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Comparative morphology of early stages of two Mediterranean *Sarcophaga* Meigen, 1826 (Diptera; Sarcophagidae) and a review of the feeding habits of Palaearctic species

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Abstract

The third instar larva of *Sarcophaga hirticrus* Pandellé, 1896 and *Sarcophaga javita* (Peris, González-Mora and Mingo, 1998) are described and figured for the first. The use of scanning electron microscopy (SEM) has been demonstrated as an effective tool for determining differences at the specific level, and is here applied. The two species are distinguished from other *Sarcophaga* spp. and the principal diagnostic character states are illustrated and discussed. Comparative information on immature stages morphology of the described Palaearctic Sarcophaga species and its feeding habits are compiled and provided in a tabulated form.

Keywords: Sarcophagidae; Immature stages; Larval morphology; Ultrastructure; Breeding habits

1. Introduction

The immature stages of the majority of dipterous families remain poorly known (Hennig, 1968). In the case of the Sarcophagidae relatively few papers have appeared dedicated to the larval morphology of the family (Aspoas, 1991; Ebejer, 2000; Kirk-Spriggs, 1999, 2000, 2003; Méndez and Pape, 2002; Zumpt, 1965). The larval stages of many species of Sarcophagidae are necrophagous and for this reason those species termed 'flesh-flies' are significant in forensic entomology, being second only to the Calliphoridae (Diptera) in terms of their usefulness. Precise knowledge and precise diagnoses of their immature stages therefore have a very practical application in estimating the post-mortem interval (Sukontason et al., 2003; Wells et al., 2001). Other species of Sarcophaginae and Miltogramminae are predatory on invertebrates (e.g. other insects, snails, earthworms, scorpions, crabs, etc.) (Méndez and Pape, 2002; Pape, 1987), and several species have been reported as being parasitic in vertebrates (mammals, turtles) (Dahlem, 1991; Valle de Sales et al., 2004).

In some cases, detailed descriptions of larvae implicated in traumatic human myiasis (Colwell and O'Connor, 2000; Zumpt, 1965) or found infesting mummified human remains have been published (Sukontason et al., 2003), but in these cases the descriptions have not been attributed to any named species, as adults were not obtained. Therefore, the breeding habits should be related to morphological and taxonomic knowledge in order to improve biological and practical considerations.

Sarcophagids larvae are easily recognised at family and generic level, but are morphologically remarkably similar subgenerically and inter-specifically (Aspoas, 1991). The use of scanning electron microscopy (SEM) to observe and photograph the morphology of dipterous larvae has enabled observation of a suite of intra-specific differences (Colwell, 1989), and to characterise some aspects (pseudocephalon, spinules, anterior and posterior spiracles, spiracular setae, rim of spiracular atrium tubercles and sensilia) not easily resolved with light microscopy (Aspoas, 1991). For this reason Cantrell (1981), indicates that it is important to describe the number of papillae forming the anterior spiracles, the shape of the peritreme of the posterior spiracles and the cephalopharingeal skeleton.

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Pape et al. (2002) cite 73 species of the genus *Sarcophaga* as occurring in the Iberian Peninsula, but detailed morphological descriptions of larvae based on SEM have been published for only five of these, namely: *Sarcophaga africa* (Wiedemann, 1824) (as *S. cruentata* Meigen, 1826), *Sarcophaga crassipalpis* Macquart, 1838, *Sarcophaga dux* Thomson, 1869, *Sarcophaga exuberans* Pandellé, 1896 and *Sarcophaga tibialis* Macquart, 1851.

Sarcophaga hirticrus Pandellé, 1896 and *Sarcophaga javita* (Peris, González-Mora and Mingo, 1998) occur sympatrically in southern Spain, where they are relatively abundant. Both species have been occasionally collected together from the terrestrial snail *Otala punctata* (Müller, 1777) (Pérez-Moreno, 2004).

In this study, the 3rd instar larvae of *S. hirticrus* and *S. javita* are described for the first time. Numerous features of the 3rd instar larvae of the two species are examined by use of SEM, in order to determine the presence of morphological character states of potential taxonomic value.

A table is provided which compiles published information about described larvae of *Sarcophaga* species and feeding habits (Table 1).

2. Materials and methods

All larval stages of *S. hirticrus* and *S. javita* were obtained from a laboratory colony maintained over several generations under constant conditions of 25 °C temperature and 70–85% relative humidity, and a photoperiod of 15:9 h L:D. The colony was originated from field-collected material obtained in Mutxamel 30SYH203506 (Alicante, SE Spain) (Pérez-Moreno, 2004), during studies of the life cycle of these species. Adult flies of *S. hirticrus* were placed into rearing cages and provided with a diet of sugar, water and pig's liver. The liver provided the protein meal for adult female as well as a medium for larviposition. Larvae of *S. hirticrus* were placed on a bed of a fine sand to facilitate the pupation. Some of these puparia were left for 24/48 h in order to allow their sclerotisation before being killed by freezing. In the case of *S. javita*, alive snails were provided to the females in order to stimulate the larviposition. The larvae and pupae were extracted with the help of a hand needle of the snail's shell.

Third instar larvae were selected for preservation. These are distinguishable from larvae of other calyptrate families in that the majority of species possess three nearly vertical, parallel, posterior spiracular openings, usually not orientated toward the opening in the peritreme. For permanent preservation, larvae were killed by immersion in cold water and slowly boiled for approximately four minutes to distend them. Following this they were preserved in 70% alcohol.

Descriptions are based on preserved larvae and/or puparia. Cephalonpharyngeal skeletons attached by a membrane to the leading ventral edge of the interior of the puparia, were removed by immersion in water for approximately 24 h and were preserved in glycerine until use. Measurements were made using a graticule mounted in an eye piece of a binocular microscope.

