Larval morphology, development and forensic importance of *Synthesiomyia nudiseta* (Diptera: Muscidae) in Europe: a rare species or just overlooked?

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Abstract

The muscid *Synthesiomyia nudiseta* (van der Wulp, 1883) is a species with forensic importance in tropical and subtropical regions of the world. This fly has recently been introduced into southern Europe and, until now, had not been recorded in forensic cases in this region. Here, morphology of all larval instars of *S. nudiseta* is documented in detail by using a combination of light and scanning electron microscopy. Literature data concerning larval morphology are revised and characters allowing identification from other forensically important Muscidae are listed. The life cycle of this species was studied at four constant temperatures: 15, 20, 25 and 30°C. Total development varied between 46.50 ± 0.97 days at 15°C and 15.39 ± 0.32 days at 30°C. Moreover, we report this species breeding in human corpses, for the first time in Europe, in forensic cases from autopsies at the Anatomical Forensic Institute of Madrid and the Institute of Legal Medicine of Alicante, Spain.

Keywords: *Synthesiomyia nudiseta*, larval morphology, scanning electron microscopy, preimaginal development, forensic entomology, postmortem interval, Europe

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Introduction

The muscid *Synthesiomyia nudiseta* (van der Wulp, 1883) is widespread in Old and New World tropics and subtropics, with 20°C annual isotherms (Skidmore, 1985). This species has

been involved in forensic investigations in different countries, such as Costa Rica, India, Malaysia, Thailand and the United States (i.e. southern USA and Hawaii) (Jirón *et al.*, 1983; Lord *et al.*, 1992; Omar *et al.*, 1994; Kulshrestha *et al.*, 1997; Goff, 2000; Lee *et al.*, 2004; Sukontason *et al.*, 2007; Kumara *et al.*, 2009). It has also been associated with human cadavers in archaeological contexts in Mexico and Peru (Huchet & Greenberg, 2010).

In Europe, *S. nudiseta* has been recorded mainly from insular territories, such as Malta, the Azores, Madeira,

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the Canary Islands and two small islands close to the coast of Alicante (SE Spain) (Pont, 1986; Ebejer & Gatt, 1999; Martínez-Sánchez *et al.*, 2005). This species was recorded for the first time on the European mainland in 1996 from Marbella (Bowden, 1997), a locality situated on the coast of southern Spain, about 70 km from North Africa (36°N 4°W). Until now, the distribution of *S. nudiseta* seemed to be restricted to insular territories and Mediterranean coastal areas because it has not been cited previously in studies about sarcosaprophagous dipteran communities in the interior of the peninsula.

This is the only known species under the genus *Synthesiomyia* Brauer & Bergenstamm, 1893 (Muscidae: Azeliinae: Reinwardtiini). The larvae are commonly found in animal and human faeces, as well as in decaying vegetable matter and garbage. However, it has been reported that this species prefers carrion as the food source of its choice and will successfully pupate in confined environments (Byrd & Castner, 2010; Byrd & Tomberlin, 2010). Facultative carnivorous larvae feed just below the surface of the flesh and, often, post-feeding larvae secrete a silky white substance in which the puparium forms, frequently with adhered debris (Siddons & Roy, 1942). This species is considered eusynanthropic because adults present a marked preference for human environments (Nazni *et al.*, 2007; Uribe-M. *et al.*, 2010) and are frequently associated with corpses discovered indoors at urban locations (Goff, 2000; Lee *et al.*, 2004; Sukontason *et al.*, 2007). Developmental studies of *S. nudiseta* are limited, and they have been performed in South America and Asia at 20, 26 and 28°C (Siddons & Roy, 1942; Rabinovich, 1970; Krüger *et al.*, 2002; Kumara *et al.*, 2009).

