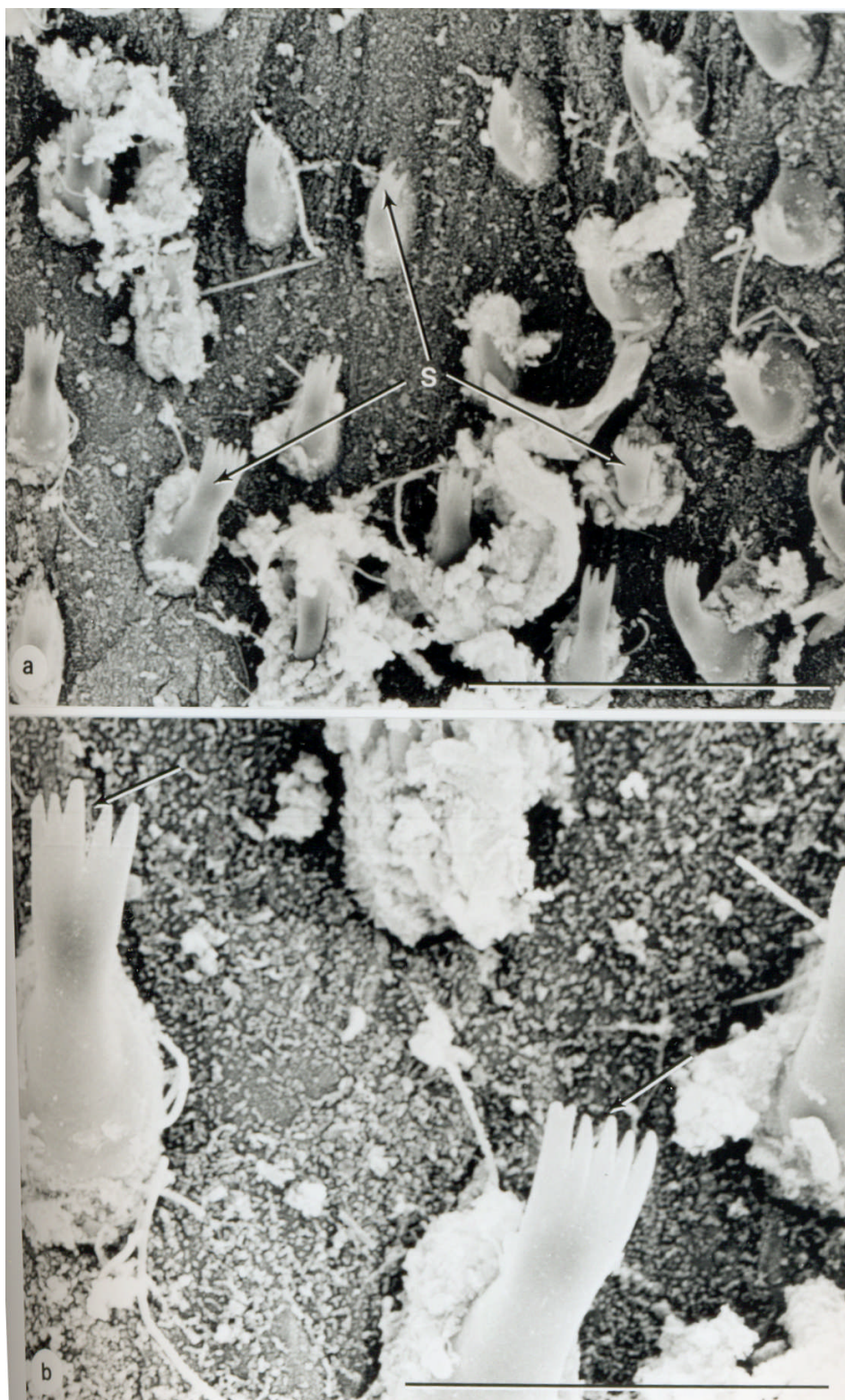
The egg stage of this species hatches in 6-7 days. The eggs are white/brownish in colour, 1.0-1.2 mm long and 0.3-0.4 mm wide, with characteristic chorionic sculpturing (Fig 25). 3rd-instar larvae have a breathing tube which can be extended to several times the length of the body; there are no lappets. There is a group of stout, highly sclerotized spinules on the front margin of the prothorax, subdivided into fine teeth at the tips (Fig 26). The prothoracic spiracles (Figs 27 & 28) are slightly recurved, with widespread spiracular openings. The

prolegs are well developed and carry well-sclerotized crochets (Fig 29 a). Ventrally, on the 6th abdominal segment in front of the prolegs, there is a short double row of anteriorly-directed crochet-like spines, and 5-6 of the posterior row are larger than the others (Fig 29 b). The anal papillae have the formula 2:2:2 (Fig 30). The posterior spiracles are similar to *E. taeniops*.