Stereoscan micrographs were taken with SEM HITACHI S3000N operated at 20 kV. Specimens of immature stages of *S. hirticrus* and *S. javita* are deposited in the Entomological Collection of the University of Alicante (CIBIO).

The character states examined are as follows: the pseudocephalon, the cephalopharyngeal skeleton, the spinules, the anterior spiracles, the spiracular atrium and the posterior spiracles.

Taxonomic nomenclature in this paper follows Pape (1996). Larval terminology used in this paper follows Teskey (1981) and Courtney et al. (2000) for ventral organ.

3. Results

3.1. *Sarcophaga hirticrus* Pandellé, 1896

3.1.1. Overall appearance (Fig. 1(A))

Newly moulted larvae are creamy white in colour. Length 9.85 ± 0.86 mm, maximum width 3.63 ± 0.31 mm ($n = 10$).

Larvae elongated, sub-cylindrical in cross-section with a flattened ventral surface, truncated posteriorly and tapering toward the anterior extreme.

Posterior surface of anal segment with a distinct cavity, which contains the posterior spiracles. The surface of thoracic and abdominal segments with bands of spinules of subtriangular form. The inter-band areas are devoid of spinules (Fig. 1(A)).

Anal segment with two postanal tubercles with apical sensillae. Surface of the postanal tubercles covered with spinules in the half basal part.

3.1.2. Pseudocephalon (Fig. 1(B) and (C))

The pseudocephalon has a pair of small antennae (a) and two palps (p) (Fig. 1(C)). Antennae appearing with two segments, the apical (dome) in conical shape. The palps are mammeliform, with concentric ribbons at the apical extreme where are placed five sesilla, the three posterior ones sited in the same protuberance and nominated maxillary palpus sensilla (mxpp).

At both sides of the mouth, between the inferior part of the palps and the maxilla, appear extensive bands of elongated, slightly corrugated overlapping oral ridges (or). Ventral organs (vo) present.

The strong maxillae (m) are smooth, widely curved and retractable (Fig. 1B). Buccal cavity displays a pair of sensilium (Fig. 1(C)).

3.1.3. Thorax (Fig. 1(D))

The prothorax is rounded by spinules one or two pointed and grouped in a variable number. Anterior spiracles (as)

Table 1
Review of morphology of immature stages and feeding habits of Palaearctic *Sarcophaga* species

Species	T	No. of lobes anterior spiracles (3rd instar)	Ecdysial scar	Cephalopharingeal skeleton description (instar)	Material from	References	Host/feedind habits
Sarcophaga aegyptica Salem, 1935	LM	14–15/single row	–	2nd and 3rd instar	Spain	Saloña Bordas and Gonzalez-Mora, 2005 (M&F)	Necrophagous
Sarcophaga africa (Wiedemann, 1824) (= *S. cruentata* Meigen, 1826)	SEM	11–13/single row	–	–	South Africa	Aspoas, 1991 (M); Zumpt, 1965 (F); Pape, 1987 (F); Berner, 1973 (F)	Myiasic, decomposing matter, feces, dead snails
Sarcophaga alba (Schiner, 1868)	LM	9–12/single row	–	3rd instar	Japan	Ishijima, 1967 (M&F)	Dead fish and mammals
Sarcophaga albiceps Meigen, 1826	LM	32–38/irregular rows	No	All instars	Kano et al., 1951	Kano et al., 1951 (M)	Dead animals, feces, garbage
				3rd instar		Ishijima, 1967 (M&F)	
Sarcophaga antilope Böttther, 1913	LM	46–52/irregular rows	No	3rd instar	Japan	Ishijima, 1967 (M&F)	Flesh in laboratory, parasite of moth
Sarcophaga argyrostoma Robineau-Desvoidy, 1830	SEM	10–11/single row	Yes	–	Egypt	Awad et al., 2003 (M&F); Povolny and Verves, 1997 (F)	Myiasic, necrophagous, parasitoids
Sarcophaga brevicornis Ho, 1934	LM	12–15/single row	No	3rd instar	Japan	Ishijima, 1967 (M); Tumrasvin and Cano, 1979 (F)	Dead animals and garbage
Sarcophaga caerulescens Zetterstedt, 1838 (=*Rovineauella scoparia* (Pandelle,1896))	LM	48–54/irregular rows	No	3rd instar	Japan	Ishijima, 1967 (M); Pape, 1987 (F)	Necrophagous, carcasses, predators of lepidopterous pupae
Sarcophaga caudagalli Böttcher,1912	LM	32–36	No	3rd instar	Japan	Ishijima, 1967 (M&F)	Chicken in laboratory
Sarcophaga crassipalpis Macquart, 1838	LM	9–14/single row	–	All instars	Japan	Kano et al., 1951 (M)	Privies and animal carcasses
		9–14/single row	No	3rd instar	Japan	Ishijima, 1967 (M&F)	
		11–12	Yes	All instars	Australia	Cantrell, 1981 (M)	
Sarcophaga dux Thomson, 1869	SEM	14–17/single row	–	All instars	Thailand	Sukontason et al., 2003 (M); Zumpt, 1965 (F)	Myiasic, carrion
Sarcophaga exuberans Pandellé,1896	SEM	8–10/single row	–	–	South Africa	Aspoas, 1991 (M); Zumpt, 1965 (F)	Myiasic,carrion
Sarcophaga harpax Pandellé, 1896	LM	40–44/irregular row	No	3rd instar	Japan	Ishijima, 1967 (M&F)	Decaying animal matter, internal parasite
Sarcophaga hirticrus Pandellé, 1896	SEM	7–10/single row	Indistinct	3rd instar	Spain	New data (M&F); Castillo, 2001 (F); Blackith et al., 1997 (F)	Carrion, snail and insect
Sarcophaga horii Kano, 1953	LM	34–37	No	3rd instar	Japan	Ishijima, 1967 (M&F)	Horseflesh in laboratory
Sarcophaga inzi Curran, 1934	LM	10/single row	–	3rd instar	Kalahari	Kirk-Spriggs, 1999 (M&F)	Dead millipedes

