CrossMark

ORIGINAL PAPER

Sarcophaga (*Liosarcophaga*) *tibialis* Macquart 1851 (Diptera: Sarcophagidae): micromorphology of preimaginal stages of a fly of medical and veterinary interest

Ángela Paños-Nicolás[1] · Mª Isabel Arnaldos[1] · Mª Dolores García[1] · Nicolás Ubero-Pascal[1]

Abstract Sarcophagids are a large family of Diptera, with a worldwide distribution. They are related to decomposing organic matter and are very interesting for health science and in forensic cases since many species produce myiasis and occur in human corpses. This family is considered difficult to study, particularly with regard to their immature stages, to which little attention has been paid. Genus *Sarcophaga* Meigen, 1826 is composed of species of very similar morphology, making very difficult to distinguish. Knowledge of the immature stages of this genus is important because such stages occupy the greater part of the life cycle, so that establishing a basis for their identification will increase their usefulness in systematic and applied sciences. This contribution presents a detailed study of the morphological features, both external and internal, of the preimaginal stages of *Sarcophaga* (*Liosarcophaga*) *tibialis* Macquart, 1851, providing a taxonomical context for the correct identification of *Liosarcophaga* species of forensic interest in the Iberian Peninsula. Both light and scanning electron microscopy were applied. Complete descriptions of every stage are provided and illustrated, and their usefulness for species comparison, taking into account our uneven knowledge of morphologically immature stages of this subgenus, is indicated. Features of the cephalopharyngeal skeleton, such as the shape of the mouth hook and the intermediate and basal sclerites, and external morphology, such as the pattern of spinose band and anterior and posterior spiracles, proved useful for separating species. Finally, tentative identification keys based on light microscopy observation to distinguish *S.* (*L.*) *tibialis* from other species of forensic interest belonging to *Liosarcophaga* subgenus are proposed for every immature stage.

Keywords Diptera · Identification keys · Ultramorphology · Immature · Cephalopharyngeal skeleton · SEM · Light microscopy

✉ Nicolás Ubero-Pascal
nubero@um.es

[1] Area of Zoology, Department of Zoology and Physical Anthropology, Biology Faculty, Murcia University, 30100 Murcia, Spain

Introduction

One of the problems in Medical and Veterinary Entomology, especially Forensic Entomology, is the difficulty of identifying the species involved since, as Barros and Antunes (2008) mention, few specialized taxonomists can identify the different species, and there is a lack of identification keys for some groups, even in the case of the most common species. One particularly complex group that remains to be studied is the family Sarcophagidae, a dipteran family involved in many forensic and myiasis cases. Sarcophagids can be looked upon in two ways because of their feeding habits. While some species are beneficial in many ecosystems, since they play an important role in the food chain as decomposers (Ferrar 1987), other species are harmful for higher animals, since they are parasitic or act as transmitters of diseases, which may have a severe economic impact in the case of livestock and increase health care costs in humans (Greene 1925; Knipling 1936; Greenberg 1971; Grassberger and Reiter 2002; Awad et al. 2003). Even those species that parasitize

Springer

invertebrates alone, e.g., some species of Miltogrammatinae, manifest this duality, because some of them may act as biological control agents of pests such as locusts and moths, but may also infest pollinators, such as bees and wasps (Ferrar 1987; Szpila 2010). At a forensic level, the necrophagous habits of some species are useful for detecting, among others, drugs and toxins in tissues (Goff and Lord 1994; Musvasa et al. 2001) but mainly for estimating the postmortem interval (PMI), the time elapsing between the death occurred, and the moment in which the corpse is found (Pape 1987; Arnaldos et al. 2004a). Such estimation can be made on the basis of several aspects, one of them being the duration of each larval stages and the age of the larvae breeding on the corpse. In this respect, knowledge of larval morphology is crucial for knowing the duration of each larval stage.

There are a number of papers in which Sarcophagidae have been discussed in relation to forensic cases, animal carrion, and myiasis both in animals and humans (Greene 1925; Judd 1956; Ali-Khan and Ali-Khan 1974; Leclercq 1976; Ruiz-Martínez et al. 1989; Aspoas 1991; Benecke 1998; Delir et al. 1999; Colwell and O´Connor 2000; Soler Cruz 2000; Castillo Miralbes 2002; Grassberger and Reiter 2002; Sukontason et al. 2003a, 2007, 2010; Romera et al. 2003; Mohammadzadeh et al. 2008; Sharma et al. 2008; Prado e Castro et al. 2010; Szpila et al. 2010; Velásquez et al. 2010; Jeffery 2011; Arnaldos et al. 2013; Bonacci et al. 2014; Rafinejad et al. 2014). Although fewer sarcophagid species seem to colonize corpses than those of other families (mainly Calliphoridae), those that do colonize them do so under the most diverse environmental conditions. For example, they colonize corpses out and indoors, in sunny or shaded sites, as well as in wet or dry ones, and can be found associated with carcasses in both the early and late stages of decomposition (Byrd and Castner 2001; Romera et al. 2003; Sukontason et al. 2003a; Wyss and Cherix 2006; Al-Mesbah et al. 2011; Raghavendra et al. 2011; Cherix et al. 2012; González-Medina et al. 2012). What all of these papers have in common is that species identification is based on adults, and it is necessary to breed immature specimens in lab. The current tendency is to try to characterize immature stages morphologically to allow species identification directly by applying microscopy techniques (Klong-klaew et al. 2012).

Following Aspoas (1991), larvae of Sarcophagidae are easily distinguishable at the family level and even at generic level but are very similar at lower levels. Traditionally, the main features that distinguish the larvae of Sarcophagidae from those of other Muscomorpha families are the spiracular cavity, surrounded by papillary projections, in which the hind respiratory spiracles are located and the open peritreme of posterior spiracles (Greene 1925; Pape 1996; Byrd and Castner 2001; Gennard 2007). However, Szpila et al. (2015) have recently questioned the representativeness of these features since they do not appear in all the species belonging to this family.

Species of genus *Sarcophaga* Meigen, 1826 are very similar morphologically and difficult to identify at species level both at larvae and adult stages. To identify larvae, the most widely used features to date have been the morphology of fore spiracles and the number of their papillae, the shape of the spiracular cavity, the shape of the peritreme of the hind spiracles, spiracular tufts, the cephalophayngeal skeleton, spinulation and the sculpturing pattern of interbands, and the pseudocephalon features (Cantrell 1981; Aspoas 1991; Pérez-Moreno et al. 2006; Velásquez et al. 2010; Ubero-Pascal et al. 2015), but the larval morphology of many species is still unknown. A critical analysis of the taxonomical usefulness of all these features has been recently given by Szpila et al. (2015). Enlarging our knowledge of distinguishing features and establishing identification keys will help in the identification of species involved in forensic cases (Greene 1925; Knipling 1936; Smith 1986; Aspoas 1991; Byrd and Castner 2001; Szpila 2010; Velásquez et al. 2010; Szpila et al. 2015; Ubero-Pascal et al. 2015).

Sarcophaga (*Liosarcophaga*) *tibialis* Macquart, 1851 is a species distributed throughout the Afrotropical Region, Madagascar, and some areas of Europe (Zumpt 1965; Pape 1996; Villet et al. 2006). The characteristics of its life cycle have been studied under certain conditions (Abasa 1970; Aspoas 1991; Musvasa et al. 2001; Villet et al. 2006; Arnaldos et al. unpublished data). It has been reported to breed in carrion and faeces and is known to cause traumatic dermal myiasis (Zumpt 1965; Aspoas 1991; Musvasa et al. 2001) making it significant for forensic purposes as well as for medico-legal ones since it can act as a vector of disease (Zumpt and Patterson 1952). Although, to date, it has not been found related to human corpses, it has been reported as very frequent in animal carcasses in Spain and, in fact, is the commonest and most abundant sarcophagid species on animal corpses in the southeast of the Iberian Peninsula, and is able to breed on the same (Castillo Miralbes 2002; Martínez Sánchez 2003; Romera et al. 2003; Arnaldos et al. 2004b). Velásquez et al. (2010) consider it to be of forensic interest and include it in the identification key they propose for mature larvae but, to the best of our knowledge, little information is available on the morphology of all its preimaginal stages.

The aim of this work is to describe in detail the micromorphology of all the immature stages of S. (L.) *tibialis* using both optical and scanning electron microscopy. These two techniques have shown to be complementary and useful for studying larval micromorphology (e.g., Ubero-Pascal et al. 2010; Szpila and Villet 2011) and will allow the most relevant features for distinguishing *Liosarcophaga* species to be established. Light microscopy will also be used to propose a tentative identification key for each preimaginal stage, but especially for the third larval stage as a contribution to extending the key for flesh flies of forensic importance proposed by Szpila et al. (2015) to the Mediterranean area.

Material and methods

Larvae of *S. (L.) tibialis* were collected in a periurban environment from a plot in the Servicio de Experimentación Agroforestal of the University of Murcia during April 2012 using a modified Schoenly trap (Arnaldos et al. 2001) baited with a piglet carcass. Larvae were kept in laboratory conditions until the adults emerged. Selected adult specimens were held in the laboratory under controlled environmental conditions (25 °C, 60 % RH, and 12 h L/D photoperiod) in a Sanyo MLR-350 growth chamber. All the studied immature specimens come from these sister colonies. The analyzed material, as well as the adults, is kept at the Forensic Entomology Laboratory at the Department of Zoology and Physical Anthropology of Murcia University (Spain).

The immature specimens selected were rinsed and euthanized in water near to boiling point—except puparium—according to EAFE guidelines (Amendt et al. 2007). For scanning electron microscopy (SEM) analysis, 55 specimens—15 larvae of each instar and 10 puparium—were fixed in McDowell fixative solution, dehydrated in series of increasing concentration of ethanol, air-dried after hexamethyldisilizane treatment, and prepared for observation following the procedural given by López-Esclapez et al. (2014). For light microscopy analysis, 60 specimens—20 larvae of each instar; pupae were not studied by this technique—were fixed in McDowell reagent and preserved in 70 % ethanol. The procedural for clearing and mounting the larvae is described by Paños et al. (2013).

The systematic classification of Sarcophagidae given by Pape (1996) has been followed. To describe the morphological characteristics of immature stages and the cephalopharyngeal skeleton, the body divisions and the terminology given by Cantrell (1981), Courtney et al. (2000), and Ubero-Pascal et al. (2015) were followed.

The descriptions of immature stages given below are mainly based on SEM observations, except for the cephalopharyngeal skeleton. However, many of the structures described by this technique are also recognizable by light microscopy once their morphology is known by SEM. As light microscopy is still the main technique used by forensic entomologists (Szpila and Villet 2011; Ubero-Pascal et al. 2010), the morphological features have been illustrated with pictures of both microscopy techniques insofar as has been possible. In fact, the identification keys proposed are only based on light microscopy observations to allow a more general use.

Results

S. (L.) tibialis larvae show a typical muscoid shape in which the three regions established by Courtney et al. (2000) can be distinguished. Larvae are whitish-yellow and slightly sclerotized in the first instar, but they become dark and hardened as the larval cycle progresses. According to Pape (1996), Sarcophagid larvae are easily distinguishable from the other Diptera of forensic interest by their posterior spiracles within an integument cavity—the spiracular cavity—moreover, second and third instar larvae show an incomplete peritreme without a distinct ecdysial scar—button. Although Sarcophagids are mostly larviparous, *S. (L.) tibialis* can lay pharate larvae that soon shed the thin chorionic envelope with no external sculpturing (Fig. 1a).

Micromophology of the first instar larva

The pseudocephalon is slightly split into two frontal cephalic lobes, each with a two-segmented antenna in dorsal position and a laterofrontal maxillary palpus (Fig. 1b and Fig. S1a, b of online resources). The mouth opens ventrally and is surrounded, from anterior to posterior position, by the ventral organs, the oral ridges, and the labial organ (Fig. 1b and Fig. S1a). The basal ring and dome of the antenna are approximately of the same length, but the proximal segment is at least twice the size of than the distal one (Fig. S2a, online resources). The lateral sensillum of the basal ring is placodea-like, and it is associated with a pit sensillum (Fig. S2a), while other pit sensilla appear around both the tip of the basal ring and the base of the dome. The sensillar area of the maxillary palpus is surrounded by three to five concentric ridges of tegument and consists of a central cluster of sensilla and, dorsally displaced, two additional coeloconic sensilla and a pit sensillum (Fig. 1c and Fig. S1c). The central cluster presents six large sensilla—three ceoloconic and three basiconic arranged alternately, one of them elongated—and at least five small coeloconic sensilla among them (Fig. 1c). The ventral organ is a rounded structure with a transversal split that is partially covered dorsally by a large peak-like projection (as long as the diameter of the whole structure) and five short finger-like projections (Fig. 1d and Fig. S1c); the split presents one pit sensillum in the middle and two placodea-like sensilla at the ends (Fig. 1d). Three longitudinal striae appear between the cephalic lobe and the ventral organ, close to the mouth opening (Fig. 1b and Fig. S2b); Draber-Monko et al. (2009) describe a similar structure in *Sarcophaga (Liopygia) argyrostoma* (Robineau-Desvoidy, 1830). In this area of the mouth opening, the tips of the mouth hooks are often visible outside the larva (Fig. 1b and Fig. S2b). Two simple oral ridges, reaching the laterodorsal area of the pseudocephalon, flank the mouth opening (Fig. 1b). The labial organ shows a rounded tip and two labial lobes disposed obliquely; each one bears a long coeloconic sensillum (Fig. S2c).