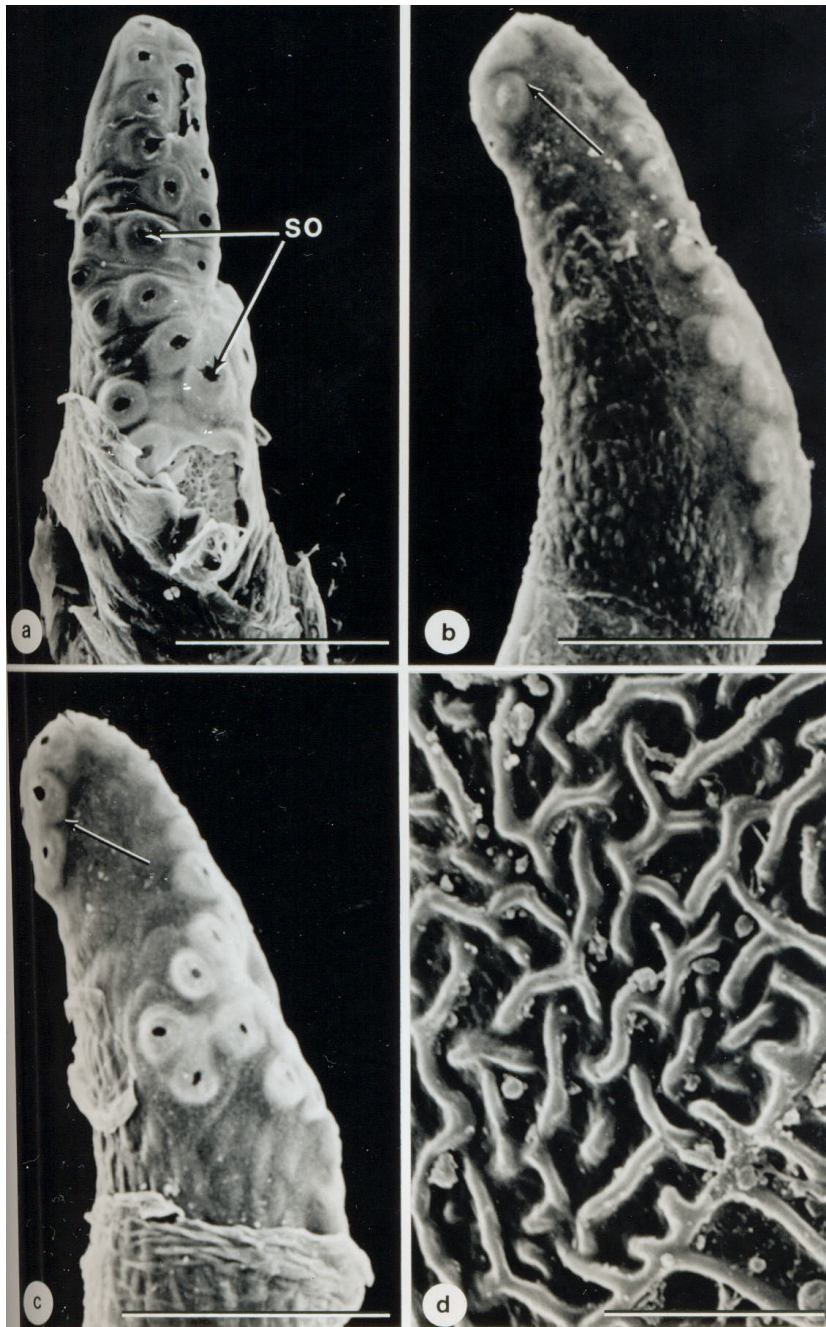
**Fig 25:** SEMs of the egg of *Lathyrrophthalmus aeneus*; (a) entire egg; arrow indicates the pole; bar = 250  $\mu\text{m}$ ; (b) sculpturing at the pole; bar = 10  $\mu\text{m}$ ; (c) sculpturing at the central part; bar = 20  $\mu\text{m}$ .



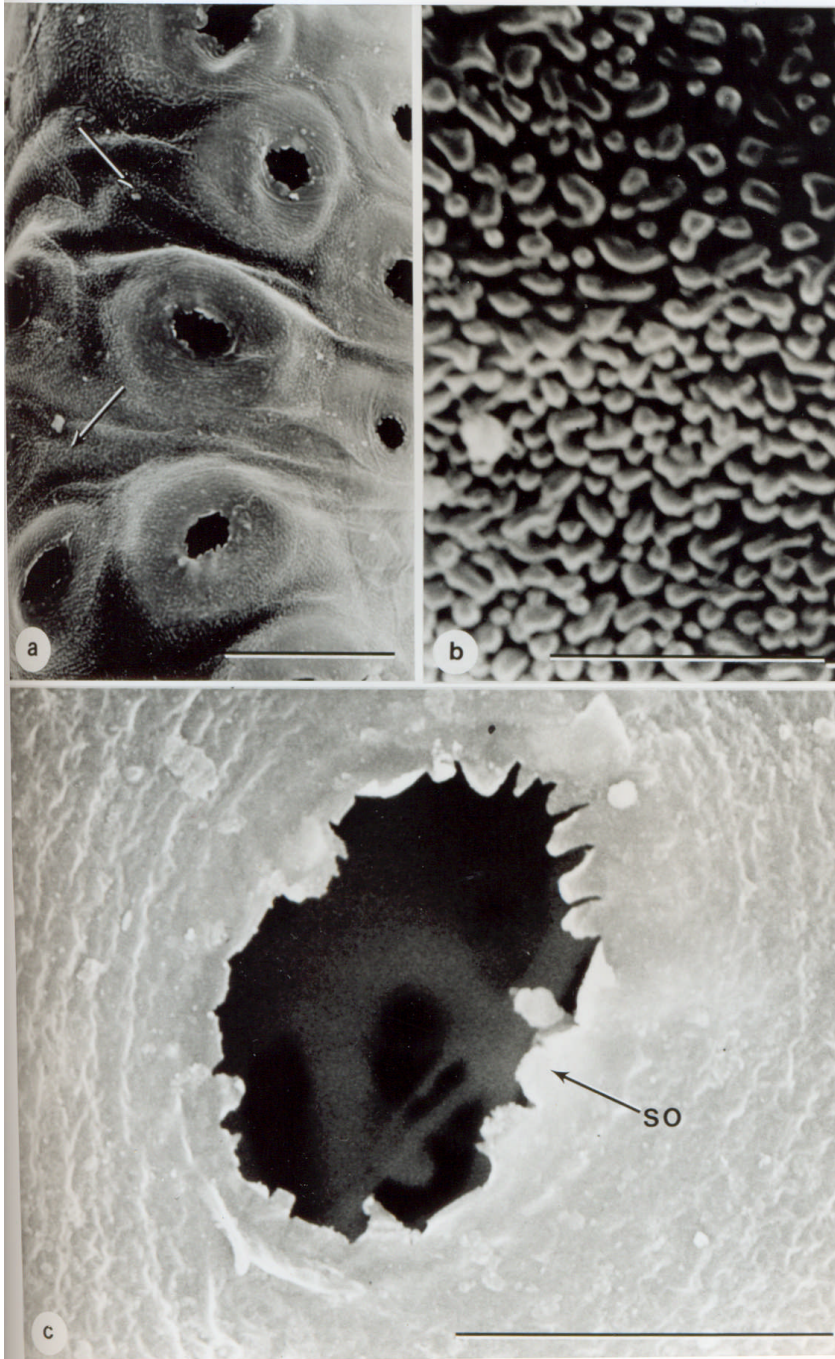
**Fig 26:** SEMs of anterior margin of the prothorax of 3rd-instar larva of *Lathyrophthalmus aeneus*: (a) setae; bar = 100  $\mu$ m; (b) higher magnification; note fine 3-7 digitate tips (arrowed); bar = 50  $\mu$ m