(*continued on next page*)

Table 1 (*continued*)

Species	T	No. of lobes anterior spiracles (3rd instar)	Ecdysial scar	Cephalopharingeal skeleton description (instar)	Material from	References	Host/feedind habits
Sarcophaga javita (Peris, Gonzalez-Mora & Mingo, 1998)	SEM	12–14/single row	Indistinct	3rd instar	Spain	New data (M)	Snail predator (unpublished)
Sarcophaga kagaensis Hori, 1954	LM	34–38/two rows	No	3rd instar	Japan	Ishijima, 1967 (M&F)	Horseflesh and chicken in laboratory
Sarcophaga kawayuensis Kano, 1950	LM	32–36/irregular rows	No	3rd instar	Japan	Ishijima, 1967 (M&F)	Dung, carcasses
Sarcophaga melanura Meigen, 1826	LM	12–15/single row	No	All instars	Japan	Kano et al., 1951 (M)	Myiasic; birds and mammals. Snails. Feaces.
		16–22/single row		3rd instar		Ishijima, 1967 (M&F); Seguy, 1941 (F); Blackith et al, 1997 (F)	
Sarcophaga misera[a] Walker,1849	LM	16–17/single row	No	3rd instar	Japan	Ishijima, 1967(M&F); Parashar and Rao, 1988 (F);	Human feces, carcasses, dead fish, snails predator
Sarcophaga okazakii Kano, 1953	LM	38–43/irregular rows	No	3rd instar	Japan	Ishijima, 1967 (M&F)	Animal matter in laboratory
Sarcophaga orchidea[a] Böttther, 1913	LM	28–34/two rows	No	3rd instar	Japan	Ishijima, 1967 (M&F)	Human feces, dead animals
Sarcophaga peregrina Robineau-Desvoidy, 1830	LM	24–28/two rows	No	All instars	Japan	Kano et al., 1951 (M)	Myiasic, necrophagous, garbage, dead snails
		24–26/two rows	No	3rd instar	Japan Australia	Ishijima, 1967 (M&F)	
		28	Yes	All instars		Cantrell, 1981 (M); Seguy, 1941 (F)	
Sarcophaga polystylata Ho, 1934	LM	14–16/single row	–	3rd instar	Japan	Ishijima, 1967 (M&F)	Dead animals
Sarcophaga pterygota Thomas, 1949	LM	24–28	No	3rd instar	Japan	Ishijima, 1967(M&F)	Decaying animal matter, carcasses, garbage
Sarcophaga schuetzei (Kramer,1909)	LM	32–36	No	3rd instar	Japan	Ishijima, 1967 (M&F)	Dead animals, parasites of lepidopterous
Sarcophaga seniorwhitei Ho,1938 (=*Trichoproctia flavinervis* (Senior-White,1924))	LM	42–46/irregular rows	No	3rd instar	Japan	Ishijima, 1967 (M&F)	Fish, chicken and horseflesh in laboratory
Sarcophaga septentrionalis (Rohdendorf, 1937)	LM	28–30/two rows	No	3rd instar	Japan	Ishijima, 1967 (M&F)	Dead animals
Sarcophaga shiritakaensis Hori, 1954	LM	46–49/irregular rows	No	3rd instar	Japan	Ishijima, 1967 (M&F)	Animal matter in laboratory
Sarcophaga similis Meade, 1876	LM	24–30/two rows	No	All instars	Japan	Kano et al., 1951 (M)	Carcasses, garbage, feaces, intestinal myiasis
				3rd instar		Ishijima, 1967 (M&F)	

Sarcophaga taenionota (Wiedemann,1819) (= *S. omega* Johnston and Tiegs, 1921)	LM	28	No	All instars	Australia	Cantrell, 1981	Carrion	
Sarcophaga tibialis Macquart, 1851	SEM	15–21/irregular double row	–	–	South Africa	Aspoas, 1991 (M); Abasa, 1972 (F); Zumpt, 1965 (F)	Myiasic, necrophagous, dead snails	
Sarcophaga tsushimae Senior-white, 1924	LM	33–36/irregular rows	No	All instars	Japan	Kano et al., 1951 (M)	Dead animals	
				3rd instar		Ishijima, 1967 (M&F)		
Sarcophaga ugamskii (Rohdendorf, 1937)	LM	14–17	No	3rd instar	Japan	Ishijima, 1967 (M&F)	Chicken in laboratory	

In column *Species* it is indicated the valid name according to Pape (1996) and, when needed, between brackets, the name of the species used by the author cited in the column *References*. Column 'T', refers to the *Technique* used for the description of the larvae; SEM (scanning electron microscopy) or LM (light microscope). In column 'References' the author that describes morphology and feeding habits are indicated ('M' to morphology and 'F' to feeding habits).

[a] Although *S. orchidea* Böttcher, 1913, is considered by Pape (1996) as junior synonym of *S. misera* Walker, 1849, we maintain this reference because it is probable that Ishijima (1967) described two taxa different to *S. misera*.

arising posterolaterally on the prothoracic segment and fan-shaped (Fig. 1(D) pupa). Each anterior spiracle contains a single row of papillae that number from 7 to 10.

The rest of the thoracic segments with an anterior band of spinules encircling each segment. These bands are constituted by seven crows of spinules without bifurcation.

3.1.4. Abdomen (Fig.1(A), (E)–(G))

Abdominal segments with anterior spinule bands. These bands encircle the segments. The inter-band areas are devoid of spinules.