The cephalopharyngeal skeleton is well sclerotized and composed of a pair of mouth hooks, a pair of dental sclerites, a labrum, an anterior labial sclerite, a pair of posterior labial sclerite, an intermediate sclerite, a pair of parastomal bars, and
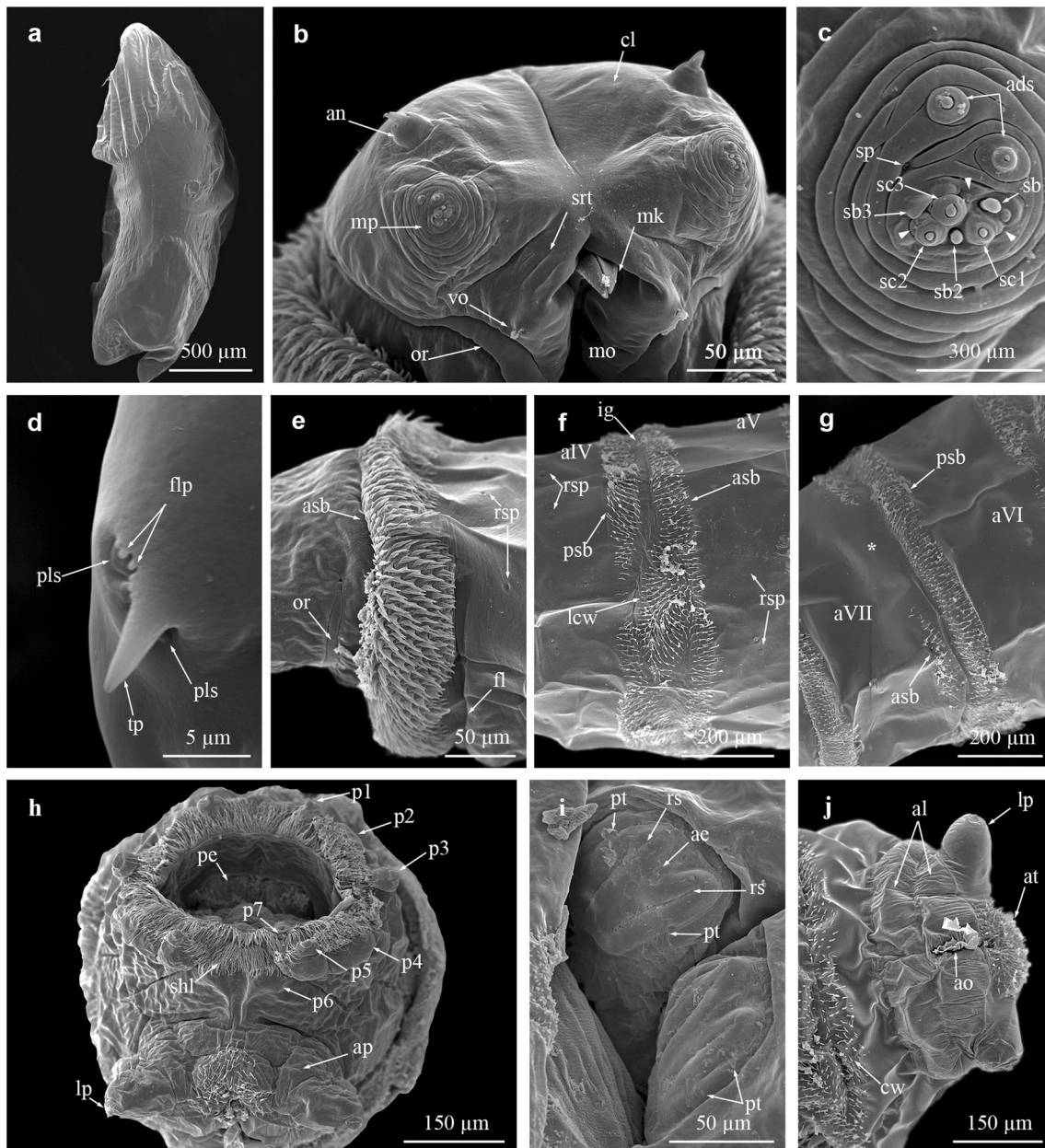
Fig. 1 Morphology of the egg and first instar larva of *S.* (*L.*) *tibialis* by SEM. **a** Egg. **b** Frontal view of pseudocephalon. **c** Maxillary palpus. **d** Ventral organ. **e** Lateral view of anterior spinose band of tI. **f** Lateral view of spinose bands between aIV and aV. **g** Dorsal view of spinose bands between aVI and aVII. **h** Posterior view of anal division. **i** Detail of posterior spiracles. **j** Ventral view of anal pad. *aIV-aVII* abdominal segments, *ads* additional sensilla, *ae* non-functional respiratory slit, *al* anal lips, *an* antenna, *ao* anal opening, *ap* anal pad, *asb* anterior spinose band, *at* anal tuft, *cl* cephalic lobe, *cw* ventral creeping welt, *fl* folding, *flp* finger-like projection, *ig* intersegmental groove, *lcw* lateral creeping welt, *lp* anal papilla, *mk* mouth hook, *mo* functional mouth opening, *mp* maxillary palpus, *or* oral ridge, *p1-p7* posterior papillae, *pe* posterior spiracles, *pls* placoid-like sensillum, *psb* posterior spinose band, *pt* peristigmatic tuft, *rs* respiratory slit, *rsp* ring of pit sensillum in interbands, *sb1-3* basiconic sensilla of maxillary palpus, *sc1-3* coeloconic sensilla of maxillary palpus, *shl* hair-like spines, *sp* pit sensillum, *srt* striae, *tp* triangular projection, *vo* ventral organ, *arrowheads* additional coeloconic sensilla of maxillary palpus, *asterisk* discontinuity anterior spinose band in dorsal area of aVII

a pair of lateral plates (Fig. 2a, b and Fig. S1d, e). The tooth of the mouth hook is twice the length of the basal part, almost rectilinear with only the distal third curved (Fig. 2a); the tip also has triangular cross section (Fig. S2b and Fig. S1c). The basal part of the mouth hook is quadrangular in shaped with the dorsal edge appearing as a double hump and the ventral edge concave (Fig. 2a). The inner surface of each basal part is dorsally expanded in a triangular process, which partially holds the labrum sclerite (Fig. S1e). At the ventral tip of the basal part of each mouth hook, a small and bar-shaped dental sclerite articulates (Fig. 2a and Fig. S1d); it is often difficult to observe from a lateral view since it is slightly displaced toward
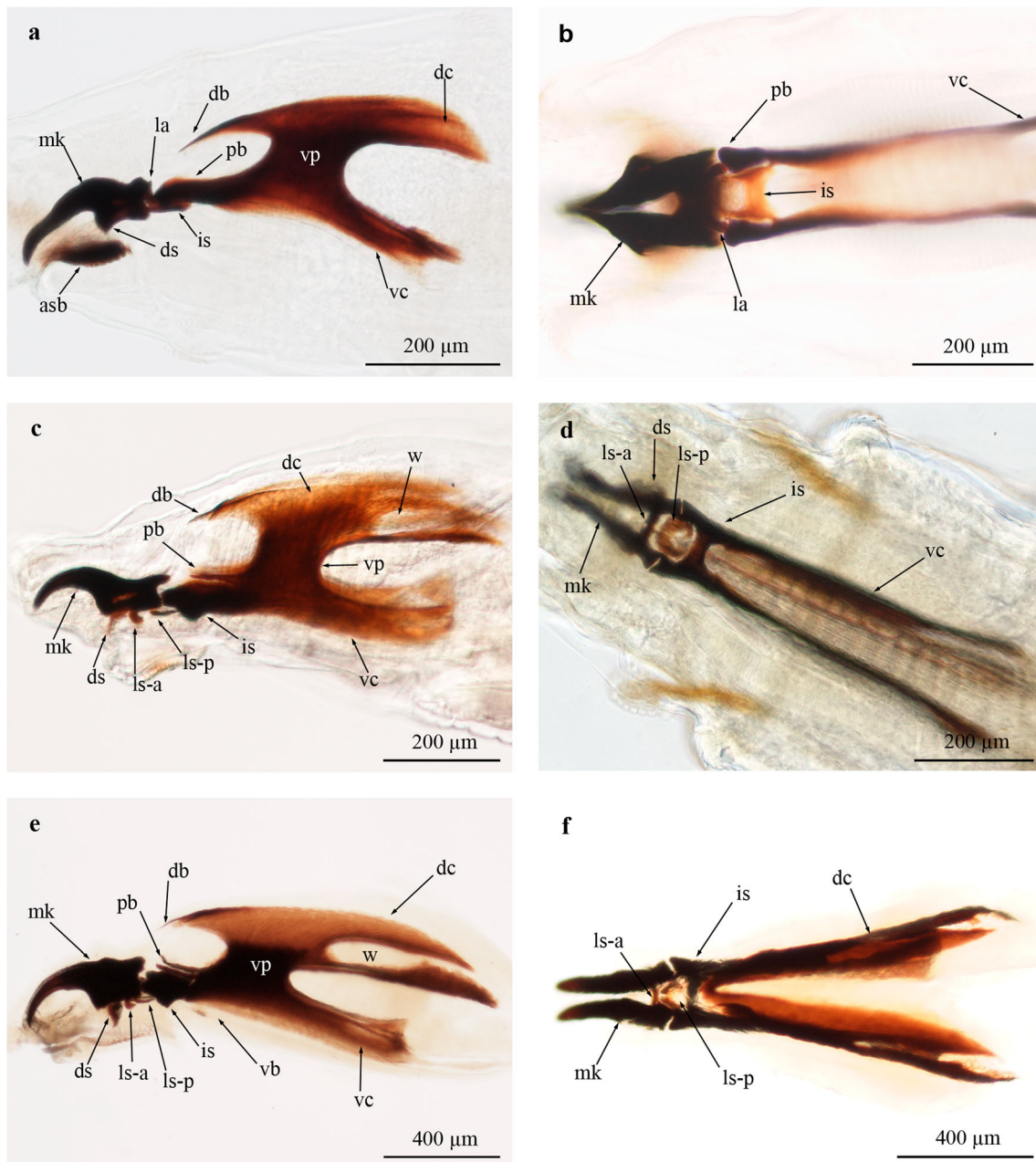
Fig. 2 Morphology of cephalopharyngeal skeleton of *S.* (*L.*) *tibialis* in the first (**a–b**), second (**c–d**), and third (**e–f**) instar larvae. **a**, **c**, and **e** General arrangement of sclerites in lateral view. **b** General arrangement of sclerites in dorsal view. **d** and **f** General arrangement of sclerites in ventral view. *asb* anterior spinose band, *db* dorsal bridge, *dc* dorsal cornu, *ds* dental sclerite, *is* intermediate sclerite, *la* labrum, *ls-a* anterior labial sclerite, *ls-p* posterior labial sclerite, *mk* mouth hook, *pb* parastomal bar, *vb* ventral bridge, *vc* ventral cornu, *vp* vertical plate, *w* window

the inner surface of the mouth hook. The pseudocephalon is usually rolled back, producing a sclerotized-like artifact under the mouth hooks (Fig. 2a and Fig. S1a), which is actually formed of well-sclerotized spines that overlap due to the folding of the anterior spinose band of first thoracic segment. The anterior labial sclerite is a transverse arch, the branches of which are closer to the inner surface of the mouth hook and the ventral tips turn backwards (Fig. S1f); it is difficult to appreciate since it is practically covered by the mouth hook. The labrum sclerite is also a transverse arch, the dorsal area of

which is wide, and the ventral tips articulate the parastomal bar (Fig. S1e–h); this sclerite is only visible in dorsal or ventral views, except when the mouth hook is slightly displaced with respect to the parastomal bars (Fig. 2a and Fig. S1d–h). The intermediate sclerite is ventral to parastomal bar (Fig. 2a and Fig. S1d), but both sclerites are sometimes so close that it is difficult to differentiate them in lateral view. The intermediate sclerite is forked anteriorly, the tips of the arms is thickened and converge, and the posterior edge of the bridge area is concave (Fig. S1f–h). The posterior labial sclerites are two

rounded structures between the forks of the intermediate sclerite; these sclerites appear diffuses due to their weak sclerotization (Fig. S1e–g). The parastomal bars are two robust sclerites fused with the lateral plates (Fig. 2a, b). A wide vertical plate—twice as wide as the ventral cornu—and a ventral and dorsal cornua without windows constitute each lateral plate (Fig. 2a). The posterior end of ventral cornu is forked (Fig. 3a) and the dorsal edge of the dorsal cornu is rounded (Fig. 2a). A thin pointed dorsal bridge fuses the lateral plates (Fig. 2a), the length of which almost reaches the level of the anterior tip of the parastomal bar. A descriptive picture interpreting the cephalopharyngeal skeleton arrangement in this larval stage is provided in Fig. S3a, b of online resources.