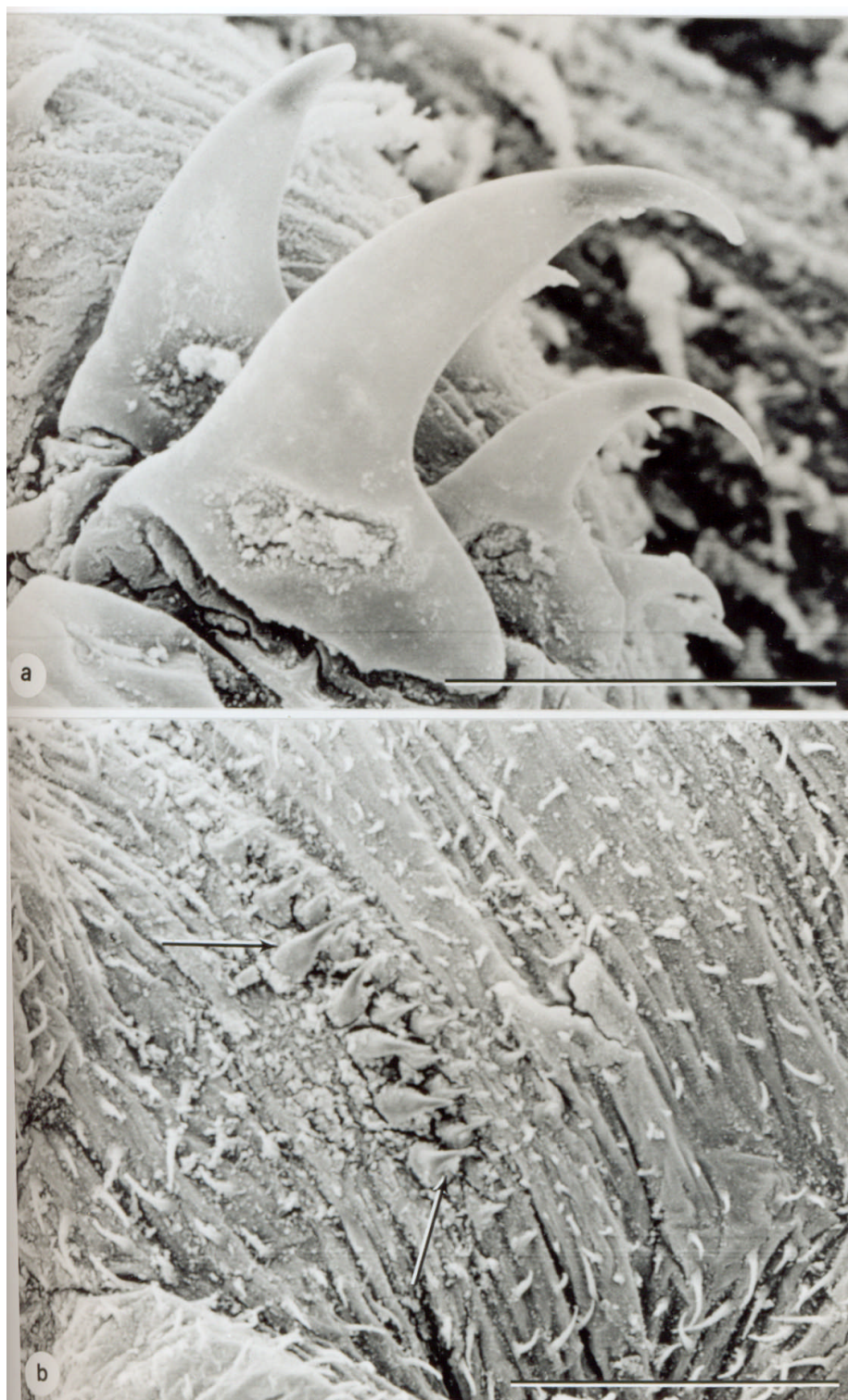
**Fig 27:** SEMs of the prothoracic spiracles of 3rd-instar larva of *Lathyrophthalmus aeneus*: (a) ventral view, showing the spiracular openings (SO) over the entire surface; (b, c) lateral views, showing the spiracular openings extending over the apices in three double rows (arrowed); bar = 250  $\mu$ m; (d) texture of the dorsal surface; bar = 10  $\mu$ m.



**Fig 28:** SEMs of prothoracic spiracles of 3rd-instar larva of *Lathyrrophthalmus aeneus*: (a) the spiracular openings on the ventral surface; bar = 50  $\mu\text{m}$ ; (b) surface texture of the area between the ventral spiracular openings; bar = 5  $\mu\text{m}$ ; (c) single spiracular opening (SO); note the irregular margin; bar = 10  $\mu\text{m}$ .



**Fig 29:** SEMs of the crochets on the ventral surface of the 3rd-instar larva of *Lathyrophthalmus aeneus*: (a) claw-like spines on the prolegs; bar = 100  $\mu$ m; (b) row (arrowed) of five anteriorly directed, large, crochet-like spines immediately anterior to the 6th abdominal prolegs; bar = 1mm.





**Fig 30:** SEM of the anal papillae of the 3rd-instar larva of *Lathyrophthalmus aeneus*: tubes (arrowed) comprising the anal papillae are arranged symmetrically either side of the anterior mid-line (AML); bar = 1 mm.



**“Eristalis” sp 1 (probably a species of Eristalodes)**

The anterior spiracles are short, dark brown and about 0.6-0.7 mm long (Fig 31). The crochets (Fig 32 a) have a broad base and very tapering end. There is a row of about 9-10 anteriorly directed crochet-like spines just anterior to the 6th abdominal prolegs (Fig. 32 b). The lappets on the anal segment are as in *E. megacephalus*.