The distribution of the abdominal spinule bands is different in dorsal and ventral position. In dorsal view the spinules are posteriorly projected and form one simple band, lacking spinules in the dorso-central area. These bands are wider towards the last body segment.

In ventral view, each band is constituted by three subbands of 3, 4–5, 3–4 rows of spinules alternately disposed. The spinules of the first subband are anteriorly projected and the spinules of the second and third subbands posteriorly. These spinules are one pointed (Fig. 1(E)).

In addition, there are rounded lateral areas in the spinules of the sides of the segments.

Abdominal segments display small fleshy protuberances laterally (Fig. 1(A)).

Posterior spiracles on the last eleventh body segment are set within a deep spiracular atrium (Fig. 1(G)). There are 12 tubercles (t) located on the rim of the spiracular atrium (Fig. 1(F)). Spinules distributed around the rim and extending as far as the outer circumference of the ring formed by the tubercles.

Spiracles consisting of three elongate slits (s) oriented vertically, with the openings disposed radially, each surrounded by an incomplete sclerotized peritreme (p). Ecdysial scar indistinct. A branch of spiracular hairs (sh), constituted by a single trunk with 2–3 branches, at both sides of the slits.

The anal segment displays two postannal tubercles whose surface is covered by spinules until the half. Each postanal tubercle has a sensilla in the apex.

3.1.5. Cephalopharyngeal skeleton

Mouthhook well developed (m); deeply pigmented, sickle-shaped, pointed in the apex and basally square. Dental sclerites (ds) present. Subhypostomal sclerite (ss) middle moon-shaped from above. Intermediate sclerite (hypopharyngeal sclerite, hs) short, wide with rounded anterior and posterior projections, H-shaped from above and not fused to the basal sclerite. Pharyngeal sclerite (ps) relatively bulky and heavily pigmented at middle, with a parastomal bar (pb), slightly incurvated apically upwards. Dorsal cornu (dc) wider and longer than ventral cornu. Dorsal cornu with a narrow elongated window apically. Ventral cornu (vc) approximately twice longer than wide, apically subtruncated and with a small and dorsoapical oval

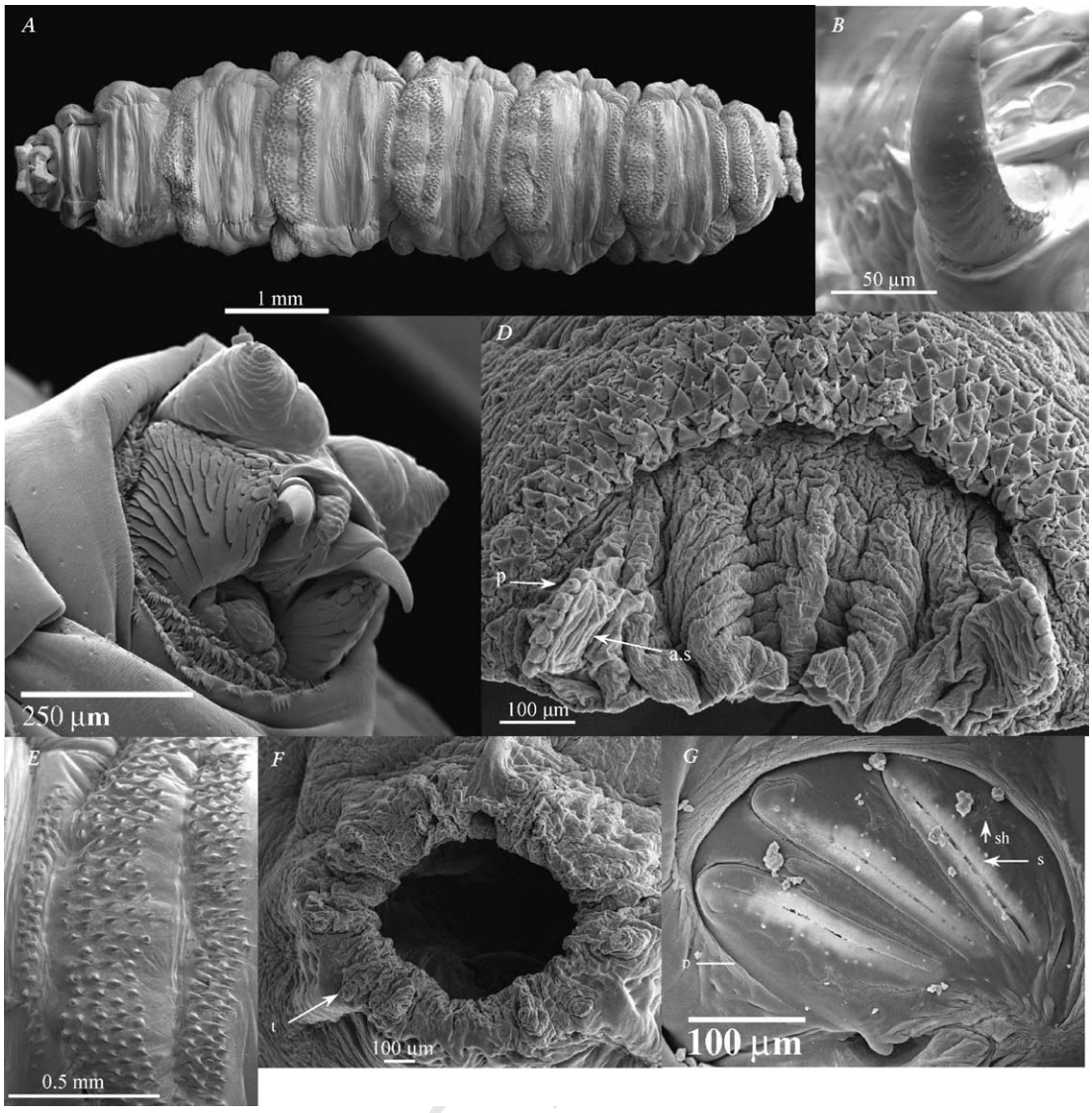

Fig. 1. *Sarcophaga hirticrus*. (A) general ventral view, (B) mouthhook, (C) pseudocephalon (a; antenna, or; oral ridges, p; palp, vo; ventral organ), (D) anterior spiracles (as, p; papillae) (puparium), (E) spinules, (F) spiracular atrium (puparium), (G) posterior spiracles (p; peritreme, s; slits, sh; spiracular hairs).

window. Dorsobasal lobe (dl) slightly marked near the apex of ventral cornu (Fig. 2).