Preimaginal stages have been described in several papers (Siddons & Roy, 1942; Skidmore, 1985; Pires de Alencar & Rios, 1992; Calderón-Arguedas *et al.*, 2005; Sukontason *et al.*, 2007; Ubero-Pascal *et al.*, 2010b), and identification keys to mature larvae of this species can be found in Ishijima (1967), Skidmore (1985) and Velásquez *et al.* (2010). Although methods of scanning electron microscopy (SEM) have been used in some studies (Pires de Alencar & Rios, 1992; El-Alfy, 1994; Ubero-Pascal *et al.*, 2010a,b), no exhaustive documentation of larval morphology has been published until now.

Due to the potential usefulness of *S. nudiseta* in forensic investigations in southwestern Europe, the aims of this paper are (i) to describe details of larval morphology for all instars using combined methods of light microscopy and SEM, (ii) to provide development data of this species under four constant temperatures, and (iii) to review the cases where *S. nudiseta* has appeared breeding in human corpses in Spain.

Materials and methods

Colony of Synthesiomyia nudiseta

Larvae were collected from a human corpse during autopsy procedures at the Institute of Legal Medicine of Alicante (IMLA, Spain) and reared on pork liver *ad libitum* until development was completed. A colony was established at the Department of Environmental Sciences and Natural Resources, University of Alicante from emerged adults. The colony was maintained at 23°C, 60–70% RH with a photoperiod of 12:12 (L:D) in mesh cages (40 × 40 × 40 cm) with sugar and water. Fresh fish was used for oviposition and pork liver as a food source for larvae.

Larval morphology study

Larvae for morphological study were obtained from the colony. They were killed by soaking in hot water ($\approx 97^\circ\text{C}$), cleaned with a fine brush and preserved in 70% ethanol. For light microscopy study, larvae were slide-mounted in Hoyer's medium using concave slides. Photographs were taken with a Nikon 8400 digital camera mounted on a Nikon Eclipse E200 microscope (Nikon Corp., Tokyo, Japan). For additional observations, larvae were prepared with methyl salicylate according to Niederegger *et al.* (2011). Preparation for SEM study involved dehydration through 80%, 90% and 99.5% ethanol, critical point drying in CO_2 , mounting on aluminium stubs with double-sided tape and coating with platinum. Images were taken with variable-pressure SEM (LEO 1455). Larval terminology follows Skidmore (1985) and Courtney *et al.* (2000) with a few modifications proposed by Szpila & Pape (2005).

Development under constant temperatures

Growth and developmental time were evaluated under four constant temperatures (15°C, 20°C, 25°C, 30°C), with 60–70% RH and a photoperiod of 12:12 (L:D). After oviposition, egg masses (± 12 h old) were introduced into an environmental chamber at one of the four temperatures proposed. These eggs were checked every 12 h until larvae hatched. Then, 150 larvae were placed in a pot with 50 g of pork liver covered with mesh. For each temperature, ten replicates were carried out. Five of the largest larvae were collected daily, killed by soaking in hot water ($\approx 97^\circ\text{C}$) for five minutes and preserved in 70% ethanol. As a preliminary trial, five replicates at 12°C were performed. In this case, eggs were incubated at 25°C and then larvae were introduced into the environmental chamber at 12°C. Larvae were collected every 24 h until the sixth day and after that, every 72 h until pupation.

To build growth curves, total length was measured using a digital vernier (0.01 mm) and the mean of the maximum length of larvae measured daily was plotted against time for each temperature regime. The minimal developmental time for larval and pupal stages was determined when the first five individuals in each replicate had changed to the next stage and it was recorded as the average of all pots. At 12°C, only the larval stage was analyzed. For practical use in forensic investigation, developmental times from oviposition to adult emergence were represented against temperature in the isomorphen-diagram, where lines represent morphological changes and areas between lines represent identical morphological stages. Normality and homogeneity of variance of data were tested. A Kruskal-Wallis test (H) was carried out to compare development between temperatures. If significant differences were found, *post hoc* pairwise comparison procedures were carried out using Dunn's method (SigmaStat for Windows 3.5).