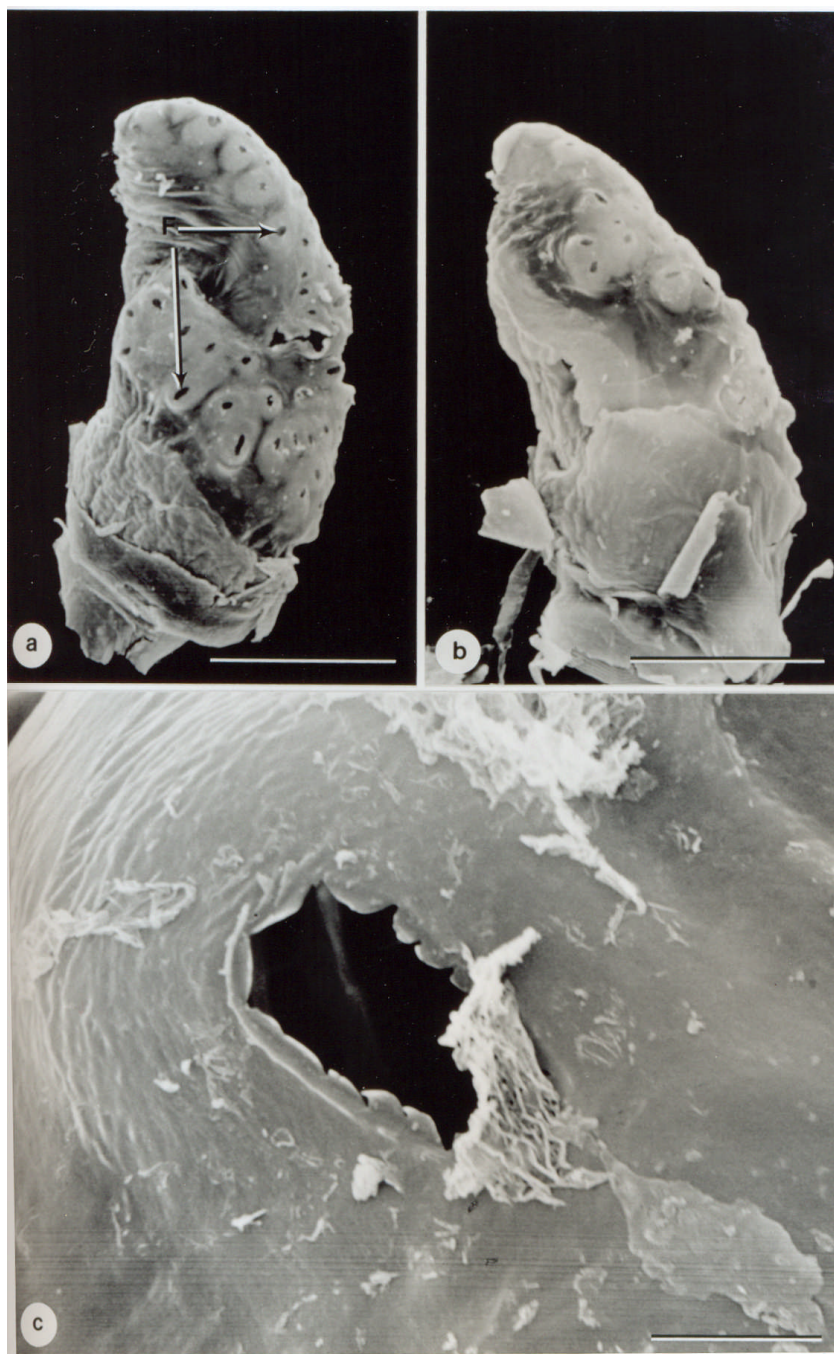
**“Eristalis” sp 2 (probably a species of Lathyrophthalmus)**

The front margin of the prothorax comprises a group of short, stout setae (Fig 33). The anterior spiracles are elongated and about 0.6-0.7 mm in length (Fig 34). The spiracular openings are recurved posteriorly in two rows. There are two rows of 6 anteriorly directed crochet-like spines on the ventral surface of the 6th abdominal segment. No lappets are developed on the anal segment.

**“Eristalis” sp 3**

The prothoracic spiracles are stout with spiracular openings concentrated on the faces (Fig 35). On the front margin of the prothorax there is a group of stout, short sclerotized setae (Fig 36 a), while on the ventral surface there is a row of 9 long, anteriorly directed crochet-like spines (Fig 36 b), just anterior to the 6th abdominal prolegs.

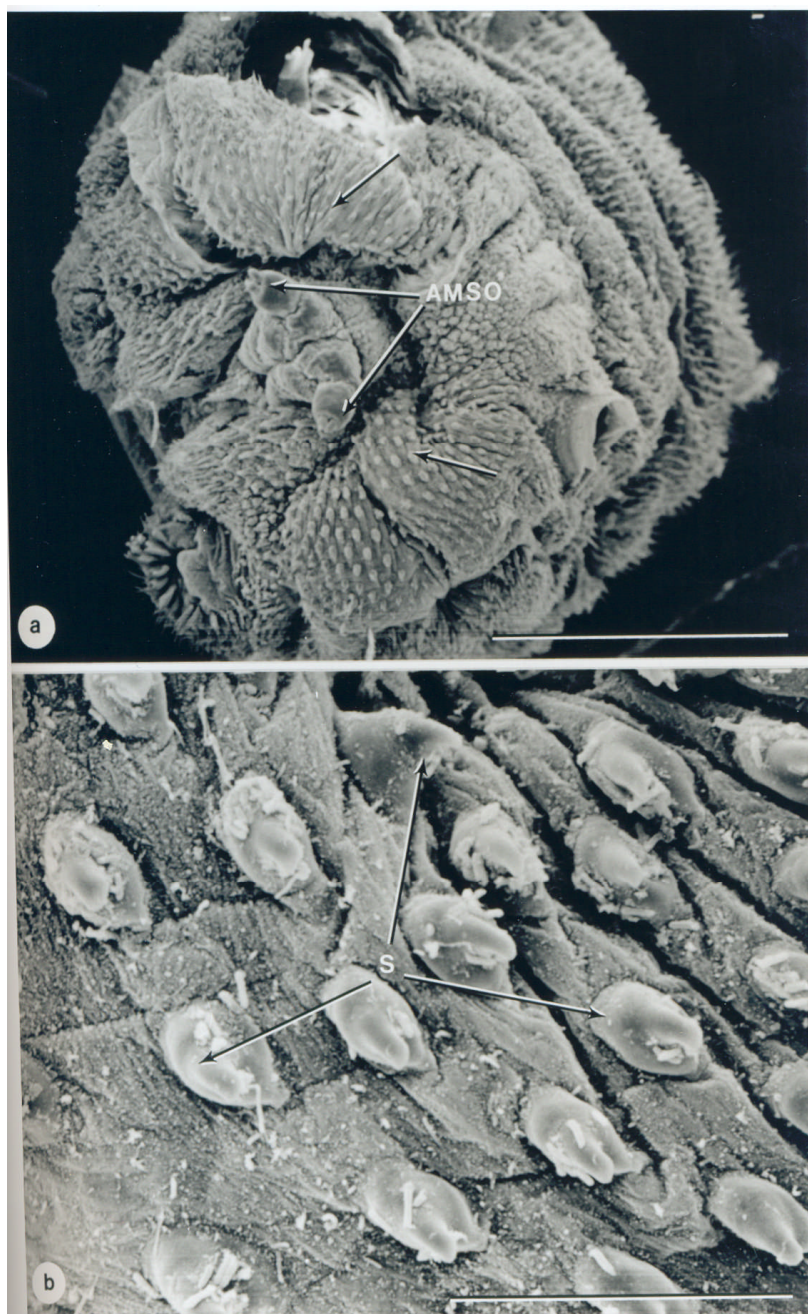
**Fig 31:** SEMs of the prothoracic spiracles of the 3rd-instar larva of "*Eristalis*" sp1: (a, b) lateral views; note that facets (F) extend laterally; bar = 250  $\mu\text{m}$ ; (c) High magnification of spiracular opening; bar = 10  $\mu\text{m}$ .