The three thoracic segments present a complete anterior spinose band, the width of which decreases from the first (tI) to the third segment (tIII). The spinose band of tI is almost twice as wide ventrally as dorsally (Fig. 1e), while in tII and

tIII, the dorsal and ventral widths are similar size. The size of the spines in the spinose band also decreases from the intersegment to the interband areas (Fig. S2d). The spines are well sclerotized, especially in the distal third (as in Fig. S1i). The general appearance of the spines varies from conical and thin—three times as long as the width of the base—in the dorsal area (Fig. S2d), to thorn-like—flattened laterally with a wide base and slightly curved at the tip—in lateral and ventral areas (Fig. 1e). Ventrally, especially in the spinose band of tI, the spines are close together and may share the same base (Fig. S2e); in segments tII and tIII, they can take on the appearance of a comb or fork. A pair composed of trichoid sensilla and Keilin's organ appears ventrally in each thoracic segment (Fig. S2f). The hairs of the Keilin's organs are thinner and longer than those in *Liopygia* species (Draber-Monko et al. 2009, Fig. 22). A ring of pit sensilla also appears in the interband area (Fig. 1e). The
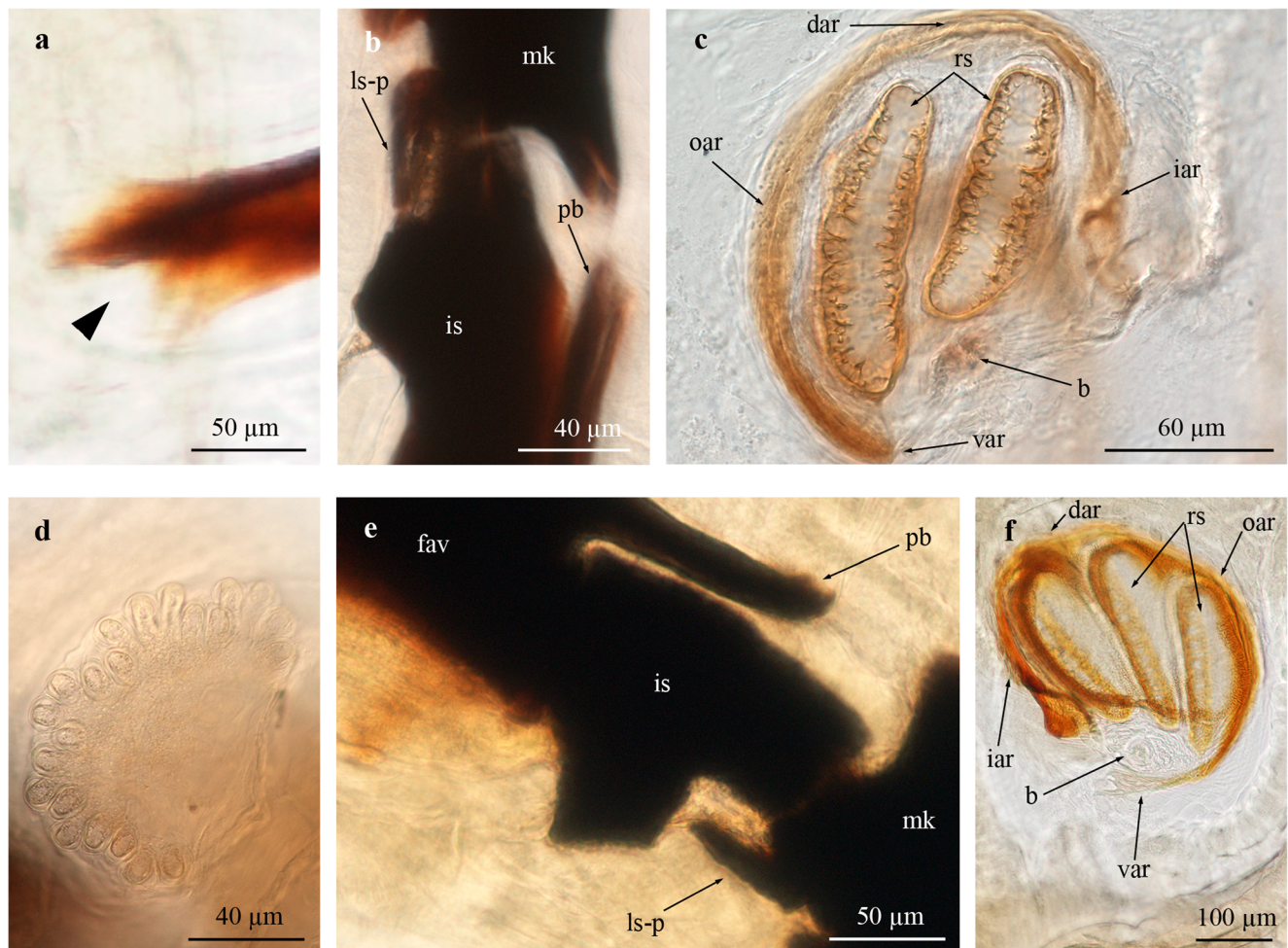
Fig. 3 Selected features to be applied at the identification keys of first (**a**), second (**b–c**), and third (**d–f**) instars larvae. **a** Posterior tip of ventral cornua. **b** Lateral view of central area of cephalopharyngeal skeleton. **c** Detail of posterior spiracle. **d** Detail of anterior spiracle. **e** Lateral view of central area of cephalopharyngeal skeleton. **f** Detail of posterior spiracle.

b button, *dar* dorsal arch of posterior spiracle, *fav* foremost arm of ventral cornu, *iar* inner arch of posterior spiracle, *is* intermediate sclerite, *ls-p* posterior labial sclerite, *mk* mouth hook, *oar* outer arch of posterior spiracle, *pb* parastombal bar, *rs* respiratory slit, *var* ventral arch of posterior spiracle, *arrowhead* tip forked

anterior spiracles, in the form of a simple transverse indent, are present on both sides of tI in a laterodorsal position, near the intersegmental groove (Fig. S2g).

The general morphology of every abdominal segment (aI to aVII), except the anal division, is very similar but shows the following pattern of spinose bands: (a) the anterior spinose bands are complete in segments aI to aVI (Fig. 1f), but the number of rows of spines decreases dorsally; in segments aVI, there is a patch without spines in the lateral area and in segment aVII, and the anal division does not have spines in the dorsal and lateral areas (Fig. 1g); (b) the posterior spinose band is incomplete in aI and aII, with only a single row of spines laterally in the latter segment, while it is complete from aIII to aVII (Fig. 1f), the number of spine rows gradually increasing in the dorsal area. Ventral creeping welts are present in all the segments, each one composed of three branches of spines (Fig. S2h), except segment aI with only two branches. Lateral creeping welts (Fig. 1f) are present from aII to the anal division. A small hole among the spines of the anterior spinose bands also appears in each segment. According to Courtney et al. (2000), these might be non-functional lateral spiracles. The spines are well sclerotized around the spinose band (Fig. S1j, k). While the spines are filiform with a swollen base in dorsal and lateral areas (Fig. S1k and Fig. S2i), even in lateral creeping welts, those of the ventral creeping welts are conical with the tip slightly curved (Fig. S1j and S2j). A ring of pit sensilla, similar to that observed in the thoracic segment, also appears in the interband area of each abdominal segment (Fig. 1f).

The spiracular field of the anal division shows the typical cavity of sarcophagids, within which the posterior spiracles lie (Fig. 1h). The edge of the spiracular cavity is surrounded by a band of hair-like spines and a ring of posterior papillae—three pairs in the dorsal arch and four pairs in the ventral arch (Fig. 1h and S1l). The odd posterior papillae are distinct bulges with a trichoid sensillum at the tip (Fig. S2k). However, the even posterior papillae are poorly developed and can be identified by the position of their pit sensilla (Fig. S2k). The seventh papilla is found in the spinose band (Fig. 1h). The dorsal vault of the spiracular cavity is indented, and the posterior spiracles appear in its deepest area (Fig. 1h). The posterior spiracle is almost quadrangular, well sclerotized, with two functional respiratory slits and one non-functional slit in between (Fig. 1i). Each respiratory slit, even the non-functional one, bears one peristigmatic tuft, except the outermost, which bears two tufts. The peristigmatic tuft consists of a wide stem usually ending in two short branches (Fig. S2l). The anal field consists only of the anal pad flanked by two anal papillae (Fig. 1h). Dorsally, the anal pad bears a small patch of filiform spines (Fig. 1h)—anal tuft according to Courtney et al. (2000)— and two membranous lips in the ventral area (Fig. 1j). The true anal opening is a simple longitudinal indent in the middle of the posterior membranous lip (Fig. 1j).

Micromophology of the second instar larva

Morphologically, the second instar larva is very similar to the first stage described above, but some differences must be highlighted. As regards the pseudocephalon (Fig. 4a and Fig. S4a of online resources), (1) the dome of the antenna is smaller than the basal ring—approximately half the length and width—(Fig. S5b, online resources); (2) the concentrical ridges of maxillary palpus are shorter and more numerous (Fig. 4b and Fig. S4b); (3) a row of scales separates the cephalic lobe and the oral ridges (Fig. 4a and Fig. S5a); (4) the ventral organ protrudes, almost cylindrical in shape, with two or three finger-like projections (Fig. 4c); (5) the oral ridges are more numerous (Fig. 4a); and (6) the longitudinal striae have disappeared.

The cephalopharyngeal skeleton is strongly sclerotized and composed of the same sclerites as in the previous larval stage, although their shape and arrangement clearly varies, especially in the intermediate area (Fig. 2c, d). The tooth and basal part of mouth hook have the same length, but while the tooth is bent, the basal part is rectangular with a prominent dorsoposterior process (Figs. 2c and 3b); the inner triangular projection of each basal part is highlighted (Fig. S4f). The dental sclerite is comma-shaped, but frequently extends ventrally, resembling a gun trigger (Fig. 2c). The anterior labial sclerite lies between the basal part of the mouth hooks in the form of a transverse arch, the ventral tips of which are slightly backwards (Fig. 2c and Fig. S4d–e). The posterior labial sclerites are kidney shaped, with two oval windows disposed obliquely (Fig. 2d and Fig. S4e). In this larval stage, the intermediate sclerite is located between the mouth hooks and basal sclerites, displacing the parastomal bars upwards, so that they only join with the basal sclerites (Fig. 2c, d). The intermediate sclerite is H-shaped with the anterior and posterior arms of the same length (Fig. 2d); the central bridge is projected ventrally and truncate obliquely in a forward direction (Fig. 3b). The basal sclerite shows a curved dorsal edge, a wide vertical plate, and the dorsal and ventral cornua are practically of the same length (Fig. 2c). The dorsal cornu shows an oval window that opens posteriorly (Fig. 2c). The ventral cornu also shows a window but is rectilinear (Fig. 2c). A descriptive picture interpreting the cephalopharyngeal skeleton arrangement in this larval stage is provided in Fig. S3c, d of online resources.

As regards the thorax, in comparison with the previous larval stage, the spinose band of tI is incomplete, because the spines in the dorsal area are separated from those in lateral areas (Fig. 4a), and a posterior secondary band of spines appears in the ventral area separated from the main spinose band by a tegumental fold (Fig. S5c). The spines in all the thoracic spinose bands are well sclerotized, especially in their distal third (Fig. S4c), and are conical in shape—three times the width of the base—(Fig. S5d), although in the dorsal area,
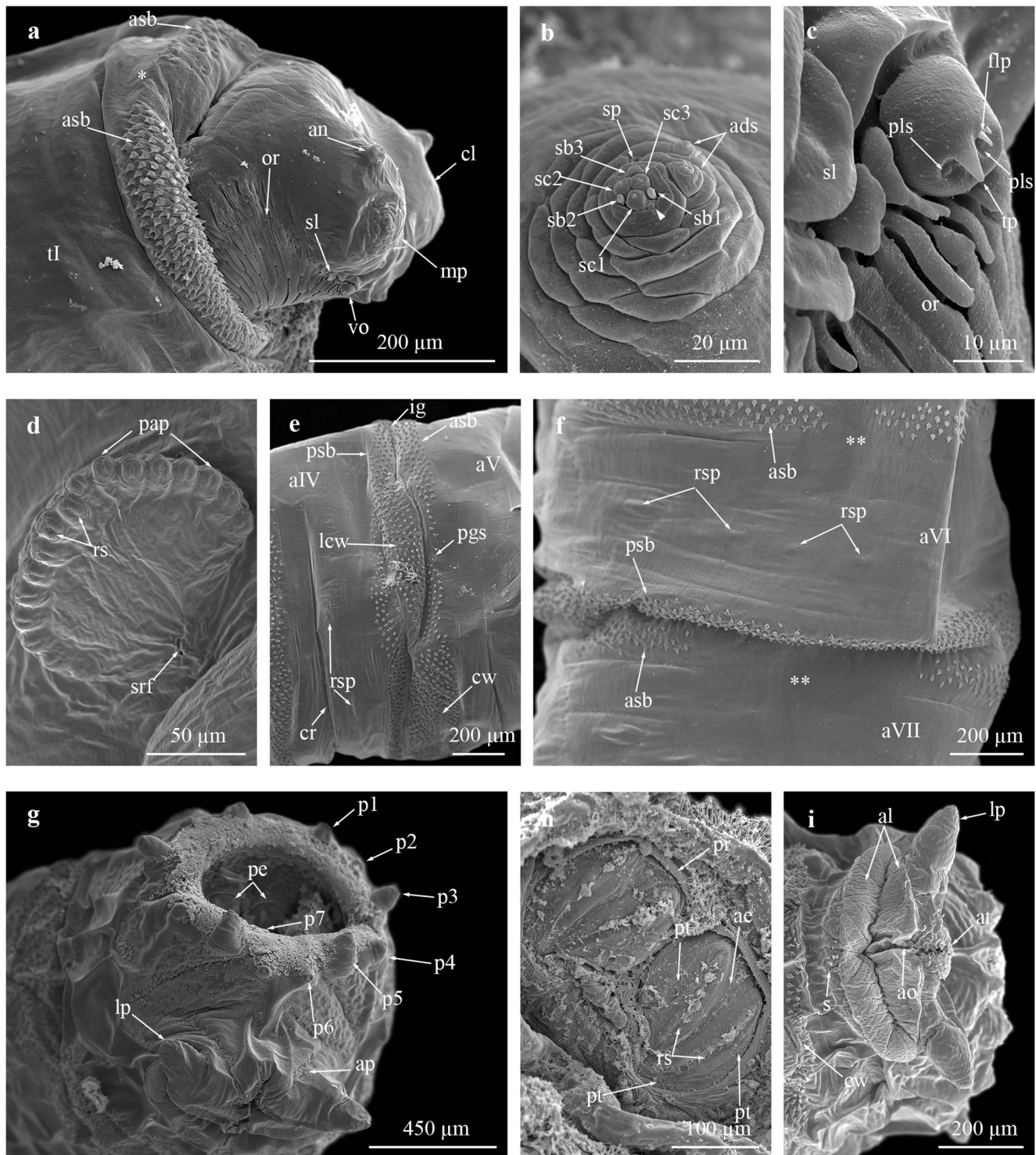
Fig. 4 Morphology of the second instar larva of *S. (L.) tibialis* by SEM. **a** Lateral view of pseudocephalon. **b** Maxillary palpus. **c** Ventral organ. **d** Anterior spiracle. **e** Lateral view of spinose bands between aIV and aV. **f** Dorsal view of spinose bands between aVI and aVII. **g** Posterior view of anal division. **h** Detail of posterior spiracles. **i** Ventral view of anal pad. *aIV-aVII* abdominal segments, *ads* additional sensilla, *ae* non-functional respiratory slit, *al* anal lips, *an* antenna, *ao* anal opening, *ap* anal pad, *asb* anterior spinose band, *cl* cephalic lobe, *cr* crevice, *cw* ventral creeping welt, *flp* finger-like projection, *ig* intersegmental groove, *lcw* lateral creeping welt, *lp* anal papilla, *mp* maxillary palpus, *or* oral ridge, *p1-p7* posterior papillae, *pap* papillae of anterior spiracle, *pe* posterior spiracles, *pgs* membranous-like globular papilla, *pls* placoid-like sensillum, *pr* peritreme, *psb* posterior spinose band, *pt* peristigmatic tuft, *rs* respiratory slit, *rsp* ring of pit sensillum in interbands, *s* spines, *sb1-3* basiconic sensilla of maxillary palpus, *sc1-3* coeloconic sensilla of maxillary palpus, *sl* scales, *sp* pit sensillum, *srf* scarf, *tI* first thoracic segment, *tp* triangular projection, *vo* ventral organ, *arrowheads* additional coeloconic sensilla of maxillary palpus, *asterisk* discontinuity of anterior spinose band in laterodorsal dis, *double asterisk* discontinuity of anterior spinose band in dorsal area of aVI y aVII

they seem globose with a small tip (Fig. S5e); in ventral and lateral areas, it is possible to find spines joined at the base, forming comb-like or forked structures (Fig. S5c). The anterior spiracle is a rounded structure (Fig. 4d), with a variable number of respiratory papillae, the most frequently 16–17 papillae, although some specimens may have 15–20 papillae. The respiratory papillae are aligned to form one main row, although one to three of the central papillae may be displaced, constituting a second alignment (Fig. 4d and Fig. S4g) that cannot always be considered as a second row. This irregular alignment of the papillae is difficult to distinguish in specimens with a low number of papillae in the anterior spiracle.