Accumulated degree-days (ADD) for larval and total development at all studied temperatures were calculated from the formula: $\text{ADD} = y(t - t_0)$, where y is the developmental time in days, t is the rearing temperature and t_0 is the minimum development threshold temperature. The t_0 was estimated from the linear regression of the development rate (developmental time⁻¹) on constant temperature.

Fig. 1. First instar of *S. nudiseta*. (A) Anterior end of body, lateral view; (B) anterior end of body, ventral view; (C) antennal complex; (D) maxillary palpus; (E) ventral organ; (F) Keilin's organ; (G) lateral sensillum; and (H) anterior spiracle. an, antennal complex; as, anterior spiracle; cir, cirri; ll, labial lobe; mp, maxillary palpus; ns1 [ns2], first [second] additional sensillum coeloconicum; or, oral ridges; sb1 [sb2/sb3], first [second/third] sensillum basiconicum; sc1 [sc2/sc3], first [second/third] sensillum coeloconicum; vo, ventral organ.

Analysis of forensic cases

A review was conducted of the cases where *S. nudiseta* was involved, in autopsies carried out at the Anatomical Forensic Institute of Madrid (IAFM, Spain) and the IMLA. Fourteen cases were processed by the IAFM, one by the Forensic Anthropology Laboratory of the General Commissariat of the Scientific Police of Madrid and eight at the University of Alicante. One part of the larval sample was preserved, and the remainder of the larval and pupal samples were reared to the adult stage in an environmental chamber under controlled conditions (23–25°C, 60–70% RH). The larvae were reared with pork liver and pupae were kept individually. Specimens of *S. nudiseta* were identified using Skidmore (1985), Pont (1991) and Velásquez *et al.* (2010). Collected specimens were deposited in the Entomological Collection of the University of Alicante (CEUA).

Results

Larval morphology

Pseudocephalon

Each lobe of the bilobate pseudocephalon is equipped with an antenna, maxillary palpus, ventral organ and labial lobe

(figs 1B–E; 2C–D and 3B–D). The antennal complex consists of an antennal dome situated on a basal ring (fig. 1C). The length of the antennal dome is similar to the height of the basal ring in the first instar and slightly shorter in the successive instars. The maxillary palpus in all instars consists of three sensilla coeloconica, three sensilla basiconica and one or more small additional sensilla, all in a tight cluster. Two typical sensilla coeloconica of possible non-maxillary origin are present laterodorsally on the surface of the maxillary palpus (figs 1D, 2B and 3C). In the second and third instars, maxillary palpus are surrounded by several rings of semi-circular folds (figs 2B and 3C). A facial mask surrounding the functional mouth opening in the first instar is composed of cirri and oral ridges (fig. 1B). Cirri are arranged in one row and have a shape of stripes tapering distally, without distinct sclerotization (fig. 4A, B). In the successive instars, oral ridges are more numerous, and sclerotized suprabuccal teeth are present in the anterior part of the buccal cavity (figs 2C, 3E, 4C–E and 5B, C). In the third instar, cutaneous teeth are present on the sides of the buccal cavity (figs 3E and 5C).

Cephaloskeleton

The cephaloskeleton of the first instar (figs 4A, B and 5A) consists of paired mouthhooks, an unpaired labrum, an

Fig. 2. Second instar of *S. nudiset*. (A) Anterior end of body, lateral view; (B) maxillary palpus; (C) anterior end of body, ventral view; (D) ventral organ; (E) posterior spiracles; (F) abdominal segments, ventral view; (G) posterior end of body, latero-dorsal view; and (H) posterior end of body, ventral view. A1 [A2/A3], first [second/third] abdominal segment; an, antennal complex; ap, anal plate; as, anterior spiracle; cr, transverse crevice; ex, extra-anal papilla; ko, Keilin's organ; ll, labial lobe; lo, labial organ; mp, maxillary palpus; ns1 [ns2], first [second] additional sensillum coeloconicum; or, oral ridges; pa, postanal papilla; paa, para-anal papilla; pre, pre-anal papilla; sa, subanal papilla; sb1 [sb2/sb3], first [second/third] sensillum basiconicum; sc1 [sc2/sc3], first [second/third] sensillum coeloconicum; sub, suprabuccal teeth; vo, ventral organ.