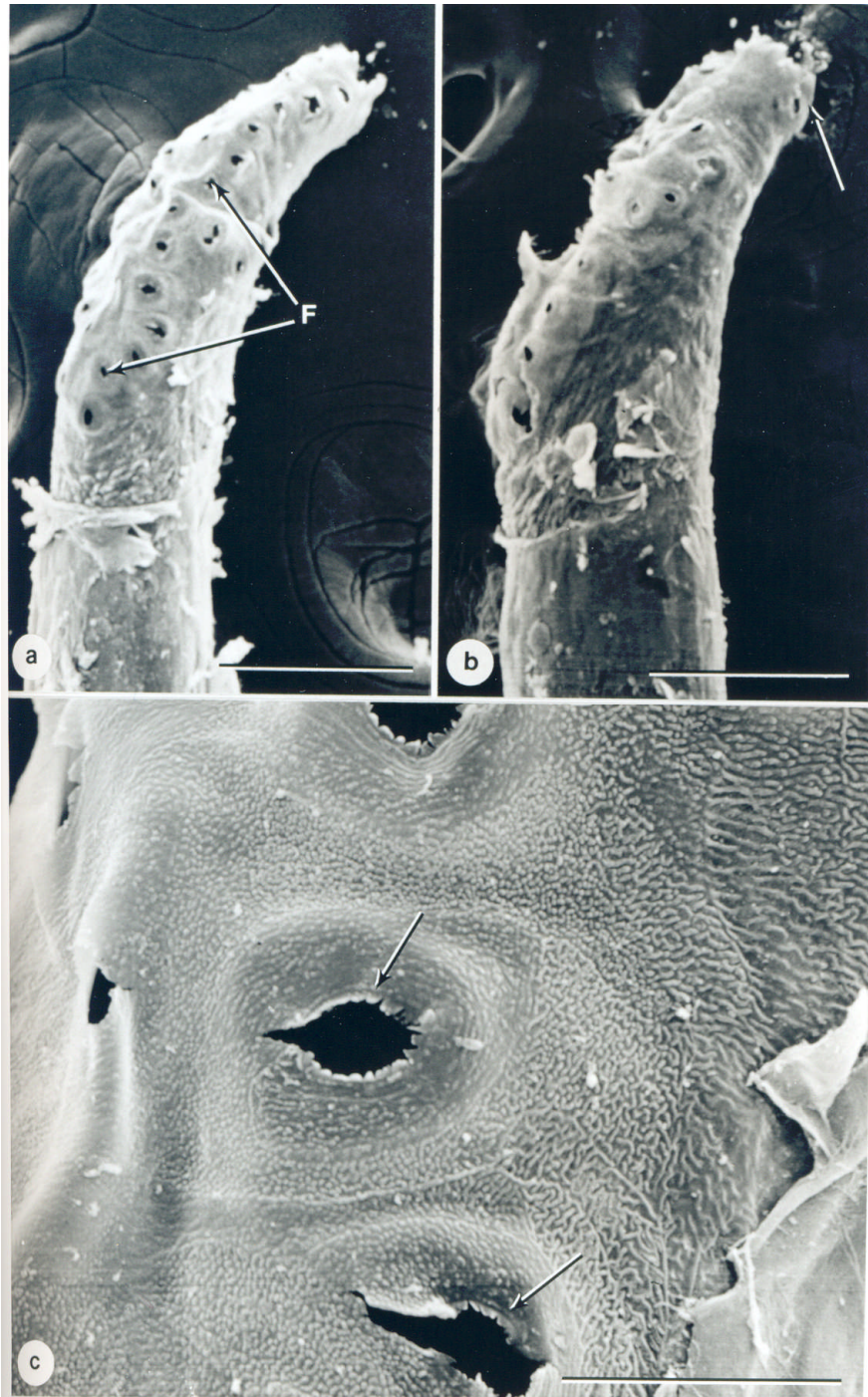
**Fig 32:** SEMs of the crochets on the ventral surface of 3rd-instar larva of "*Eristalis*" sp1: (a) proleg crochet; note the highly sclerotized tips (arrowed) of the claw-like spines; PC primary crochet; SC secondary crochet; bar = 100  $\mu$ m; (b) row (arrowed) of anteriorly directed crochet-like spines just in front of 6th abdominal segment; bar = 200  $\mu$ m.



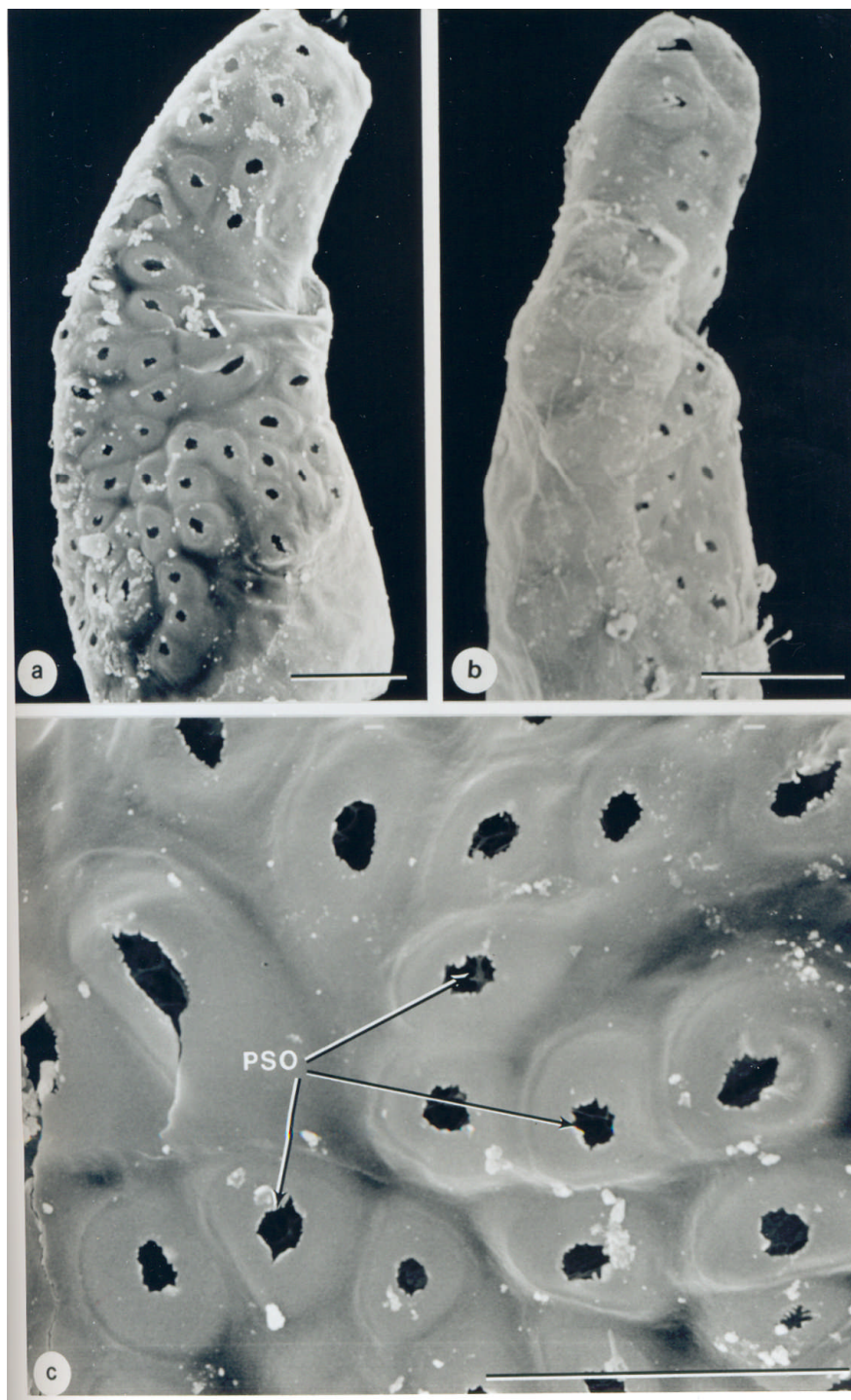
**Fig 33:** SEMs of the prothoracic segment of 3rd-instar larva of "*Eristalis*" sp1: (a) anterior margin of prothorax (arrowed); note the antenno-maxillary sense organ (AMSO); bar = 1 mm; (b) anterior margin showing group of short, stout, backwardly directed setae (S); bar = 100  $\mu$ m.