The spinose pattern of the abdominal segments of the first instar larvae is retained in this larval stage (Fig. 4e, f), but the shape of the spines differs slightly. They are conical with a curved tip (Fig. S5f), while some of them may seem globose (Fig. S5g); both types of spines a well sclerotized distal third (Fig. S4h, i). Instead of the lateral hole observed in first instar larvae, a membranous-like globular papilla appears (Fig. 4e and Fig. S5g). The ring of pit sensilla in the interbands is more prominent in the ventral area and in the last segments (Fig. 4e, f). Regarding anal division (Fig. 4g), all the posterior papillae are prominent, but the sixth and seventh pairs are considerably smaller than the other pairs (Fig. S5i). The hair-like spines of the spiracular cavity edge are swollen at the base (Fig. S5j). The posterior spiracles are round with two functional respiratory slits and one non-functional slit, between them; all of them are straight and converge ventrally (Fig. 4h and Fig. S4i). The peristigmatic tufts of the respiratory slits show a wide base and short branches, but in the non-functional slit is paintbrush-like (Fig. S5k). The peritreme surrounding each spiracular plate is well sclerotized and incomplete, with a button (Fig. 3c). Following Cantrell (1981), the outer, inner, and dorsal arches of the peritreme are well differentiated, and all of them are curved (Fig. 3c); the ventral arch is poorly developed or undistinguishable. The anal papillae are long and divergent; the anal tuft only surrounds the anal opening area, and some spines appear near the anterior membranous anal lip; this lip are wider than the posterior one (Fig. 4i).

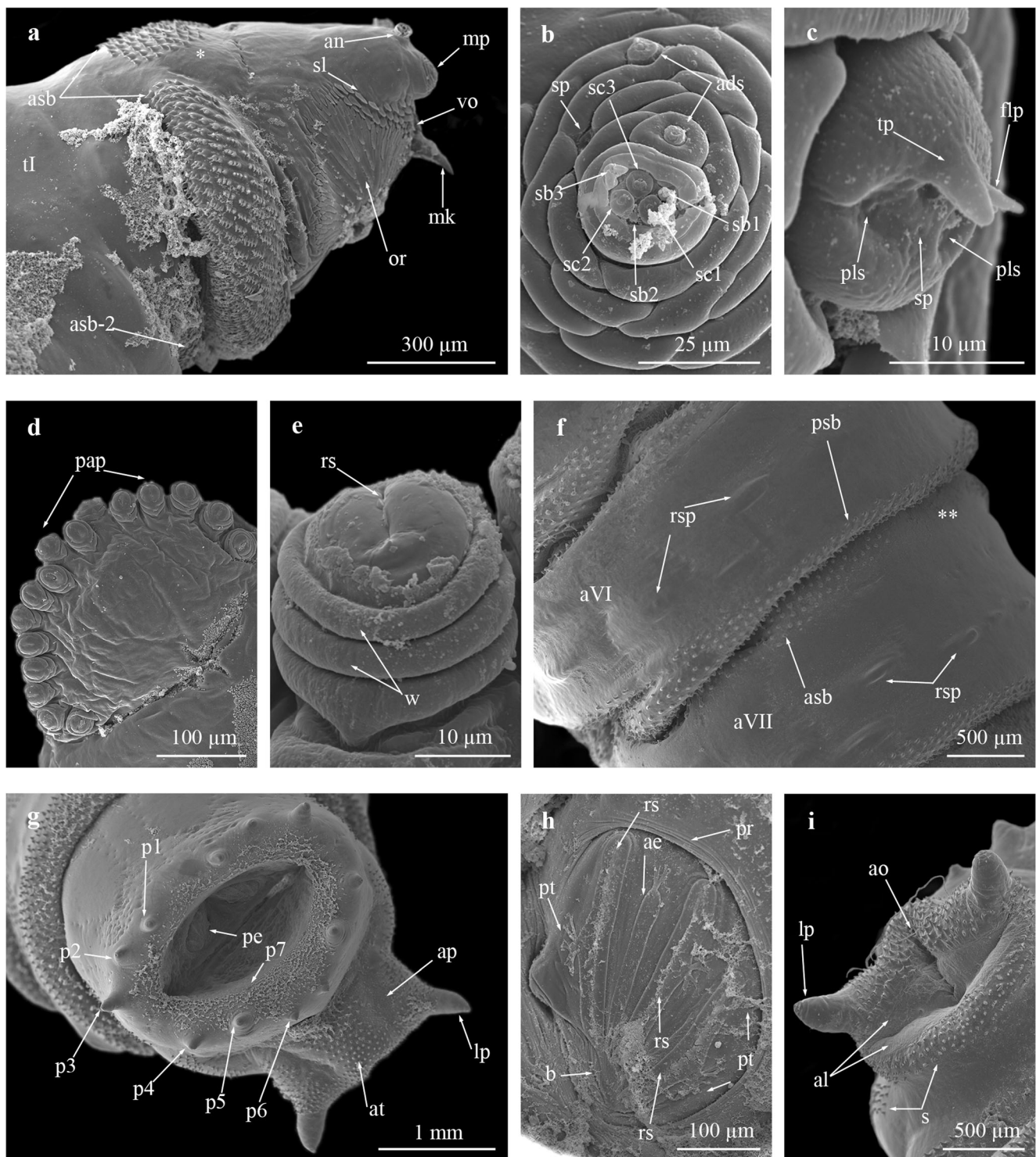**Micromophology of the third instar larva**

The morphology of second and third instar larvae is very similar, but, in general, the latter is more robust and bigger (Fig. 5a and Fig. S7b, online resources). Of note is the fact that, in the pseudocephalon, the antenna, maxillary palpus, and ventral organ are arranged in the same way (Fig. 5b, c, Fig. S6a, online resources, and Fig. S7a). However, there are also some differences such as (1) the cephalic lobes of the pseudocephalon are clearly separated, but not divergent (Fig. S7b); (2) the basal ring of the antenna is three times wider than the dome (Fig. S7a); there are four rows of scales between the cephalic lobes and oral ridges, which are also

more numerous (Fig. 5a and Fig. S7c); and the labial lobe is larger than the labial organ, which is rounded (Fig. S7d).

The cephalopharyngeal skeleton in this larval stage is very similar to that of the second instar larvae (Fig. 2e, f and Fig S6d–g). The only differences concern the shape of some sclerites: (a) the mouth hook shows a thick tooth with a semicircle section (Fig. 2e and Fig. S7e), and the basal part with a wide dorsoposterior projection (Fig. 2e); (b) the central bridge of intermediate sclerite is strongly prolonged ventrally and truncate obliquely in a forward direction (Fig. 2e and Fig. S3e); (c) the window of each posterior labial sclerites is oblique and wider (Fig. S6e); (d) the distal end of the dorsal bridge shows several tips (Fig. 2c); (e) the dorsal cornu is longer than the ventral cornu (Fig. 2e); (f) there is a ventral bridge between the fore arms of the ventral cornua (Fig. 2e and S6b); and (g) the optic depression in older larvae is clearly pigmented. A descriptive picture interpreting the cephalopharyngeal skeleton arrangement in this larval stage is provided in Fig S3e, f of online resources.

The pattern of the spinose band in the thoracic segment of the second instar larvae, even the incomplete spinose band in tI (Fig. 5a), is retained in this larval stage. The spines are poorly sclerotized, conical in shape, and as long as the base is wide (Fig. S6c and Fig. S7f). Interestingly, the spines on tI are well sclerotized (Fig. S6b) and usually differ in size or shape: (a) They are three times longer than the width of the base and grouped the shape of a comb consisting of up to four units in lateral areas and in the secondary ventral branch (Fig. S7g) or (b) they may be bifid and trifid in ventral area of the main branch (Fig. S6b). The anterior spiracles retain the semicircular shape and both the number and arrangement of respiratory papillae as in second instar larvae (Fig. 5d and Fig. 3d), but each papilla shows three tegumental folds (Fig. 5e).

The pattern of spinose bands, the shape of spines, and the presence of membranous-like globular papilla near the lateral creeping welt in the abdominal segments are the same as the preceding larval stage (Fig. 5f and Fig. S7h–j). However, in this larval stage, the spines are weakly sclerotized (as in Fig. S6c), and both the longitudinal row of muscle scars and the ring of pit sensilla in the interband are clearly visible in all segments. This latter structure is especially noticeable because it is associated with tegumental thickening (Fig. 5f), especially in the three last segments; however, the thickenings are smaller than in S. (L.) cultellata (Ubero-Pascal et al. 2015, Fig. 13b). The dorsal and lateral areas, as well as the space between the spiracular cavity and the anal pad, present tegumental sculpturing in the form of randomly distributed indented patches (Fig. 5g). The spiracular cavity is elliptic (Fig. 5g), and the spines of the perimeter band are fine hairs with a wide swollen base (Fig. S7k), not reaching the circle formed by the posterior papillae (Fig. S5g). The posterior papillae are smooth and glabrous (Fig S6l); the seventh pair

Fig. 5 Morphology of the third instar larva of *S. (L.) tibialis* by SEM. **a** Lateral view of pseudocephalon. **b** Maxillary palpus. **c** Ventral organ. **d** Anterior spiracle. **e** Detail of respiratory papilla of anterior spiracle. **f** Laterodorsal view of spinose bands between aVI and aVII. **g** Posterior view of anal division. **h** Detail of posterior spiracles. **i** Ventral view of anal pad. *aVI-aVII* abdominal segments, *ads* additional sensilla, *ae* non-functional respiratory slit, *al* anal lips, *an* antenna, *ao* anal opening, *ap* anal pad, *asb* anterior spinose band, *asb-2* second branch of anterior spinose band, *at* anal tuft, *b* button, *flp* finger-like projection, *lp* anal papilla, *mk* mouth hook, *mp* maxillary palpus, *or* oral ridge, *p1-p7* posterior papillae, *pap* papillae of anterior spiracle, *pe* posterior spiracles, *pls* placoid-like sensillum, *pr* peritreme, *psb* posterior spinose band, *pt* peristigmatic tuft, *rs* respiratory slit, *rsp* ring of pit sensillum in interbands, *s* spines, *sb1-3* basiconic sensilla of maxillary palpus, *sc1-3* coeloconic sensilla of maxillary palpus, *sl* scales, *sp* pit sensillum, *srf* scarf, *tI* first thoracic segment, *tp* triangular projection, *vo* ventral organ, *w* wrinkles, *asterisk* discontinuity of anterior spinose band in laterodorsal area, *double asterisk* discontinuity of anterior spinose band in dorsal area of aVI y aVII

is observable by light microscopy (Fig. S6h). The posterior spiracles are rounded, and each one presents three straight functional respiratory slits, besides the non-functional one (Fig. 5h and Fig. S6i). Each respiratory slit shows a peristigmatic tuft, in the shape of a short stem and up to five branches disposed in a fan-shape, except in the non-functional slit that is paintbrush-shape (Fig. 5h). The peritreme is well sclerotized, with clearly differentiated rounded dorsal and outer arches and a straight inner arch; a straight ventral arch is also distinguishable but is slightly sclerotized, its free tip reaching the line drawn by the inner arch (Fig. 3f). A not-sclerotized scar is clearly observable by both microscopy techniques used (Fig. 5h and Fig. 3f), similar to the button of Calliphoridae (Ubero-Pascal et al. 2012: Fig. 12b). The anal pad is fully covered with conical spines, except the anal papillae, which are smooth and glabrous (Fig. 5i).

Micromophology of the puparium

The puparium is barrel-like and dark brown colored. Except for the pseudocephalon, which is collapsed (Fig. 6a), the morphological features of third instar larvae can be distinguished—at least the way in which they are arranged. The anterior spiracles are easily observed (Fig. 6a), since they preserve the rounded shape, and the respiratory papillae can still be counted despite being degenerated (Fig. 6b). Although the interbands are very wrinkled, the spines are flattened dorsoventrally and the fleshy tegumental structures are collapsed (Fig. 6c, d), while the pattern of the spinose bands is retained (Fig. 6e); the position of the ring of pit sensilla, the membranous-like globular papilla, and the muscular scars are also distinguishable (Fig. 6c, e, f). The spiracular and anal fields are joined by a wide keel, which is enhanced due to the shape of the dorsal area of the anal pad (Fig. 6g). The edge of the spiracular cavity is clearly oval, in contrast to its shape in the third instar larvae (Figs. 5g and 6g). The tegumental sculpturing, spine distribution, and arrangement of posterior papillae in anal division are also clearly distinguishable, but the anal pad and posterior papillae are completely collapsed (Fig. 6g). Of note are the posterior spiracles, which almost totally conserved, even the peristigmatic tufts (Fig. 6h, i).