3.2. *Sarcophaga javita (Peris, González-Mora and Mingo, 1998)*

3.2.1. *Overall appearance*

Length 11.46 ± 1.73 mm, maximum width 4.6 ± 0.42 mm ($n = 10$). Larvae are creamy white in colour, elongated, sub-cylindrical in cross-section with a flattened ventral surface, truncated posteriorly and tapering toward the anterior extreme.

The surface of thoracic and abdominal segments displays bands of spinules rounded in form. The inter-band areas are devoid of spinules.

3.2.2. *Pseudocephalon (Fig. 3(A), (B) and (D))*

The pseudocephalon has a pair of small antennae (a) in dorsal position and two palps (p) in inferior position (Fig. 3(A)).

The antennae are located in a depression and are formed by two segments, the apical (dome) sharpened at its end.

The palps are mammeliform, with concentric ribbons at the apical extreme. In the apical part there are three protuberance nominated maxillary palpus sensilla (mxpp) and exist two more situated at different levels (Fig. 3(D)). Ventral organs (vo) present.

At both sides of the mouth appear bands of little deep subparallel oral ridges (or). The base of the buccal cavity displays a pair of sensilium each of them with one setae and one hole (Fig. 3D).

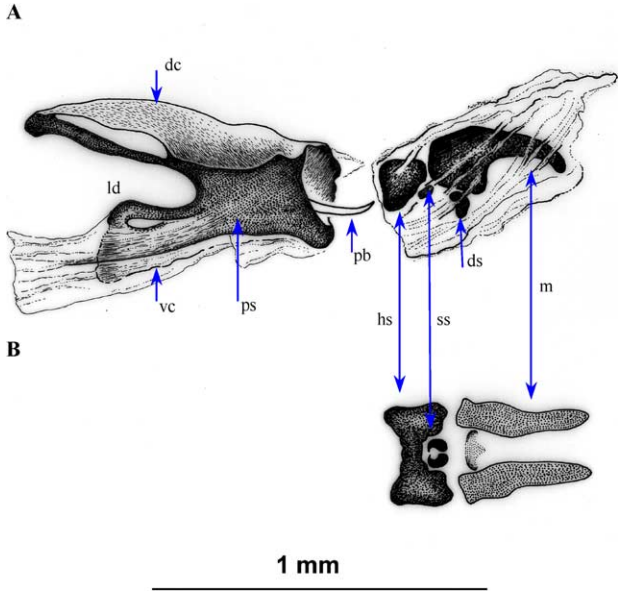

Fig. 2. Cephalopharyngeal skeleton of *S. hirticrus* ((A) lateral view, (B) dorsal view); *pb*: parastomal bar, *dc*: dorsal cornu, *vc*: ventral cornu, *ds*: dental sclerite, *ps*: pharyngeal sclerite, *hs*: hypopharyngeal sclerite, *ss*: subhypostomal sclerite, *dl*: dorsobasal lobe, *m*: mouthhook.

3.2.3. Thorax (Fig. 3(C), (E)–(G))

The prothorax is anteriorly surrounded by a band of acute spinules (Fig. 3(C)), grouped in a variable number and some of them with two apical points. Anterior spiracles fan-shaped. Each anterior spiracle contains a single row of papillae that number from 12 to 14 (Fig. 3(G)). The rest of the thoracic segments, with an anterior band of spinules encircling each segment. These spinules have a single end (Fig. 3(E) and (F)).

3.2.4. Abdomen (Figs. 3(H), 4(A)–(D))

Abdominal segments with anterior spinule bands one pointed and anteriorly projected. One of these bands narrower than the others and with the spinules posteriorly projected. Inter-band areas are devoid of spinules.

The posterior spiracles on the last eleventh body segment are set within a deep spiracular atrium. There are 12 tubercles (t) located on the rim of the atrium (Fig. 4(A)) each one with an apical setae (Fig. 4(D)). Spiracular atrium tapestried internally with spinules finished in a hook, sometimes grouped and none of them bifid (Fig. 4(C)). These spinules extended only as far as the inner circumference of the ring of tubercles. Posterior spiracles posterodorsal in the atrium, on a spiracular plate. Spiracles consisting of three elongate slits (s), oriented vertically, with the openings disposed radially, each with an incomplete sclerotized peritreme (p) (Fig. 3(H)). Ecdysial scar indistinct.

The anal segment (Fig. 4(B)) displays two postannals tubercles. The internal margin of these fleshy projections is covered with little sharp-pointed spinules. The apical stream of these fleshy projections shows a sensilia. A group of spinules appears at both sides of the insertion zone of the fleshy projections.