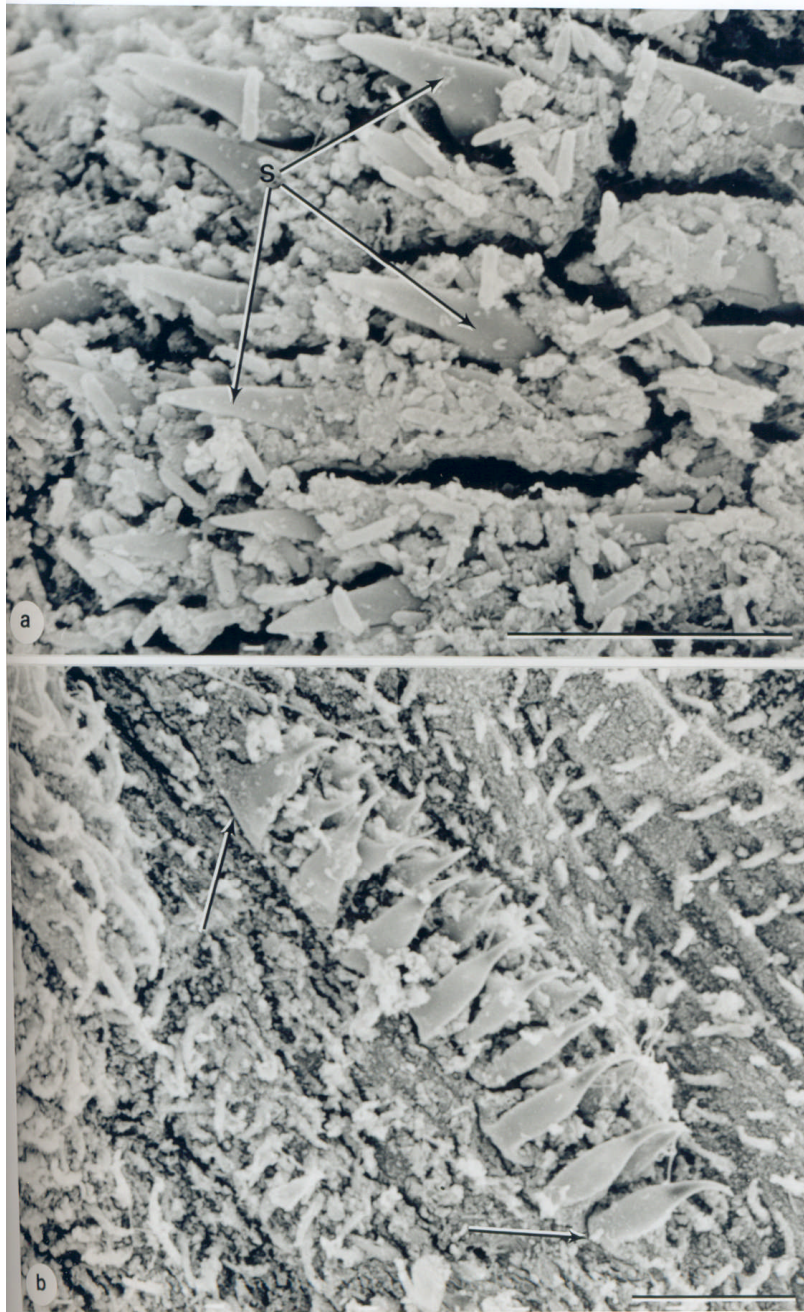
**Fig 34:** SEMs of prothoracic spiracles of the 3rd-instar larva of "*Eristalis*" sp2: (a, b) lateral surface showing the distribution of the facets (F) of the spiracular openings; note that facets extend over the apex of the dorsal surface (arrowed); bar = 250  $\mu\text{m}$ ; (c) the 'eye shape' of the spiracular opening (arrowed); bar = 50  $\mu\text{m}$ .



**Fig 35:** SEMs of the prothoracic spiracle of the 3rd-instar larva of "*Eristalis*" sp3: (a) ventro-lateral surface; (b) lateral surface; (c) shape and arrangement of spiracular openings (PSO). Bars = 100  $\mu$ m.



**Fig 36:** SEMs showing forms of the surface body spines of the 3rd-instar larva of “*Eristalis*” sp3: (a) front margin of the prothorax, showing sclerotized setae (S); bar = 50  $\mu$ m; (b) row (arrowed) of 8-9 anteriorly directed crochet-like spines just anterior to the 6th abdominal prolegs; bar = 100  $\mu$ m.



## Discussion

The morphology of eristaline larvae has been a popular topic for many authors, perhaps because the 3rd instar is so big. Most researchers have used binocular microscopy for examining the fine structure, and only Perez-Bañon *et al.* (2003) used SEM. Virtually all previous studies have dealt mainly with the 3rd instar, and very little is known about the first instars. Descriptions of 3rd instars and puparia of Eristalini have been published in detail by a number of authors (e.g. Wahl 1900, 1901; Hase 1926, Dixon 1960, Hartley 1961, Dolezil 1972,

Rotheray 1993), but only Perez-Bañon *et al.* (2003) dealt with the same species as in Egypt (their material was from Spain and Greece).