Discussion

The complete micromophology of the immature stages of *S. (L.) tibialis* is described and illustrated for the first time using SEM and light microscopy. These data not only aim to increase our overall knowledge of Sarcophagidae from a biological point of view but also for forensic, medical, and veterinary practice since they may ensure the proper identification of entomological evidence. Moreover, according to Grzywacz et al. (2015), the morphology of immature is a very useful

source of data, not only for a taxonomic purpose, but also for systematic studies.

In a review of the current morphological knowledge of this group of Diptera, Ubero-Pascal et al. (2015) pointed out the uneven data available for both immature stages and the microscopic techniques used. The morphology of the immature stages of subgenus *Liosarcophaga* is poorly studied, preventing successful implementation in the common practical of forensic and health sciences. In fact, *Sarcophaga* (*Liosarcophaga*) *dux* Thomson, 1869 is the only species belonging to this group in which egg, larva, and pupa micromorphologies have been described by SEM and light microscopy (Greene 1925; Aspoas 1991; Sukontason et al. 2003b, 2005, 2006, 2010, 2014). Some features of the third instar larvae of *S. (L.) tibialis* may be also found in Aspoas (1991). In the case of *Liosarcophaga* species related to medical, veterinary, and forensic cases, but also taking into account those species that occasionally occur in carrion, such as *Sarcophaga* (*Liosarcophaga*) *aegyptica* (Salem, 1935), *Sarcophaga* (*Liosarcophaga*) *jacobsoni* (Rohdendorf, 1937), *Sarcophaga* (*Liosarcophaga*) *marshalli* (Parker, 1923), *Sarcophaga* (*Liosarcophaga*) *namibia* Reed, 1974, *Sarcophaga* (*Liosarcophaga*) *portschinskyi* (Rohdendorf, 1937), or *Sarcophaga* (*L.*) *teretirrostris* Pandellé, 1896 (Martínez-Sánchez et al. 2000; Romera et al. 2003; Prado e Castro et al. 2010), the preimaginal stages are scarcely described or remain unknown (Kirk-Spriggs 2003; Saloña-Bordas and González-Mora 2005; Saloña-Bordas et al. 2007; Richet et al. 2011). This situation hinders a global comparison that allows the main features to be established for unequivocal identification of *Liosarcophaga* species of forensic interest. However, as far as possible, we have tried to establish what specific features are useful for taxonomical application, until further studies improve our micromorphological knowledge of immature stages in this subgenus.

Morphological correlation between immature stages of S. (L.) tibialis

The features retaining the same shape and arrangement in all the larval stages have been noted and also what features change for each instar. It is interesting that the pattern of spinose bands in all the segments are retained in all larval stages, except for the first thoracic segment, in which the spinose band becomes discontinuous dorsolaterally in the second and third instar larvae. The shape and arrangement of the maxillary palp, antenna, sensilla ring in interbands, and posterior papillae, including the number and type of sensilla, are also preserved in all larval stages, as is the number of respiratory slits in the anterior spiracles in the two latter stages. However, each instar can be differentiated according to the shape and arrangement of the oral ridges, cephalopharyngeal skeleton, anal lips, and posterior spiracles, as well as the degree of
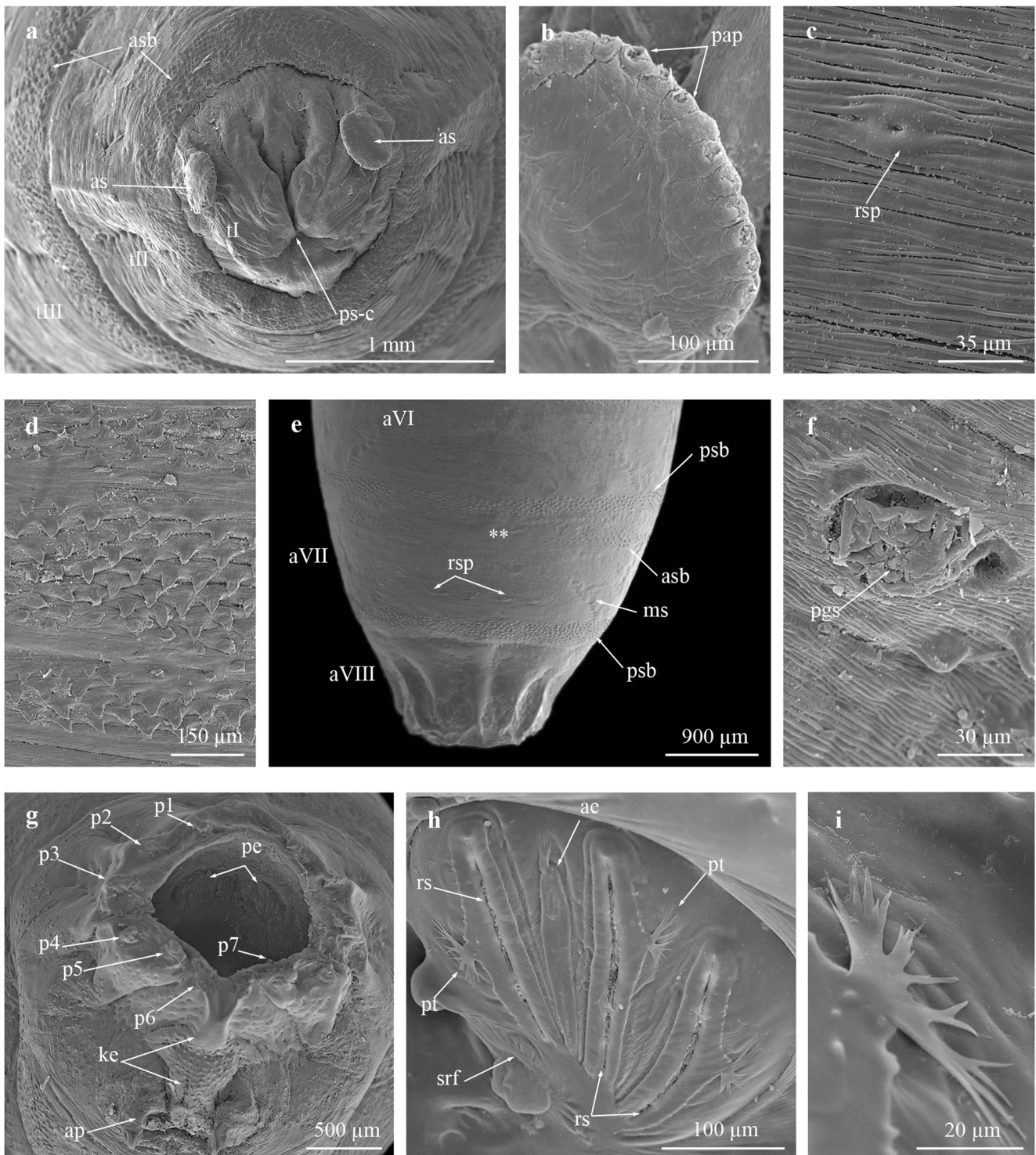
Fig. 6 Morphology of the pupa of *S.* (*L.*) *tibialis* by SEM. **a** Frontal view of pseudocephalon collapsed. **b** Anterior spiracle. **c** Detail of the ring of pit sensillum and body wrinkles. **d** Rows of spines of ventral creeping welt. **e** Dorsal view of segments aVI–aVIII. **f** Detail of membranous-like globular papilla. **g** Posterior view of anal division. **h** Detail of the posterior spiracle. **i** Detail of peristigmatic tufts. *aVI–aVIII* abdominal segments, *ae* non-functional respiratory slit, *ap* anal pad, *as* anterior spiracle, *asb* anterior spinose band, *ke* keel, *ms* muscle scarf, *p1-p7* posterior papillae, *pap* papillae of anterior spiracle, *p*, posterior spiracles, *pgs* membranous-like globular papilla, *psb* posterior spinose band, *ps-c* pseudocephalon collapsed, *pt* peristigmatic tuft, *rs* respiratory slit, *rsp* ring of pit sensillum in interbands, *srf* scarf, *tI-tIII* thoracic segments, *double asterisk* discontinuity of anterior spinose band in dorsal area of aVI y aVII

sclerotization and shape of the spines. The puparia show the main features of the third instar larvae, although the fleshy structures are collapsed.

Morphological comparison of eggs of Liosarcophaga species

Although the sarcophagids are larviparous, or ovolarviparous according to Pimsler et al. (2014), oviposition has been reported in some species of this group, especially when they are breeding in laboratory conditions (Knipling 1936; Aspoas 1991; Sukontason et al. 2005; Saloña-Bordas et al. 2007; Pimsler et al. 2014). S. (L.) tibialis is included among these species. Abasa (1970) showed that S. (L.) tibialis lay larvae covered by a translucent embryonic envelope, which they quickly shed. In fact, the embryonic layer of this species seems to be a simple layer with no chorionic sculpturing, in contrast to the multilayered and net-like surface of the egg chorion of S. (L.) dux or Blaesoxipha (Gigantotheca) plinthopyga Wiedemann, 1830 (Sukontason et al. 2005; Pimsler et al. 2014). The eggs of S. (L.) aegyptica have been described by light microscopy (Saloña-Bordas et al. 2007), but the morphological differences described in the chorion surface between hatched and unhatched eggs means that comparison showed not be made with the species noted above until a SEM analysis has been made.

Morphological comparison of the first instar larvae of Liosarcophaga species

Unlike for Liopygia species (Ubero-Pascal et al. 2015), the morphological data obtained by SEM analysis of first instar larvae are limited to S. (L.) dux, which, unfortunately, is poorly described or pictured, preventing comparison of all the features given for S. (L.) tibialis in this study (see

Table 1). Notwithstanding, the shape of the mouth hook and posterior spiracles may be useful for distinguishing these species of this instar (Table 1). The triangular cross section of the tooth of the mouth hooks and the close position of their tips when they are outside of the larvae—like an owl's beak—seem to be characteristic of S. (L.) tibialis, at least with our current level of knowledge (see Ubero-Pascal et al. 2015 for an overview). Interestingly, both species share some features with a similar morphology, such as the shape of the ventral organ, spines of first thoracic segments, and Keilin's organ, which are clearly different from those of Liopygia species, e.g., S. (L.) cultellata (Table 1). However, S. (L.) tibialis also shares features with Liopygia species, such as the spinose pattern, with S. (L.) cultellata, and the striae close to mouth opening, with S. (L.) argyrostoma (Draber-Monko et al. 2009; Ubero-Pascal et al. 2015).

On the basis of the light microscopy data, the pattern of spinose bands allows us to differentiate S. (L.) aegyptica from S. (L.) tibialis, since the former shows a complete anterior spinose band in all the segments (Saloña-Bordas et al. 2007). This fact supports the views of Knipling (1936) and Cantrell (1981) concerning the taxonomic utility of this feature by itself, although it could be more useful in combination with other features to differentiate it from other sarcophagids of forensic or health science interest, such as S. (L.) cultellata or S. (L.) argyrostoma (Draber-Monko et al. 2009; Ubero-Pascal et al. 2015). According to Ubero-Pascal et al. (2015), further studies are needed before the usefulness of this feature can be confirmed, because in many sarcophagids of forensic interest, such as S. (L.) dux, it is still unknown. The number of posterior papillae differs between S. (L.) tibialis and S. (L.) aegyptica, although this feature must be treated with caution, as already stated by Ubero-Pascal et al. (2015). According to Saloña-Bordas et al. (2007), S. (L.) aegyptica shows six pairs of posterior

Table 1 Comparison of morphological features of larvae I of *Liosarcophaga* species and *Sarcophaga (Liopygia) cultellata* obtained by SEM

Feature	S. (L.) tibialis	S. (L.) dux[a]	S. (L.) cultellata[b]
Mouth hook	Triangular cross section	Blade-like cutting margin[b] (Fig. 1d)	Oval cross section (Fig. 1e)
Striae near mouth opening	Yes (three)	nd	No (Fig. 1e)
Triangular projection of ventral organ	Long	Long (Fig. 1b)	Short (Fig. 1d)
Dorsal spines in tI	Conical shape	nd; seem to be conical shape (Fig. 1b)	Trident shape (Fig. 1h)
Setae of Keilin's organ	Thin and long	Thin and long (Fig. 1e)	Wide and short
Anterior spinose band in aVII segment	Without spines in dorsal area	nd	Without spines in dorsal area (Fig. 5e)
Ring of sensilla in abdominal interbands	Pit sensillum	nd	Pit and trichoid sensilla alternates on fleshy protuberances (Fig. 5a)
Anal papillae	Prolonged	nd	Rounded (Fig. 5f, j)
Posterior spiracle	Quadrangular	Circular (Fig. 1f)	Circular

nd no data available

[a] Data from Sukontason et al. (2003b)

[b] Data from Ubero-Pascal et al. (2015)

papillae but, in our opinion, *Sarcophaga* species usually have seven pairs, since the seventh pair may often go unnoticed by light microscope because of their small size.