3.2.5. Cephalopharyngeal skeleton

Cephalopharyngeal skeleton deeply pigmented. Mouthhook (m) sickle-shaped, pointed apically. Dental sclerite (ds) present. Subhypostomal sclerite (ss) middle moon-shaped from above. Intermediate sclerite (hypopharyngeal sclerite, hs) short, longer than wide, H-shaped from above and not fused to the basal sclerite, with anterior arms tapering apically. Pharyngeal sclerite (ps) heavily pigmented with a pointed parastomal bar (pb). Extreme of pharyngeal sclerite incurved downwards. Dorsal cornu (dc) wider and longer than ventral cornu. Dorsal cornu with a narrow elongated apical window and opened in the apical extreme. Ventral cornu (vc) approximately twice longer than wide and apically truncated. Window reduced to a small split dorso-apically. Dorsobasal lobe (dl) slightly marked near the apex of ventral cornu (Fig. 5).

4. Discussion

Within the Sarcophagidae, larvae of some species of the genera *Oxysarcodexia* and *Ravinia* (Lopes and Leite, 1987), *Sarcophaga* (Aspoas, 1991; Kirk-Spriggs, 1999, 2000, 2003; Sukontason et al., 2003) and *Wohlfahrtia* (Ruiz-Martínez et al., 1989), have been described using of SEM. The increase of this kind of study is a consequence that the knowledge of the morphology of the immature stages receiving more attention due to their use in medico-forensic entomology.

The larvae of the two species described here, possess the morphological character states defined by Dahlem (1991) for the Sarcophagidae: 'deeply recessed posterior spiracles, mature larvae having three nearly vertical, parallel posterior spiracular slits arising from a ventral ecdysial scar (which is frequently indistinct or absent) and by the spiracular slits usually not pointing toward the opening in the peritreme'.

The cephalopharingeal skeleton is very similar in the two species studied as it is common in other saprophagous diptera (Rotheray and Gilbert, 1999), but there are some differences. The mouthhooks of *S. hirticrus* are proportionally larger and more curved than those in *S. javita*. Other authors have noted a limit in the mouthhooks retraction into the cephalic segment in other species such as *Wohlfahrtia magnifica* (Ruiz-Martínez et al., 1990). This character state should be analysed in other *Sarcophaga* species.

In *S. hirticrus* the ventral cornu of the basal sclerite has a small window, which is reduced to a small split in *S. javita*, whereas the parastomal bar is straighter in *S. javita*.

The presence of a central hook, as in *W. magnifica*, is possibly consistent with their parasitic nature (Ruiz-Martínez, 1990). It should also be interesting to describe the surface, ornamentation, shape and size of the mouthhooks in

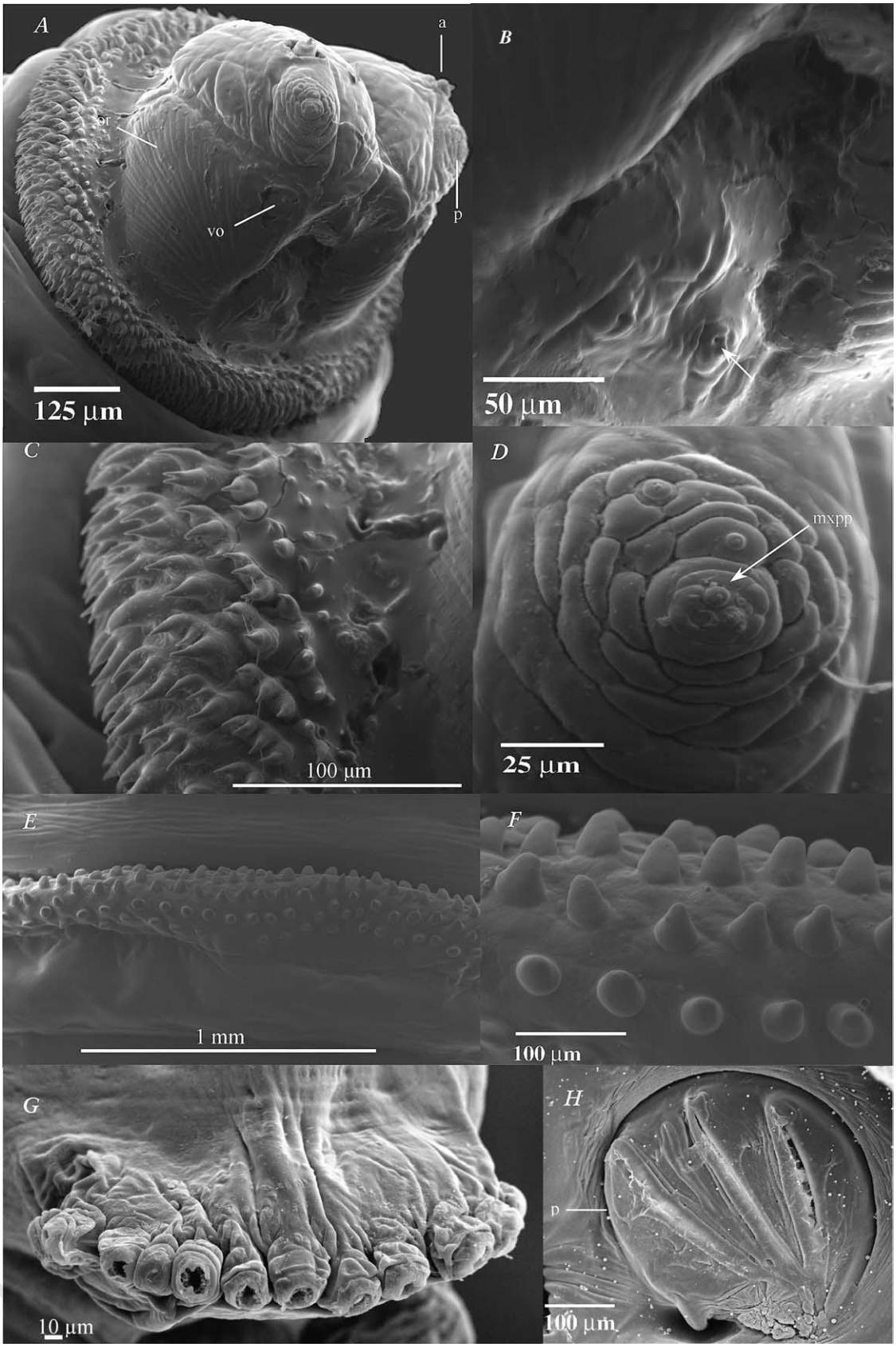

Fig. 3. *S. javita*. Pseudocephalon: (A) general view (*a*; antenna, or; oral ridges, *p*; palp, vo; ventral organ), (B) oral cavity amplified, (C) prothoracic spinules, (D) palp (*mxpp*; maxillary palpus sensilla). Thorax: (E) dorsal spinules, (F) spinules amplified, (G) anterior spiracle, (H) posterior spiracle (p; peritreme).