unpaired intermediate sclerite and a basal sclerite with paired parastomal bars and paired vertical plates connected by a dorsal bridge, with ventral and dorsal cornua. The mouthhooks are shaped like a slightly up-curved rod, with a widened basal part. The mouthhooks are not equipped with teeth apically. The labrum is shaped like a knife blade

with a broader basal part and narrower apical part and is fused with the tips of the parastomal bars. Each parastomal bar is long and, in the anterior part, equipped with a short process directed posteriorly. The H-shaped intermediate sclerite is elongated, and the crossbeam is broad. The vertical plate is slightly broader than the width of the dorsal and

Fig. 3. Third instar of *S. nudiseta*. (A) Anterior end of body, lateral view; (B) anterior end of body, ventral view; (C) maxillary palpus; (D) ventral organ; (E) facial mask; (F) anterior spiracle; (G) Keilin's organ; and (H) lateral spinulation of second thoracic segment. an, antennal complex; as, anterior spiracle; cut, cutaneous teeth; ko, Keilin's organ; ll, labial lobe; lo, labial organ; mp, maxillary palpus; ns1 [ns2], first [second] additional sensillum coeloconicum; or, oral ridges; sb1 [sb2/sb3], first [second/third] sensillum basiconicum; sc1 [sc2/sc3], first [second/third] sensillum coeloconicum; sub, suprabuccal teeth; vo, ventral organ.

ventral cornua. The dorsal cornua are shorter than the ventral ones. The dorsal bridge has few perforations and is less sclerotized than the vertical plates and the ventral and dorsal cornua.

The cephaloskeleton of the second instar (figs 4C, D and 5B) consists of three main parts, paired mouthhooks, an unpaired intermediate sclerite and a basal sclerite. The mouthhooks are F-shaped with a robust basal part and slender distal part slightly curved ventrally. Paired suprabuccal teeth are present anteriorly to the mouthhooks; each consists of about three teeth. The suprabuccal teeth are not connected to the mouthhooks. Below the anterior ventrolateral extension of the mouthhooks, paired angular dental and paired tooth-like accessory stomal sclerites are present. The intermediate sclerite is elongated, H-shaped with a broad crossbeam

in lateral view directed anteroventrally. A longitudinal incision is present on the dorsal surface of the intermediate sclerite, forming a bar-like structure that does not reach the basal sclerite. Anterodorsally, an angular epistomal sclerite lies freely between the anterior arms of the intermediate sclerite, and a pair of labial sclerites lie anteroventrally. The epistomal sclerite is equipped with two rounded perforations. The dorsal bridge is well perforated. A necklace structure with two rounded perforations is present in the antero-ventral part of the vertical plate. Vertical plates are wider than the width of the dorsal and ventral cornua.

The cephaloskeleton of the third instar (figs 4E and 5C), as in the second instar, consists of three main parts and additional sclerites. Among the additional sclerites, paired oral bars with serrated tips and anterior ribbons adjoining

Fig. 4. Larvae of *S. nudiseta*. (A) First instar, cephaloskeleton, lateral view; (B) first instar, cephaloskeleton, ventral view; (C) second instar, cephaloskeleton, lateral view; (D) second instar, cephaloskeleton, ventral view; (E) third instar, cephaloskeleton, lateral view; and (F) third instar, posterior spiracles.

dorsally, a pair of dental sclerites connected ventrally, paired accessory stomal sclerite, paired labial sclerite and an epistomal sclerite equipped with four rounded perforations are found. Posteriorly to the accessory stomal sclerites, a pair of supplementary accessory stomal sclerites is present in mature larva. Paired suprabuccal teeth, each consisting of about three teeth are not connected with mouthhooks. Between suprabuccal teeth and the dental sclerite, a row of cutaneous teeth is present on both sides of the buccal cavity. The dorsal bridge is finely perforated.