The cephalopharyngeal skeleton of this instar has been described or pictured for at least five *Liosarcophaga* species of forensic interest and, in general, is very similar morphologically (Saloña-Bordas et al. 2007; Sukontason et al. 2010; Richet et al. 2011). Interestingly, the general shape of the mouth hook could characterize this subgenus, at least to distinguish it from *Liopygia* species of forensic interest: The tooth of the mouth hook is longer than the basal part, and its proximal part is humped dorsally and is rectilinear ventrally, with only the tip curved. Although some features of the cephalopharyngeal skeleton of *S. (L.) dux* and *S. (L.) aegyptica* may easily differentiate them from *S. (L.) tibialis* (Saloña-Bordas et al. 2007; Sukontason et al. 2010, 2014), such as the shape of the dental sclerite, we think that this feature should be treated with caution because it could be wrongly identified. In our opinion, the dental sclerite described in *S. (L.) dux* seems to be a typical tegumental artifact due to the piling up of spines of the anterior spinose band of the first thoracic segment when the pseudocephalon is retracted. In *S. (L.) tibialis*, this artifact in the form of a dark ventral area also appears and shows a variable position, depending on the degree to which the pseudocephalon rolls back, while the dental sclerite is a small structure articulating with the mouth hook. However, comparing the pictures of the cephalopharyngeal skeleton in profile with those available in the literature, some features, such as the length ratio between the dorsal bridge and parastomal bar, the width ratio between dorsal and ventral cornua, the shape of the dorsal edge of the dorsal cornu, the posterior end of the ventral cornu, and the tip of the dorsal bridge, may help to distinguish the species of *Liosarcophaga* (Saloña-Bordas et al. 2007; Sukontason et al. 2010; Richet et al. 2011).

At present, the morphological data available for this larval stage is insufficient to allow the unequivocal differentiation of *Liosarcophaga* species. The shape of the mouth hook and the pattern of the spinose bands are useful for distinguishing *S. (L.) tibialis* from *S. (L.) dux* and *S. (L.) aegyptica*, respectively, but unfortunately, these features cannot be applied to differentiate between the latter species because neither feature is known on either species. Table 1 shows that morphological differences in this larval stage may be established when the species are described in detail, such as between *S. (L.) tibialis* and *S. (L.) cultellata* despite their belonging to different subgenera. Until further studies provide new morphological data, the cephalopharyngeal skeleton seems to be the only structure that can help in differentiating species at this larval stage. For this reason, a tentative taxonomical key is presented based only on the features of the cephalopharyngeal skeleton mentioned above.

1a. Dorsal bridge pointed at the tip (Fig. 2a) …2

1b. Dorsal bridge blunt at the tip (Richet et al. 2011, Fig. 110 F) …*S. (L.) teretirrostris*
2a. Length of dorsal bridge at least two thirds the length of the parastomal bar (Fig. 2a) …3
2b. Length of dorsal bridge half the length of the parastomal bar length (Saloña-Bordas et al. 2007, Fig. 9) …*S. (L.) aegyptica*
3a. Dorsal cornu wider than ventral cornu. Posterior end of ventral cornu forked (Fig. 3a) …4
3b. Width of dorsal and ventral cornua approximately equal. Posterior end of ventral cornu not forked (Richet et al. 2011, Fig. 108E and 108 J) …*S. (L.) dux/S. (L.) jacobsoni*
4a. Dorsal margin of dorsal cornu rounded (Fig. 2a) …*S. (L.) tibialis*
4b. Dorsal margin of dorsal cornu rectilineal (Richet et al. 2011, Fig. 110D) …*S. (L.) portschinskyi*

Morphological comparison of second instar larvae of Liosarcophaga species

The second instar larvae of Sarcophagidae, as noted by Ubero-Pascal et al. (2015), have been barely studied due to their similar morphology with the third instar larvae, a fact that has been confirmed in *Liosarcophaga* species. The available data concerning the external morphology of *S. (L.) dux* and *S. (L.) aegyptica* do not allow these species to be unequivocally distinguished from *S. (L.) tibialis* (Sukontason et al. 2003b, 2010; Saloña-Bordas and González-Mora 2005; Saloña-Bordas et al. 2007), since the data are scarce, imprecise, and of low taxonomic value. Even the data from SEM analysis identifies only minimal morphological differences (Table 2). In contrast, detailed descriptions by SEM allow several differences to be established between *S. (L.) tibialis* and *S. (L.) cultellata* (Table 2), highlighting the usefulness of this source of data. Although the anterior spiracle of *S. (L.) tibialis* is more rounded than in *S. (L.) dux* and *S. (L.) aegyptica*, this difference is almost negligible taking into account that in all three species, the range of the number and the alignment of the main papillae overlap; these features are even shared with *S. (L.) cultellata* (Ubero-Pascal et al. 2015). The shape of the outermost slit of posterior spiracle shows certain variability that may help in distinguishing species, since it is strongly curved in *S. (L.) dux* and slightly curved in *S. (L.) tibialis*, *S. (L.) aegyptica*, and *S. (L.) jacobsoni* (Sukontason et al. 2003a; Saloña-Bordas et al. 2007; Richet et al. 2011), while it is straight in other *Liosarcophaga* species of little forensic or health science interest, such as *Sarcophaga (Liosarcophaga) pleskei* Rohdendorf, 1937. The pattern of the spinose band allows *S. (L.) aegyptica* to be distinguished from *S. (L.) tibialis* based on the same features as for the first instar larvae (Saloña-Bordas et al. 2007).

Table 2 Comparison of morphological features of larvae II of *Liosarcophaga* species and *Sarcophaga* (*Liopygia*) *cultellata* obtained by SEM

Feature	S. (L.) tibialis	S. (L.) dux[a]	S. (L.) cultellata[b]
Ventral organ	Cylindrical	Rounded (Fig. 2b)	Rounded (Fig. 6d)
Anterior spiracle	15–20 papillae, single irregular row	14–17 papillae, single regular row (Fig. 2d)	15–17 papillae, single regular row (Fig. 9a)
Anterior spiracle: shape	Rounded	Fan-shaped (Fig. 2d)	Fan-shaped (Fig. 9a)
Anterior spinose band of tI	Discontinuous dorsolaterally	nd	Continuous (Fig. 6a)
Ring of sensilla in abdominal interbands	Pit sensilla, not in tegumental thickening	nd	Pit and trichoid sensilla alternates in tegumental thickening (Fig. 9e)
Rounded spines in abdominal spinose bands	No	nd	Yes (Fig. 9c)
Longitudinal rows of spines in anal division	No	nd	Yes (Fig. 9f)
Spines around of anal pad	Very few, two patch restricted	nd	Very abundant (Fig. 9i)
Anal lips	Wider than the width of anal pad	nd	Shorter than the width of anal pad (Fig. 9i))
Posterior spiracle	Outer slit slightly curved	Outer slit strongly curved (Fig. 2f)	Outer slit straight (Fig. 9h)

nd no data available

[a] Data from Sukontason et al. (2003b)

[b] Data from Ubero-Pascal et al. (2015)

The descriptions available in the literature of the cephalopharyngeal skeleton of *S.* (*L.*) *dux* and *S.* (*L.*) *aegyptica* are so succinct and general that they do not allow these species to be distinguished from *S.* (*L.*) *tibialis*. What is more, in our opinion, it is not clear that some structures are well identified and the description is not always consistent with that observed in the figures, such as the dental sclerite and the dorsal bridge—anterodorsal process (Saloña-Bordas and González-Mora 2005; Saloña-Bordas et al. 2007). However, analysis and comparison of the pictures available in Saloña-Bordas et al. (2007), Sukontason et al. (2010), and Richet et al. (2011) suggest that the length ratio between the tooth and basal part of mouth hook, the degree of development of the intermediate sclerite bridge, and the shape of dorsal bridge can be useful for taxonomic purposes.

Based on the features observed in all the species treated, the following tentative identification key is presented.

1a. Bridge of intermediate sclerite well developed and prominent (Fig. 3b)… *S.* (*L.*) *tibialis*
1b. Bridge of intermediate sclerite poorly developed (Sukontason et al. 2010, Fig. 1b)…2
2a. Dorsal bridge short and pointed, not reaching the anterior tip of intermediate sclerite (Sukontason et al. 2010, Fig. 1b). Outermost slit of posterior spiracle strongly curved… *S.* (*L.*) *dux*
2b. Dorsal bridge slender, reaching the anterior tip of the intermediate sclerite (Saloña-Bordas et al. 2007, Fig. 15). Outermost slit of posterior spiracle slightly curved…3
3a. Tooth of mouth hook almost straight and as long as basal part (Richet et al. 2011, Fig. 109C)… *S.* (*L.*) *jacobsoni*

3b. Tooth of mouth hook sickle-shaped and shorter than the basal part (Saloña-Bordas et al. 2007, Fig. 15)… *S.* (*L.*) *aegyptica*

Morphological comparison of third instar larvae of Liosarcophaga species

Many features have been proposed to distinguish the third instar larvae of sarcophagids (see an overview in Pérez-Moreno et al. 2006; Singh et al. 2012, and Ubero-Pascal et al. 2015), and recently, Szpila et al. (2015) have critically analyzed their taxonomical usefulness. However, there is no complete information that incorporates all these features for the individual species described to date, and this is a severe problem to compare species based on data from literature. If all these features were given for one species, it would comprise a full description of body larvae. This situation has led to a proposal that a whole description of immature stages should be the rule in future morphological studies to enable taxonomic comparisons to be made (Kirk-Spriggs 2003; Singh et al. 2012; Ubero-Pascal et al. 2015), since as more species are described, more features will be needed for their correct differentiation.

The third instar larvae of *Liosarcophaga* are known in few species, but the information available from some of them is confined to light microscopy pictures, as in *S.* (*L.*) *jacobsoni* (Richet et al. 2011), or is uneven as regards the features described and the microscopy techniques used, as in *S.* (*L.*) *dux* and *S.* (*L.*) *aegyptica* (Aspoas 1991; Sukontason et al. 2003b, 2010, 2014; Saloña-Bordas and González-Mora 2005; Saloña-Bordas et al. 2007; Velásquez et al. 2010; Richet et al. 2011; Szpila et al. 2015). Besides, some features of *S.*

(*L.*) *dux* are controversial and should be carefully treated, because descriptions given by Aspoas (1991)—identified as *S. exuberans* Pandellé, 1896, syn. *sensu* Pape (1996)—and Sukontason et al. (2003b) by SEM do not agree as regards some features, such as the number of papillae in the anterior spiracle, the area covered by the band of spines at the edge of spiracular cavity, and the shape of the peristigmatic tufts (Table 3). Additionally, the 13 papillae that can be counted in the pictures of Richet et al. (2011) for *S.* (*L.*) *dux* fall between the number given by Aspoas (1991) and Sukontason et al. (2003b). In contrast, the features given or pictured for *S.* (*L.*) *tibialis* by Aspoas (1991), Velásquez et al. (2010), and Richet et al. (2011) have been confirmed in this study.

The arrangement of the papillae of the anterior spiracle in an irregular double row is one of the features proposed in the literature for distinguishing *S.* (*L.*) *tibialis* from other *Liosarcophaga* species and even from other sarcophagids (Aspoas 1991; Velásquez et al. 2010). This arrangement is clearly different in those species that show two or more regular rows (Sukontason et al. 2003a, Fig. 2; Szpila et al. 2015, Fig 3f–h). However, Szpila et al. (2015, Fig. 3i, j) proposed that the papillae—lobes—of the anterior spiracles of species belonging to *Liosarcophaga* are arranged in one, sometimes slightly irregular row, picturing this feature from *S.* (*L.*) *aegyptica*. This feature is also observable in *S.* (*L*) *dux* (Sukontason et al. 2003b, Fig. 2d;

2010, Fig. 2a). In our opinion, this feature alone may not support the differentiation of *Liosarcophaga* species from other sarcophagids of forensic interest, such as the *Sarcophaga* (*s.str.*) *carnaria* group, as proposed by Szpila et al. (2015), due to the great intra-specific variability of this feature in *S.* (*L.*) *tibialis*. In our study, the arrangement of papillae in the anterior spiracle of this species has been found to vary from one irregular row to two irregular rows, involving especially the papillae of the central area; moreover, this variability can also be observed between the anterior spiracles of a given specimen. Interestingly, the shape of the anterior spiracle may also help to distinguish *S.* (*L.*) *tibialis*, where it is rounded or semicircular, while in the remaining *Liosarcophaga* species, it is typically fan-shaped. The combination of shape and papillae arrangement of the anterior spiracle might be useful for differentiating *S.* (*L*) *tibialis* from other sarcophagids of forensic interest. On the other hand, the range of papillae numbers of the anterior spiracle overlaps among *Liosarcophaga* species, so that it is not a taxonomically useful feature in this subgenus (Sukontason et al. 2003b, 2010; Saloña-Bordas et al. 2007), except the observations of Aspoas (1991) and Kirk-Spriggs (2003) for *S.* (*L.*) *dux* and *S.* (*L.*) *namibia*, respectively.