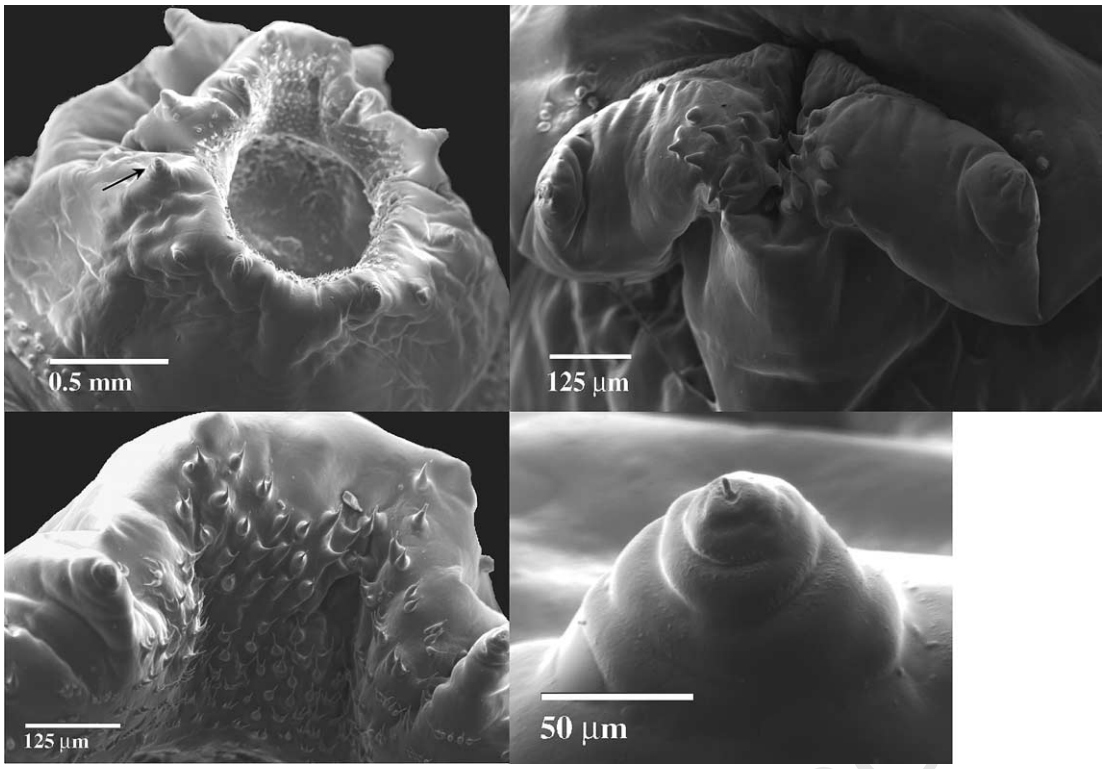

Fig. 4. *S. javita*. (A) tubercles of external border of the spiracular atrium, (B) postanal tubercles (C) internal atrium spinules, (D) tubercle amplified showing a setae.

detail in order to establish their possible relationship with feeding habits. In this sense *Wohlfahrtia magnifica* (Schiner, 1862), *S. dux*, *Sarcophaga argyrostoma* Robineau-Desvoidy, 1830 and *S. hirticrus* have an inferior cutting margin of the mouthhook (Ruiz-Martínez, 1990; Sukontason et al., 2003; Awad et al., 2003) in order to assist its penetration into carrion or wounds of animals, whereas *Ravinia belforti* show a mouthhook with delicate pits and ridges (Leite and Souza, 1987).

In the pseudocephalon of *S. hirticrus,* the palps and the antennae, are situated on a conical prominence. This morphology is also appreciated in other species such as *Sarcophaga forceps* Blackith and Blackith, 1988 (Kirk-Spriggs, 2000), however, this prominence is less pronounced in *S. javita*. Bearing in mind the differences found in the pseudocefalon of the species of Sarcophagidae, it would be interesting to illustrate this segment from the same angle always in order to facilitate comparison between species.

Although the morphology of the pseudocephalon has not usually been considered, according to Kirk-Spriggs (2003), the disposition and morphology of the oral ridges of the two species here described seem to be useful inter-specific character states.