All characters used by previous authors have been used here to describe the different larval instars of common Egyptian species using scanning electron microscopy. Some specimens were not identified to the species level and were only rarely collected from the field, and we did not succeed in rearing them to the adult stage: consequently it was impossible to clarify their identification. They seem distinct, based on comparing various characters of the 3rd instar with those of *E.taeniops*, *E.megacephalus* and *L.aeneus*).

We added more characters to the description of the 3rd instar of *L.aeneus* than those mentioned by Hartley (1961). The biology and morphology of the adult stage of this species have been studied by Abou-Elela *et al.* (1979) and Abou-Elela & Taher (1979). The fine structure of the egg chorion is an important diagnostic feature.

The structure of the integument of the 1st instar is completely different from that of the 2nd and 3rd instars. In the 3rd instar the lateral, ventral and dorsal lips are coated with special previously undescribed kinds of setae which are large, strong and supported with many fine spines along their length. They are found only around the mouth opening, and help the larvae to protect themselves from ingesting large-sized particles and therefore prevent blockage. The posterior respiratory process of the 1st instar is not as highly developed as that of 3rd instar, and can be used to identify different instars.

The prothoracic spiracles of both 1st and 2nd instars are not developed. In the 3rd instar of all studied species, they consist of many spiracular openings each with a single slit. They are lined inside with a fine network in all species; this may be a barrier to protect the opening from blockage. The shape and the way the spiracles are arranged differ from one species to another. In *E.taeniops*, the prothoracic spiracle is short, and consists of 32 spiracular slits the margin of which is surrounded by a distinctive frame. In *E.megacephalus*, the spiracle is long, and consists of 35 spiracular slits with irregular margins. The prothoracic spiracles in *L.aeneus* and "*Eristalis*" sp1 differ in shape from the previous two species. In *L.aeneus*, there are usually three rows of openings bent back over the top, while in "*Eristalis*" sp1 there is a group of openings arranged laterally: the region around each slit is wrinkled in both species.

The prothoracic region showed different kinds of setae in different species; these setae are not found anywhere else on the body. *E.taeniops* bears two rows of large, stout recurved spinules divided at the top into two or three teeth, followed by groups of shorter and undivided spinules. In *E.megacephalus*, the front margin of the prothorax is complex, bearing groups of setae of different shape and length: some are long and hooked, followed by shorter ones, while others are very long and smooth. In *L.aeneus*, the setae are very thick, each divided into very fine teeth at the top like a comb. In "*Eristalis*" sp1 the setae are elongated and directed backwards without any divisions at the top.

The shapes of the sensilla of the 1st instar differ from those of the 2nd and 3rd instars. In the first instar, they consist of a central long seta surrounded by many short ones: this is the only type of sensillum found on the body surface. In contrast, there are different types of sensilla on the body surface in both 2nd and 3rd instars. Some are sessile, while others are borne on papillae: furthermore, the number of setae on each sensillum differs from one sensillum to another within a species. The shape and structure of sensilla were found to be the same in all the cristaline species described here. The lappets differ among species: they are long in *E.taeniops* in comparison to *E.megacephalus*, absent in both *L.aeneus* and "*Eristalis*" sp2, while those of "*Eristalis*" sp1 are similar to *E.megacephalus*.

The posterior spiracles showed a different structure in the 1st from both 2nd and 3rd instars. Hartley (1961) suggested that the spiracles of the early stages are similar to those of 3rd instar, but usually less elaborate and there are only two slits instead of three on the posterior spiracles. Data from the present study showed that the structure of the posterior spiracles of the first instar at the rear end of the breathing tube consist of three to four straight slits on each side



of the spiracular disc, with undeveloped scars. Furthermore, there are four pairs of leaf-like setae, two short and two long, surrounding the spiracular disc on the periphery. In 2nd and 3rd instars, the posterior spiracles are different from the 1st instar, but are similar in all the species studied. The dorsal scars develop on the spiracular disc surrounded by four rounded spiracular openings instead of slits as in the 1st instar, and there are four pairs of hydrophobic plumose setae around the spiracular plate which spread out over the surface of the water and allow the submerged larvae to maintain contact with atmospheric oxygen.

The anal papillae in both *E.taeniops* and *E.megacephalus* are similar, and have the formula 2:3:2 on each side, not recorded before for either species. They have the formula 2:2:2 in *L.aeneus*.

From the results of this study, *E.megacephalus* looks to be closer to *E.taeniops* than to other species., a conclusion also reached by Perez-Bañon *et al.* (2003). They share the small crochet-like ventral spines next to the 6th abdominal prolegs, and in both species there is only one distinctive row of 8-9 spines. In *L.aeneus* these spines are found in two rows, one consisting of 5-6 large spines. There are 9-10 in “*Eristalis*” sp1, and 6 in “*Eristalis*” sp2, whilst in “*Eristalis*” sp3 there are 9 spines of a very distinctive type. Thus these crochets can be used to separate these species, and can also be used to discriminate the four European species *Eristalinus sepulchralis*, *L.aeneus*, *E.megacephalus* and *E.taeniops* (Perez-Bañon *et al.*, 2003).

### Acknowledgements

Thanks to the British Council and Overseas Development Administration (ODA) for financing this work. Special thanks to Dr Stephen T Moss (Portsmouth University, Hampshire, UK) for his assistance and advice during the SEM work. My gratitude to Dr Richard C Ray and Dr Eric May for their kind help and research facilities during my stay in the UK in 1999.