The distance between the posterior spiracles (spiracle distance factor (SDF), according to Erzinçlioglu 1985) and some features of the peritreme have been proposed by Sukontason

Table 3 Comparison of morphological features of larvae III of *Liosarcophaga* species and *Sarcophaga* (*Liopygia*) *cultellata* obtained by SEM

Feature	S. (L.) tibialis	S. (L.) dux	S. (L.) namibia[3]	S. (L.) cultellata[4]
Anterior spinose band of tI	Discontinuous	nd.	Continuous (Fig. 1)	Continuous (Fig. 10a)
Anterior spiracle: shape	Rounded	Fan-shaped (Fig. 2D)[1]	Fan-shaped (Fig. 8)	Fan-shaped (Fig. 13a)
Anterior spiracle: papillae	15–20, two irregular rows	14–17, single row[1,b] 8–10, single row[2,b]	9–11, single row	15–17, single row
Ring of tubercles in abdominal interbands	Developed in segments 10-11.	Developed in segments 9–10[2]	Well developed in all segments (Fig. 9)	Well developed in all segments (Fig. 13b)
Warts in abdominal segments	No	nd	No	Yes
Band of spines at edge of spiracular cavity	Not reaching the posterior papillae	Not reaching the posterior papillae (Fig. 3B)[1,b] Reaching the posterior papillae (Fig. 10)[2,b]	Not reaching the posterior papillae (Fig. 13)	Reaching the posterior papillae (Fig. 13f)
Posterior papillae	Smooth	Smooth (Fig. 10)[1]	nd	Wrinkled (Fig. 13 g)
Peristigmactic tufts	Multibranched.	Dichotomic base (Fig. 3D)[1,b] Radiate (Fig. 18)[2,b]	Without[b]	Wide base and radiate (Fig. 13 h)
Knob of inner arch of peritreme	Large	Small (Fig. 3C)[1]	nd	Large (Fig. 13 h)
Anal lips	Non plate-shaped	nd	Mandibular-shaped (Fig. 13)	Plate-shaped (Fig. 13j)

[1] Data from Sukontason et al. (2003b)

[2] Data from Aspoas (1991)

[3] Data from Kirk-Spriggs (2003)

[4] Data from Ubero-Pascal et al. (2015)

nd no data available

[a] Controversial characters from divergent description by Aspoas (1991) and Sukontason et al. (2003a)

[b] This feature must be treated with caution because Kirk-Spriggs (2003) does not present a photograph of posterior spiracles

et al. (2010) for distinguishing *S.* (*L.*) *dux* from other species of sarcophagids not belonging to *Liosarcophaga*. SDF is not useful at present for *Liosarcophaga* species because *S.* (*L.*) *tibialis* and *S.* (*L.*) *aegyptica* show the same value as *S.* (*L.*) *dux*: one third of the spiracle's width (Saloña-Bordas et al. 2007; Sukontason et al. 2010). Despite the low taxonomic reliability that Szpila et al. (2015) give to some features of the posterior spiracles, such as the level of sclerotization and the shapes of peritreme and inner respiratory slit, they are sometimes the only characters available in the literature for taxonomical comparison. Therefore, and bearing in mind this consideration, we think their analysis in *Liosarcophaga* subgenus is appropriate. The straight shape and disposition of the respiratory slits and the almost imperceptible inner projection of the peritreme between the respiratory slits are common features in *Liosarcophaga* species (Sukontason et al. 2010; Richet et al. 2011); only *S.* (*L.*) *aegyptica* has a slightly curved innermost respiratory slit (Saloña-Bordas et al. 2007, Fig. 2d). However, other features explicitly described or observed directly from the photographs available in the literature can also vary morphologically (Saloña-Bordas et al. 2007; Sukontason et al. 2010; Richet et al. 2011); for example, (1) the shape of spiracle, which is rounded or quadrangular in *S.* (*L.*) *aegyptica* and kidney- or D-shaped in the remaining species; (2) the inner arch of the peritreme, shorter than the innermost respiratory slit in all the species except *S.* (*L.*) *dux*, in which it has the same length; (3) the ventral knob of the inner arch of the peritreme, large in *S.* (*L.*) *tibialis* and *S.* (*L.*) *aegyptica*, but small in the remaining species; (4) the ventral arch, which may be long, reaching the inner arch area, as in *S.* (*L.*) *tibialis* and *S.* (*L.*) *dux*, or short, not exceeding the innermost respiratory slit, as in the remaining species; and (5) the button, not sclerotized but noticeable only in *S.* (*L.*) *tibialis* and *S.* (*L.*) *aegyptica*. These features may also differ from those in other species of non-forensic interest, such as *S.* (*L.*) *pleskei*, which even has specific features, such as the inner arch of the peritreme that is longer than the innermost respiratory slit (Richet et al. 2011).

Only six pairs of posterior papillae are mentioned in the species in which this feature has been described (Aspoas 1991; Kirk-Spriggs 2003; Sukontason et al. 2003a, b, 2010; Saloña-Bordas and González-Mora 2005), but, in our opinion, the real number is seven pairs, as we mentioned for the previous larval stages. In fact, the seventh pair of papillae can be observed in the pictures of *S.* (*L.*) *dux* (Aspoas 1991, Fig. 10; Sukontason et al. 2003b, Fig. 3b). The size of posterior papillae and the distance between some of them have been used for characterizing or distinguishing species (Saloña-Bordas et al. 2007; Velásquez et al. 2010), but these features are not explicitly described for all the species and the available pictures do not permit a correct comparison, so that treating these features at this moment would not be appropriate in our opinion.

Sukontason et al. (2003b) proposed that the little wrinkles covering the intersegmental spines might be a distinctive

feature of *S.* (*L.*) *dux*; however, in our opinion, this may be an artifact because whether or not it appears in *S.* (*L.*) *tibialis* depends on the specimens; moreover, these wrinkles appear in the species described by Aspoas (1991, Figs. 3, 5, 7, and 8). The spinose pattern and the sculpturing of the interbands were proposed by Ubero-Pascal et al. (2015) as features of taxonomical value for *Liopygia* species but, unfortunately, as also occurs with *Liosarcophaga* species, it was not possible to contrast them because they have not been described for several species. In fact, two of the characters proposed in the above paper for differentiating the *Liopygia* subgenus, including *S.* (*L.*) *cultellata*, are based on the spinose pattern and the interbands sculpturing. Szpila et al. (2015) emphasized the taxonomical value of these structures but, in our opinion, SEM analysis or, at least, light microscopy slides are essential for their correct morphological interpretation, since they are slightly sclerotized in some sarcophagids. In line with this, the discontinuous anterior spinose band of tI could be a distinctive feature of *S.* (*L.*) *tibialis*, and although it is clearly visible by light microscopy, it cannot be taxonomically contrasted since it has not been described for *S.* (*L.*) *dux* o *S.* (*L.*) *aegyptica*. As in the previous larval stage, the pattern of the spinose band allows *S.* (*L.*) *tibialis* to be differentiated from *S.* (*L.*) *aegyptica* (Saloña-Bordas et al. 2007); it has not been described in *S.* (*L.*) *dux*. Unfortunately, the interbands of *Liosarcophaga* species only show a ring of tubercles, with a degree of development in the abdominal segment that varies between *S.* (*L.*) *tibialis* and *S.* (*L.*) *dux* (Table 3), but it has not been described in *S.* (*L.*) *aegyptica*. In our opinion, the use of these last features in a tentative identification key is not appropriate until they are better known in all the species involved.

The taxonomical usefulness of the cephalopharyngeal skeleton has also been questioned by Szpila et al. (2015), due to the artifacts or distortion that usual microscopy techniques may produce. According to this paper, each microscopy technique has its "pros" and "cons" depending on which sclerite is to be studied. Until further studies using the most suitable techniques of microscopy provide evidences that confirm, or not, the description in the literature, such descriptions are the only data available for comparing our morphological observations. Taking all of this into account, the cephalopharyngeal skeleton of *S.* (*L.*) *tibialis* is critically compared to assess its taxonomical value for distinguishing *Liosarcophaga* species. The available descriptions of this structure are imprecise since they refer to the features given for the second instar larvae or are merely pictures and photographs (Kirk-Spriggs 2003; Saloña-Bordas et al. 2007; Sukontason et al. 2010; Richet et al. 2011). The general shape of the cephalopharyngeal skeleton of *Liosarcophaga* species is very similar, although the strongly pigmented optic depression in the pictures of *S.* (*L.*) *dux* and *S.* (*L.*) *aegyptica* does not allow us to determine the shape of the vertical plate and the joints with the parastomal bar and intermediate sclerite (Saloña-Bordas et al. 2007; Sukontason et al.

2010). The shape of the mouth hook, especially the dorsal edge of the basal part and the sickle degree of the tooth, and the window of the dorsal cornu might help to differentiate some species, but the position of these structures in the pictures does not allow correct comparison. However, the bridge of the intermediate sclerite seems to be clearly different in *S.* (*L.*) *tibialis*, since it can be as long as the height of the intermediate sclerite and truncate obliquely in a forward direction, while in *S.* (*L.*) *dux*, it is also long but rounded, and in *S.* (*L.*) *aegyptica*, it is short and rounded. The illustration of the cephalopharyngeal skeleton of *S.* (*L.*) *jacobsoni*, which is sagittal sectioned breaking the bridge of the intermediate sclerite, hinders knowledge of its morphology (Richet et al. 2011)

The following identification keys are proposed for the third instar larvae of *Liosarcophaga* based on features observable by light microscopy. Many of the features indicated above are not taken into consideration because they are unknown in some of the species treated. Except for *S.* (*L.*) *tibialis*, the data have been obtained from the relevant scientific literature. The features used to compare the species refer, as far as possible, to photographs of those articles. The species compared belong to the sarcosaprophagous fauna that occur in the Iberian Peninsula. It is very probable that forthcoming studies will provide new data that improve this proposal, and also, some features may lose their relevance.

> 1a. Anterior spiracles rounded with respiratory papillae arranged in an irregular double row (Fig. 3d), some specimens may also show a single irregular row. Bridge of intermediate sclerite truncate obliquely in a forward direction (Fig. 3e)...... *S.* (*L.*) *tibialis*
> 1b. Anterior spiracles fan-shaped with respiratory papillae arranged in a single row (Sukontason et al. 2010, Fig. 2a). Bridge of intermediate sclerite round 2
> 2a. Posterior spiracles with the ventral arch of peritreme absent or short, not reaching the inner arch area (Saloña-Bordas and González-Mora 2005, Fig. 2). Bridge of intermediate sclerite shorter than height of the intermediate sclerite (Saloña-Bordas et al. 2007, Fig. 17)...... *S.* (*L.*) *aegyptica*
> 2b. Posterior spiracles with long ventral arch of peritreme, reaching the inner arch area (Sukontason et al. 2010, Fig. 2b). Bridge of intermediate sclerite as same long as the height of the intermediate sclerite (Sukontason et al. 2010, Fig. 1c)...... *S.* (*L.*) *dux*

Morphological comparison of puparium of Liosarcophaga species

The puparium usually retains the morphological features of the third instar larvae (Sukontason et al. 2006; Ubero-Pascal et al. 2015), so Table 3 can also be used for distinguishing

species as long as such features are not degenerated, collapsed, or not hidden by the tegumental wrinkles. The shape of the anterior spiracle and the arrangement of their papillae, the pteristgmatic tufts, and the knob of the inner arch of peritreme can be used to differentiate species morphologically described by SEM, such as *S.* (*L.*) *tibialis* and *S.* (*L.*) *dux*. Interestingly, Sukontason et al. (2006) described a button and "*tapering cavities containing convoluted structures*"— lateral papillae according to Ubero-Pascal et al. (2015)—in *S.* (*L.*) *dux*, but these features also appeared in *S.* (*L.*) *tibialis* and even in *S.* (*L.*) *cultellata*. On the other hand, Greene (1925) proposed that the keel between the spiracular cavity and the anal pad may be a useful taxonomical character. In fact, *S.* (*L.*) *tibialis* presents a wide keel that allows it to be differentiated from *S.* (*L.*) *dux* and *S.* (*L.*) *aegyptica*.

References

Abasa RO (1970) Reproductive biology of *Sarcophaga tibialis* (Diptera: Sarcophagidae). I. Life history with notes on prepupation mortality and pupation habits. Ann Entomol Soc Am 63:466–469

Ali-Khan FEA, Ali-Khan Z (1974) Two cases of human *Sarcophaga* (Diptera: Sarcophagidae) myiasis in Quebec, with descriptions of the larvae. Can J Zool 52:643–647

Al-Mesbah H, Al-Osaimi Z, El-Azazy OME (2011) Forensic entomology in Kuwait: the first case report. Forensic Sci Int 206:e25–e26

Amendt J, Campobasso CP, Gaudry E, Reiter C, LeBlanc HN, Hall MJR (2007) Best practice in forensic entomology—standards and guidelines. Int J Legal Med 121:90–104

Arnaldos I, Romera E, García MD, Luna A (2001) An initial study on the succession of sarcosaprophagous Diptera (Insecta) on carrion in the southeastern Iberian peninsula. Int J Legal Med 114:156–162

Arnaldos MI, Sánchez F, Álvarez P, García MD (2004a) A forensic entomology case from the southeastem Iberian Peninsula. AAIJFMT 5:22–25

Arnaldos MI, Romera E, Presa JJ, Luna A, García MD (2004b) Studies on seasonal arthropod succession on carrion in the southeastern Iberian Peninsula. Int J Legal Med 118:197–205

Arnaldos MI, González-Mora D, Begoña I, García MD (2013) Nuevo sarcofágido en la comunidad sarcosaprófaga: caso de *Wohlfahrtia bella* (Macquart, 1839) (Diptera, Sarcophagidae). Bol Asoc Esp Ent 37:99–101

Aspoas BR (1991) Comparative micromorphology of third instar larvae and the breeding biology of some Afrotropical *Sarcophaga* (Diptera: Sarcophagidae). Med Vet Entomol 5:437–445

Awad A, Abel-Salam S, Abou El-Ela R, Abdel-Aal A, Mohamed D (2003) Ultrastructure comparation of the sensory morphology of the first and third instar larvae of *Parasarcophaga argyrostoma* (Robineau-Desvoidy) (Diptera: Sarcophagidae). Egypt J Biol 5: 148–154

Barros CJ, Antunes C (2008) Key to the adults of the most common forensic species of Diptera in South America. Rev Bras Entomol 52:390–406

Benecke M (1998) Six forensic entomology cases: description and commentary. J Forensic Sci 43:797–805

Bonacci T, Greco S, Cavalcanti B, Brandmayr P, Vercillo V (2014) The flesh fly *Sarcophaga* (*Liopygia*) *crassipalpis* Macquart 1839 as an

invader of a corpse in Calabria (Southern Italy). J Forensic Sci Criminol 1:404

Byrd JH, Castner JL (2001) Insects of forensic importance. In: Byrd JH, Castner JL (eds) Forensic Entomology: The Utility of Arthropods in Legal Investigations. CRC Press, Boca Raton, pp 39–126

Cantrell BK (1981) The immature stages of some Australian Sarcophaginae (Diptera: Sarcophagidae*). J Aust Entomol Soc 20: 237–248

Castillo Miralbes M (2002) Estudio de la entomofauna asociada a cadáveres en el alto Aragón (España) Monografías SEA, vol 6

Cherix D, Wyss C, Pape T (2012) Occurrences of flesh flies (Diptera: Sarcophagidae) on human cadavers in Switzerland, and their importance as forensic indicators. Forensic Sci Int 220:158–163