The number of papillae in the anterior spiracles of these two species at L3 instar (7–14) is similar to that of other species of the same genera such as *Sarcophaga melanura*, *S. crassipalpis*, *Sarcophaga africa*, *Sarcophaga iota* (Johnston and Tiegs, 1921), *S. dux*, *Sarcophaga nodosa* Engel, 1925, *Sarcophaga inzi* Curran, 1934, and in species of other genera such as *Wohlfahrtia virgil* (Walker, 1849) and *Oxysarcodexia confusa* Lopes, 1946 (Leite and Lopes, 1987; Walker, 1920). This indicates that the exclusive use of this criterion is insufficient for a correct identification of the larvae, even at generic level. Kano and Sato (1951), indicate in *Sarcophaga* a slight variability in the number of papillae between individuals or even on each side of the same pupae. This variability also occurs in other genera such as

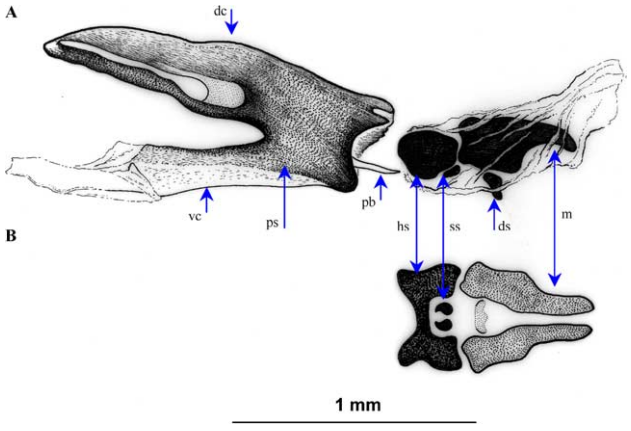

Fig. 5. *S. javita* cephalopharyngeal skeleton ((A) lateral view, (B) dorsal view); *pb*: parastomal bar, *dc*: dorsal cornu, *vc*: ventral cornu, *ds*: dental sclerite, *ps*: pharyngeal sclerite, *hs*: hypopharyngeal sclerite, *ss*: sub-hypostomal sclerite, *m*: mouthhook.

Wohlfahrtia, *Goniophyto* and *Ravinia* varying from 1 to 8 (Walker, 1937; Maurice et al., 1948; Kano and Sato, 1951; Ishijima, 1967; Ruiz-Martínez et al., 1990). However, the arrangement of the papillae in regular or irregular rows is constant at species level (Table 1) being numerous and disposed in several files in the Japanese species (Kano and Sato, 1951).

It is not possible to establish a clear relationship between the number and distribution of the papillae in the anterior spiracles and the feeding habits (Table 1).

The arrangement of the spinules in the spiracular atrium of *S. hirticrus* (as far as the outer circumference of the ring of tubercles) is similar to that found in *S. exuberans* (Aspoas, 1991), whereas in *S. javita*, *S. tibialis* and *S. dux* (Aspoas, 1991; Sukontason et al., 2003) these spinules are extended only as far as the inner circumference of the ring.

The disposition and shape of the slits on the posterior spiracles as well as the presence of an ecdysial scar are a useful tool in the identification of the Sarcophaginae species (Cantrell, 1981), with these characters being repeatedly described.

Due to the uniformity of morphological structures, many more species would need to be examined before any generic separation could be undertaken on the basis of larval morphology (Cantrell, 1981). However, differences in some characters of 3rd instar larva have been useful in the elaboration of keys at specific levels. The main characters used in the existing keys are: number of papillae in the anterior spiracles; pigmentation, arrangement and hardness of the spinules on the body segments and cephalopharyngeal skeleton (James and Gassner, 1947). Kano and Sato (1951) add the following characters: arrangement of papillae in the anterior spiracles, tubercles on upper border of anal segment, inner projections of peritreme and posterior spiracles (width and height, scar). Ishijima (1967), completes the keys adding: distance between posterior spiracles and morphology of slits. After using SEM, new characters have been incorporated into the species diagnosis: morphology of tubercles on the atrium rim and size of the fleshy projections on the anal segment (Lopes and Leite, 1987); number of rows, orientation and density of spinules in the body segments and spiracular hairs in posterior spiracles (Aspoas, 1991). Awad et al. (2003) take into account new ultrastructure characters: sensillar numbers and types, sizes and locations of the antennal-maxillary sensory complex.

As a general reflection from the preceding compilation and the new data provided in our descriptions, it can be concluded that the most useful morphological characters for diagnosis at specific level are: the structures of both, anterior and posterior spiracles, the morphology of the pseudocephalon (including oral ridges, antenna, maxillary palp, sensilla and ventral organ) as proposed by Kirk-Spriggs (2003) and the morphology and distribution of the spinules and sesilla in the body segments.

The immature stages of other species of *Sarcopaga* such as *Sarcophaga peregrina*, *S. melanura, S. africa, Sarcophaga misera* and *S. tibialis* have been described and related to snails and other feeding sources (Seguy, 1941; Ishijima, 1967; Berner, 1973; Parashar and Rao, 1988; Pérez-Moreno, 2004). Apparently, none of the character states of *Sarcophaga* larvae and puparia seem to be correlated with particular features of the breeding habits. In order to find a relationship between the feeding site and the larval morphology, similarities between those species should be sought in species with a specific feeding habit. Unfortunately, feeding habits of the genus *Sarcophaga* are very varied and there are not enough detailed descriptions realised by SEM to reach a conclusion (Table 1).

Kirk-Spriggs (2003) considers that in the case of the necrophagous *Sarcophaga namibia* Reed, 1974 the extensive overlapping oral ridges of the facial mask are consistent with saprophagy. The same character state occurs in *S. hirticrus*, also a necrophagous species. In this sense, it would be interesting to include the description of the oral ridges in future descriptions.

The early stage characters in other Diptera Cyclorrhapha as Syrphidae, have been informative phylogenetically because their larvae are interpreted to be conservative in evolution (Rotheray and Gilbert, 1999; Pérez-Bañón et al., 2003). The detailed description of early stage morphology in *Sarcophaga* species using SEM, could also be used in the future together with other character sets such as DNA sequences to estimate *Sarcophaga* phylogeny.

5. Uncited references

Ali-khan, 1974. Barfoot, 1969. Beaver, 1972. Blackith et al., 1928. Coupland and Baker, 1994. Saloña Bordas and Gónzalez-Mora, 2005. Tumrasvin and Kano, 1979.

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