### References

- Abou-Elela RG & Taher MO (1979) On the morphology of the fly *Eristalis aeneus* (Scopoli). I. The head and mouth parts. *Journal of the College of Science, University of Riyadh* 11: 45-57
- Abou-Elela RG, Taher MO & Nazer IO (1979) On the biology of *Eristalis aeneus* (Scopoli) (Cyclorrapha: Diptera) in Saudi Arabia. *Journal of the Faculty of Science, University of Riyadh* 9: 73-86
- Becher E (1882) Über die ersten Stände einiger Syrphiden und eine neue *Myiolepta* Art. *Wiener entomologische Zeitung* 1: 249-254
- Beling Th (1888) Beitrag zur metamorphose einiger zweiflügeliger Insekten aus den Familien Tabanidae, Empidae und Syrphidae. *Verhandlungen der Zoologisch-Botanische Gesellschaft in Wien* 38: 1-4
- Dewedar AM, El Houssini M, Bahgat MMM, Diab AM & Butler E (1993) The Gravel Bed Hydroponic system for sewage water treatment: performance, characteristics and efficiency of a pilot experiment at Abu-Attwa, Ismailia, Egypt. *Egyptian Society of Applied Microbiology, Proceedings of the VIII Conference of Microbiology, Cairo, Egypt.*
- Dixon TJ (1960) Key to and descriptions of third instar larvae of some species of Syrphidae (Diptera) occurring in Britain. *Transactions of the Royal Entomological Society of London* 112: 345-79
- Dolezil Z (1972) Developmental stages of the tribe Eristalini (Diptera, Syrphidae). *Acta entomologica Bohemoslovaca* 69: 339-350
- Dunavan D (1929) A study of respiration of the rat-tailed maggot, *Eristalis arbustorum* L. *Annals of the entomological Society of America* 22: 731-753
- Gäbler H (1930) Die postembryonale Entwicklung des Tracheensystems von *Eristalis tenax* L. *Zeitschrift für Morphologie und Ökologie der Tiere* 19: 427-492
- Gäbler H (1932) Beitrag zur Kenntnis der *Eristalis*-Larven (Dipt.). *Stettin entomologische Zeitung* 93: 143-147
- Gilbert F (1993) *Hoverflies*. Naturalists handbooks 5, revised edition. Richmond Publishing Co. Ltd. 67 pp.
- Hartley JC (1961) A taxonomic account of the larvae of some Syrphidae. *Proceedings of the zoological Society of London* 136: 505-573
- Hase A (1926) Beiträge zur Kenntnis der Lebensweise der *Eristalis*-Larven (Diptera). *Zoologischer Anzeiger* 68: 33-51
- Kruger F (1926) Biologie und morphologie einiger Syrphiden-larven. *Zeitschrift für Morphologie und Ökologie der Tiere* 6: 83-149

- Mahmoud M, Bahgat M, Zalat S & Dewedar A (1999) *Eristalinus* larvae and the role of bacteria in their feeding. *Journal of the Union of Arab Biologists* 11A (Zoology): 417-433
- Maier CT (1982) Larval habitats and mate-seeking sites of flower flies (Diptera: Syrphidae, Eristalinae). *Proceedings of the Entomological Society of Washington* 84(3): 603-606
- Metcalf CL (1913) The Syrphidae of Ohio. *Bulletin of the Ohio State University* 17(#31): 1-123
- Mohamed SK (1975) Ecological and taxonomic studies on the family Syrphidae in Egypt. MSc Thesis, Faculty of Science, Ain Shams University, Cairo.
- Perez-Bañon C, Rojo S, Ståhls G & Marcos-Garcia MA (2003) Taxonomy of European *Eristalinus* (Diptera: Syrphidae) based on larval morphology and molecular data. *European Journal of Entomology* 100: 417-428
- Rotheray GE (1993) Colour guide to hoverfly larvae (Diptera: Syrphidae) in Britain and Europe. *Dipterists Digest* No. 9
- Sack P (1921) Beitrag zur Entwicklung einiger Syrphiden (Dipt.). *Senckenbergiana* 3: 158-163
- Sack P (1931) Syrphidae (Diptera) der deutschen limnologischen Sunda-expedition. *Archiv für Hydrobiologie*, Suppl. 8: 585-592
- Shaumar N & Mohamed SK (1977) Keys for the identification of species of family Syrphidae (Diptera) in Egypt. *Bulletin de la Société Linnéenne de Lyon* 46: 373-380
- Shaumar N & Kamal S (1978) The Syrphidae of Egypt. *Bulletin de la Société Linnéenne de Lyon* 47: 79-84
- Smart J (1948) A handbook for the identification of insects of medical importance. 2<sup>nd</sup> ed. British Museum of Natural History, London
- Wahl B (1900) Über das Tracheensystem und die Imaginalscheiben der Larve von *Eristalis tenax* L. *Arbeiten aus dem zoologischen Institut der Universität Wien* 12: 45-99
- Wahl B (1901) Über die Entwicklung der hypodermal Imaginalscheiben im Thorax und Abdomen der Larve von *Eristalis*. *Arbeiten der zoologischen Institut der Universität Graz* 6: 171-201
- Weise B (1938) Die Atmung der Larven und Puppen der Schwebfliegen aus der Verwandtschaft der Eristalinae unter Berücksichtigung ihrer Metamorphose. *Zeitschrift für wissenschaftliche Zoologie* 151: 467-514

### الملخص العربي

التركيب السطحي الدقيق للأطوار الغير ناضجة لمجموعة إريستالينس (رتبة ذات الجناحين - فصيلة ذباب السرفيس) في مصر

سامي زلط<sup>1</sup> و منى على محمود<sup>2</sup>

- 1- قسم علم الحيوان - كلية العلوم - جامعة قناة السويس - الإسماعيلية - حالياً: جامعة طيبة - المملكة العربية السعودية
- 2- مركز بحوث التقنية الحيوية - جامعة قناة السويس - الإسماعيلية - مصر

تم خلال هذا البحث دراسة الشكل الظاهري والتركيب الدقيق لطور اليرقات والعذارى لعدد من أنواع ذباب الإريستالينس في مصر (رتبة ذات الجناحين - فصيلة ذباب السرفيس) والتي تعيش في المياه المعالجة لمحطات الصرف الصحي وذلك باستخدام الميكروسكوب الإلكتروني الماسح. تم دراسة طور البيضة والأطوار اليرقية الثلاثة بصورة تفصيلية في ذبابة "إريستالوديس نابوبس"، وطور البيضة والطور اليرقي الثالث في ذبابة "لايثروفثاليميس إينيس"، والطور اليرقي الثالث في ذبابة "إريستالوديس ميجاسيفاليس" وثلاثة أنواع أخرى من ذباب إريستالينس. أوضحت الدراسة أن دراسة التركيب الدقيق للقشرة الخارجية للبيض تعتبر كافية لتمييز وتعريف الأنواع المختلفة، وأن التركيب الخارجي لجدار الجسم في طور اليرقي الأول يختلف بصورة كبيرة عن طور اليرقي الثاني والثالث. أيضا أوضح التركيب الدقيق سواء من الناحية الجانبية أو البطنية أو الظهرية أن الشفاه في منطقة الفم في طور اليرقات مغطاة بأشواك كبيرة ومميزة ومدعمة بعدد كبير من الأشواك الدقيقة على طول الشفاه، وربما تقوم تلك الأشواك بحماية اليرقات من بلغ الجزئيات الغذائية الكبيرة والتي يمكن أن تسبب مشاكل في فم اليرقات. أيضا وضح أن الزوائد التنفسية الخلفية في طور اليرقي الأول تختلف إختلافاً كبيراً عن طور اليرقي الثالث، ويمكن الإعتماد على تركيب تلك الزوائد بصورة كبيرة في تعريف الأطوار اليرقية المختلفة في هذا النوع من الذباب. وأخيراً، أظهرت الدراسة أن الفتحات التنفسية في الصدر الأمامي لكل من طور اليرقي الأول والثاني غير نامية بصورة كافية.