Thoracic and abdominal segments

Pair of trichoid sensilla of Keilin's organ are present on the ventral side of the thoracic segments (figs 1F, 2A and 3A, G). Anterior spiracles consist of four to six lobes (figs 2A, and 3A, F), except for the first instar, where a simple aperture is present (fig. 1A, H). Openings of posterior spiracles are surrounded by four branched spiracular tufts (figs 2E, 6D and 7C). Slits of the posterior spiracles are straight in the first, slightly curved in the second and S-shaped in the third instar. The spiracular disc is surrounded by four pairs of papillae, located on the surface of the anal division in the first instar (fig. 6C, E) and on the small protuberances in the second (fig. 2G) and third (fig 7E, F) instars. The anal plate is triangular, relatively small and surrounded with well-developed papillae (figs 2G, H; 6C, F and 7D–F). It is hardly visible in the lateral view (figs 2G and 6E) and in the ventral view does not reach the base of para-anal papilla (figs 2H, 6F and 7D). Subanal papillae of all instars are equipped with a small sensory papilla in the middle. Small sensilla coeloconica are present on thoracic and abdominal segments (fig. 1G). In the middle of the ventral surface of

abdominal segments 1–7, transverse crevice is present (figs 2F, 6A, B and 7A, B, D). Spinulation pattern (figs 2F, 3H, 6A, B and 7A, B, D) found in our study are in accordance with the description by Siddons & Roy (1942).

Growth and duration of developmental stages

Faster growth was observed when temperatures increased. Larval growth at all temperatures showed sigmoidal curves (fig. 8). The longest larvae always appeared the day before prepupa, at 25°C and 30°C on day 5, at 20°C on day 6, at 15°C on day 13, and at 12°C on day 15. After this, length decreased slightly when larvae entered the post-feeding stage and abandoned the food to pupate. The maximum length reached by the larvae showed significant differences between temperatures ($H=93.40$, $df=4$, $P<0.001$). The largest larvae were obtained at 15°C (17.47 ± 0.63 mm) and the smallest at 12°C (14.60 ± 0.74 mm). The largest individual was 19.34 mm.

The duration of each developmental stage under all temperature regimes is presented in table 1 and illustrated in fig. 9. The increase of temperature had an effect on the duration of developmental stages, which decreased drastically. Total development varied between 46.50 ± 0.97 days at 15°C and 15.39 ± 0.32 days at 30°C. There were significant differences in larval duration ($H=162.41$, $df=4$, $P<0.001$), pupal duration ($H=143.012$, $df=3$, $P<0.001$) and total development ($H=143.019$, $df=3$, $P<0.001$). The rate of larval development increased with temperature ($y=0.0059x-0.0179$; $R^2=0.95$). The t_0 was 3°C and degree-days varied between 154.00 and 183.96 DD. For total development, linear regression ($y=0.003x-0.0219$; $R^2=0.98$) resulted in a t_0 of 7.3°C and degree-days ranged from 309.75 to 358.05DD (table 1).

Analysis of the cases studied

Table 2 shows a summary of the 22 cases, from the IAFM and IMLA, where *S. nudiseta* was present. Of these, 13 were found in the centre of the country (Madrid), eight in the southeast (Alicante) and one in the northwest (Ourense). A total of 17 cases were dated from autumn, two from spring, two from summer and one from winter. With the exception of one case, *S. nudiseta* was always present indoors, and most corpses were mummified to some degree. Larvae of *S. nudiseta* were collected from different regions of the corpses, including the natural openings of the head, torso, genitals and legs. In several cases, pupae and empty pupae were located attached to the clothes and hair (fig. 10A–C). Regarding succession, analyzed cases suggest that the arrival time of *S. nudiseta* could be different according to environmental conditions. It is the first colonizer in autumn but arrives after *Calliphora vicina* Robineau-Desvoidy, 1830 during colder months, whereas in summer it arrives together with, or after *Chrysomya albiceps* (Wiedemann, 1819), *Lucilia sericata* (Meigen, 1826) and *Sarcophaga* sp. Meigen, 1826. Next, a case from IMLA that has been considered the most representative of the usefulness of this species in southern Europe is presented.