Colwell D, O´Connor M (2000) Scanning electron microscopy of Sarcophagid (Diptera) larvae recovered from a case of human cutaneus myiasis. J Med Entomol 37:854–859

Courtney GW, Sinclair BJ, Meier R (2000) Morphology and terminology of Diptera larvae. In: Papp L, Darvas B (eds) Contributions to a Manual of Palaearctic Diptera: General and Applied Dipterology, Vol. 1. Science Herald, Budapest, p 85–161

Delir S, Handjani F, Emadt M, Ardehali S (1999) Vulvar myiasis due to Wohlfahrtia magnifica. Clin Exp Dermatol 24:279–280

Draber-Monko A, Malewski T, Pomorski J, Los M, Slipinski P (2009) On the morphology and mitochondrial DNA barcoding of the flesh fly Sarcophaga (Liopygia) argyrostoma (Robineau-Desvoidy. 1830) (Diptera: Sarcophagidae)—an important species in Forensic Entomology. Ann Zool 59:465–493

Erzinçlioglu Z (1985) Immature stages of British Calliphora and Cynomya, with a re-evaluation of the taxonomic characters of larval Calliphoridae (Diptera). J Nat Hist 19:69–96

Ferrar P (1987) A guide to the breeding habits and immature stages of Diptera Cyclorrhapha. E.J. Brill/ Scandinavian Science Press, Leiden-Copenhagen

Gennard D (2007) Forensic entomology: An Introduction. John Wiley & Sons, Chichester

Goff ML, Lord WD (1994) Entomotoxicology: a new area for forensic investigations. Am J Forensic Med Pathol 1:511–517

González-Medina A, González Herrera L, Jiménez Ríos G (2012) Sarcophaga species (Diptera, Sarcophagidae) recovered from human corpses during autopsies in Granada (Spain). Int J Legal Med 126(suppl 1):S184

Grassberger M, Reiter C (2002) Effect of temperature on development of Liopygia (= Sarcophaga) argyrostoma (Robineau-Desvoidy) (Diptera: Sarcophagidae) and its forensic implications. J Forensic Sci 47:1332–1336

Greenberg B (1971) Flies and disease, volume I: ecology, classification, and biotic association. Princeton University Press, Princeton

Greene CT (1925) The puparia and larvae of Sarcophagid flies. Proc US Nat Mus 66:1–26

Grzywacz A, Hall MJR, Pape T (2015) Morphology successfully separates third instar larvae of Muscina. Med Vet Entomol. doi:10.1111/mve.12117

Jeffery TA (2011) Morphological and molecular techniques for the differentiation of myiasis-causing Sarcophagidae. Theses and Dissertations. Paper 10390 http://lib.dr.iastate.edu/etd/10390

Judd WW (1956) Results of a survey of Calyptrate flies of medical importance conducted at London, Ontario, during 1953. Am Midl Nat 56:388–405

Kirk-Spriggs AH (2003) The immature stages of Sarcophaga (Liosarcophaga) namibia Reed (Diptera: Sarcophagidae) from the southwestern seaboard of Africa. Cimbebasia 18:39–47

Klong-klaew T, Sukontason K, Sribanditmongkol P, Moophayak K, Sanit S, Sukontason KL (2012) Observations on morphology of immature Lucilia porphyrina (Diptera: Calliphoridae), a fly species of forensic importance. Parasitol Res 111:1965–1975

Knipling EF (1936) A comparative study of the first-instar larvae of the genus Sarcophaga (Calliphoridae, Diptera), with notes on the biology. J Parasitol 22:417–454

Leclercq M (1976) Entomologie et médecine légale: Sarcophaga argyrostoma Rob.-Desv. (Dipt. Sarcophagidae) et Phaenicia sericata Meig. (Dipt. Calliphoridae). Bull Ann Soc R Belge Entomol 112:120–125

López-Esclapez R, García MD, Arnaldos MI, Presa JJ, Ubero-Pascal N (2014) Are the evidences of forensic entomology preserved in ethanol suitable for SEM studies? Micron 62:43–51. doi:10.1016/j.micron.2014.03.003

Martínez Sánchez AI (2003) Biología de la comunidad de Dípteros necrófilos en ecosistemas del sureste de la península ibérica. PhD Universidad de Alicante

Martínez-Sánchez A, Rojo S, Marcos-García M-A (2000) Sarcofágidos necrófagos y coprófagos asociados a un agroecosistema de dehesa (Diptera, Sarcophagidae). Bol Asoc Esp Ent 24:171–185

Mohammadzadeh T, Hadadzadeh R, Esfandiari F, Sadjjadi SM (2008) A case of gingival myiasis caused by Wohlfahrtia magnifica. Iran J Arthropod-Borne Dis 2:53–56

Musvasa E, Williams KA, Muller WJ, Villet MH (2001) Preliminary observations on the effects of hydrocortisone and sodium methohexital on development of Sarcophaga (Curranea) tibialis Maquart (Diptera: Sarcophagidae), and implications for estimating post mortem interval. Forensic Sci Int 120:37–41

Paños A, Arnaldos MI, García MD, Ubero-Pascal N (2013) Ultrastructure of preimaginal stages of Piophila megastigmata McAlpine, 1978 (Diptera, Piophilidae): a fly of forensic importance. Parasitol Res 112:3771–3788. doi:10.1007/s00436-013-3567-0

Pape T (1987) The Sarcophagidae (Diptera) of Fennoscandia and Denmark. Fauna Entomologica Scandinavica, vol 19. E.J. Brill/ Scandinavian Science Press Ltd, Copenhagen

Pape T (1996) Catalogue of the Sarcophagidae of the world (Insecta: Diptera). Memoirs on Entomology International, vol 8. Associated Publishers, Gainesville

Pérez-Moreno S, Marcos-García MA, Rojo S (2006) Comparative morphology of early stages of two Mediterranean Sarcophaga Meigen, 1826 (Diptera; Sarcophagidae) and a review of the feeding habits of Palaeartic species. Micron 37:169–179

Pimsler ML, Pape T, Johnston JS, Wharton RA, Parrott JJ, Restuccia D, Sanford MR, Tomberlin JK, Tarone AM (2014) Structural and genetic investigation of the egg and first-instar larva of an Egg-laying population of Blaesoxipha plinthopyga (Diptera: Sarcophagidae), a species of forensic importance. J Med Entomol 51:1283–1295

Prado e Castro C, García MD, Arnaldos MI, González-Mora D (2010) Sarcophagidae (Diptera) attracted to piglet carcasses including new records for Portuguese fauna. Graellsia 66:285–294

Rafinejad J, Akbarzadeh K, Rassi Y, Nozari J, Sedaghat MM, Hosseini M, Alipour H, Ranjbar A, Zeinali D (2014) Traumatic myiasis agents in Iran with introducing of new dominant species, Wohlfahrtia magnifica (Diptera: Sarcophagidae). Asian Pac J Trop Biomed 4:451–455

Raghavendra R, Randle CP, Bucheli SR (2011) Identification of a death-scene maggot using standardized molecular methods: Sarcophaga bullata Parker 1916 (Sarcophagidae) out-numbers blowflies (Calliphoridae) on an urban cadaver in Southeastern Texas. J Forensic Res 2:133. doi:10.4172/2157-7145.1000133

Richet R, Blackith RM, Pape T (2011) Sarcophaga of France (Diptera: Sarcophagidae). Pensoft, Sofia-Moscow

Romera E, Arnaldos MI, García MD, González-Mora D (2003) Los Sarcophagidae (Insecta Diptera) de un ecosistema cadavérico en el sureste de la Península Ibérica. An Biol 25:49–63

Ruiz-Martínez I, Soler-Cruz MD, Benítez-Rodríguez R, Pérez-Jiménez JM, Díaz-López M (1989) Postembyonic development of Wohlfahrtia magnifica (Schiner, 1862) (Diptera: Sarcophagidae). J Parasitol 75:531–539

Saloña-Bordas MI, González-Mora D (2005) Primera cita de *Liosarcophaga aegyptica* (Salem, 1935) (Diptera: SArcophagidae) de la Península Ibérica, con descripción de sus fases larvarias II y III, purpario y adultos. Bol SEA 36:251–255

Saloña-Bordas MI, Moneo-Pellitero J, Herrero-Dávila D (2007) New observations of *Liosarcophaga aegyptica* (Salem, 1935) (Diptera, Sarcophagidae) reared from colonies collected on the University Campus of Lejona (Vizcaya, Northern Spain). Bol SEA 40:377–383

Sharma J, Panchakssharappa G, Achatya R (2008) Primary oral myasis: a case report. Med Oral Patol Oral Cir Bucal 13(11):E714–E716

Singh D, Garg R, Wadhawan B (2012) Ultramorphological characteristics of immature stages of a forensically important fly *Parasarcophaga ruficornis* (Fabricius) (Diptera: Sarcophagidae). Parasitol Res 110:821–831

Smith KGV (1986) A Manual of Forensic Entomology. British Museum (Natural History), London & Cornell University Press, Ithaca

Soler Cruz MD (2000) El estudio de las miasis en España durante los últimos cien años. Ars Pharm 41:19–26

Sukontason K, Sukontason KL, Piangjai S (2003a) Scanning electron microscopy of third-instar sarcophagid (Diptera: Sarcophagidae) recovered from a mummified human corpse in Thailand. Rev Inst Med Trop Sao Paulo 45:95–98

Sukontason K, Sukontason KL, Piangjai S, Chai Wong T, Boonchu N, Kurahashi H, Vogtsberger RC (2003b) Larval ultrastructure of *Parasarcophaga dux* (Thomson) (Diptera: Sarcophagidae). Micron 34:359–364

Sukontason KL, Sukontason K, Vogtsberger RC, Piangjai S, Boonchu N, Chaiwong T (2005) Ultramorphology of eggshell of flesh fly *Liosarcophaga dux* (Diptera: Sarcophagidae). J Med Entomol 42:86–88

Sukontason KL, Piangjai S, Bunchu N, Chaiwong T, Sripakdee D, Boonsriwong W, Vogtsberger RC, Sukontason K (2006) Surface ultrastructure of the puparia of the blow fly, *Lucilia cuprina* (Diptera: Calliphoridae), and flesh fly, *Liosarcophaga dux* (Diptera: Sarcophagidae). Parasitol Res 98:482–487

Sukontason K, Narongchai P, Kanchai C, Vichairat K, Sribanditmongkol P, Bhoopat T, Kurahashi H, Chockjamsai M, Piangjai S, Bunchu N, Vongvivach S, Samai W, Chaiwong T, Methanitikorn R, Ngern-klun R, Sripakdee D, Boonsriwong W, Siriwattanarungsee S, Srimuangwong C, Hanterdsith B, Chaiwan K, Srisuwan C, Upakut S, Moopayak K, Vogtsberger RC, Olson JK, Sukontason KL (2007) Forensic entomology cases in Thailand: a review of cases from 2000 to 2006. Parasitol Res 101:1417–1423

Sukontason K, Bunchu N, Chaiwong T, Moophayak K, Sukontason KL (2010) Forensically important flesh fly species in Thailand: morphology and developmental rate. Parasitol Res 106:1055–1064

Sukontason KL, Sanit S, Klong-klaew T, Tomberlin JK, Sukontason K (2014) *Sarcophaga* (*Liosarcoaphaga*) *dux* (Diptera: Sarcophagidae): a flesh fly species of medical importance. Biol Res 47:14

Szpila K (2010) The first instar of European Miltogramminae (Diptera: Sarcophagidae). Nicolaus Copernicus University Press, Toruń

Szpila K, Villet MH (2011) Morphology and identification of first instars of African blow flies (Diptera: Calliphoridae) commonly of forensic importance. J Med Entomol 48:738–752

Szpila K, Voss JG, Pape T (2010) A new dipteran forensic indicator in buried bodies. Med Vet Entomol 24:278–283. doi:10.1111/j.1365-2915.2010.00883.x

Szpila K, Richet R, Pape T (2015) Third instar larvae of flesh flies (Diptera: Sarcophagidae) of forensic importance—critical review of characters and key for European species. Parasitol Res 114: 2279–2289. doi:10.1007/s00436-015-4421-3

Ubero-Pascal N, Arnaldos I, López-Esclapez R, García MD (2010) Microscopy and forensic entomology. In: Méndez-Vilas A, Díaz J (eds) Microscopy: Science, Technology, Applications and Education. Formatex, Badajoz, pp 1548–1556

Ubero-Pascal N, López-Esclapez R, García MD, Arnaldos MI (2012) Morphology of preimaginal stages of *Calliphora vicina* Robineau-Desvoidy, 1930 (Diptera: Calliphoridae): a comparative study. Forensic Sci Int 219:228–243

Ubero-Pascal N, Paños A, García MD, Presa JJ, Torres B, Arnaldos I (2015) Micromorphology of immature stages of *Sarcophaga* (*Liopygia*) *cultellata* Pandellé, 1896 (Diptera: Sarcophagidae), a forensically important fly. Microsc Res Tech 78:148–172

Velásquez Y, Magaña C, Martínez-Sánchez A, Rojo S (2010) Diptera of forensic importance in the Iberian Peninsula: larval identification key. Med Vet Entomol 24:293–308

Villet MH, MacKenzie B, Muller WJ (2006) Larval development of the carrion-breeding fleshfly, *Sarcophaga* (*Liosarcophaga*) *tibialis* (Diptera: Sarcophagidae), at constant temperaturas. Afr Entomol 14:357–366

Wyss C, Cherix D (2006) Traité d'Entomologie Forensique. Collections Sciences Forensiques. Preses Polytechniques et Universitaires Romandes, Lausanne

Zumpt F (1965) Myiasis in Man and Animals in the Old World. A textbook for physicians, veterinarians and zoologists. Butterworths, London

Zumpt F, Patterson P (1952) Flies visiting human faeces and carcasses in Johannesburg, Transvaal. S Afr J Clin Sci 3:92–106