IMLA Case: 794/2010

The fully clothed body of a woman was found sitting on the couch in an apartment in Torreveja on 4 November 2010. The corpse was mummified. The body showed no signs of injury or criminal violence and death had been due to natural causes.

The species collected with the most advanced developmental stage was *Ch. albiceps*, with empty puparium; however, they were low in number and the majority of adults emerged two days later in the laboratory. In contrast, pupae of *S. nudiseti* were the most abundant sample; and, together with *M. scalaris*, they emerged three days after being collected. No adult emerged from the single pupa of *C. vicina*. According to several authors (Marchenko, 2001; Al-Misned *et al.*, 2003; Grassberger *et al.*, 2003), *Ch. albiceps* completes its cycle in approximately 19 days at 20°C. Data from this study (table 1, fig. 9) and results obtained by Rabinovich (1970) indicate that *S. nudiseti* needs about 26–27 days to complete its development at 20°C. For *M. scalaris*, information on development at 20°C is highly variable, but pupae of this species

Discussion

The updated distribution of *S. nudiset* from European mainland includes littoral southeast Iberian Peninsula, from the provinces of Alicante (Martínez-Sánchez, 2003), Murcia (Ubero-Pascal *et al.*, 2010b), Granada (J. Marín, personal communication) and Málaga (Bowden, 1997). Recently, this species has also been collected from Lisbon, Portugal (Prado e Castro *et al.*, 2012) and Naples, Italy (Lebrun & Mayer, 2011). After a review of forensic cases, we realized that the first appearance of *S. nudiset* in a corpse was in 2004 in Madrid (central Spain). What is more, the record of Ourense (north-west Spain) confirms that this species has established itself in the Mediterranean Basin and is expanding its distribution

Fig. 6. First instar of *S. nudiseta*. (A) Thoracic and abdominal segments, ventral view; (B) abdominal segments, lateral view; (C) posterior end body, posterior view; (D) posterior spiracle; (E) posterior end of body, lateral view; and (F) posterior end of body, ventral view, A1 [A2], first [second] abdominal segment; ap, anal plate; ex, extra-anal papilla; pa, postanal papilla; paa, para-anal papilla; pre, pre-anal papilla; ps, posterior spiracle; sa, subanal papilla; st, spiracular tufts; T3, third thoracic segment.

Fig. 7. Third instar of *S. nudiseta*. (A) Thoracic and abdominal segments, lateral view; (B) abdominal segments, ventral view; (C) posterior spiracle; (D) posterior end of body, ventral view; (E) posterior end of body of young larva, posterior view; and (F) posterior end of body of mature larva, ventral view. A1 [A6/A7], first [sixth/seventh] abdominal segment; ap, anal plate; ex, extra-anal papilla; pa, postanal papilla; paa, para-anal papilla; pre, pre-anal papilla; ps, posterior spiracle; sa, subanal papilla; st, spiracular tufts; T3, third thoracic segment.

Table 1. Duration (mean $\pm$ SD) of developmental stages and accumulated degree-days for *S. nudiseta*, for each temperature regime.

Stage	Minimum duration (days)				
	12°C	15°C	20°C	25°C	30°C
Egg	–	2.00 $\pm$ 0.50	1.00 $\pm$ 0.50	1.00 $\pm$ 0.50	0.50 $\pm$ 0.50
Larva	19.80 $\pm$ 0.45 ^a	15.33 $\pm$ 0.76 ^a	9.56 $\pm$ 0.69 ^b	7.00 $\pm$ 0.59 ^c	6.78 $\pm$ 0.42 ^c
Pupa	–	28.87 $\pm$ 0.35 ^a	15.73 $\pm$ 0.89 ^b	9.73 $\pm$ 0.45 ^c	8.07 $\pm$ 0.25 ^d
Egg to adult	–	46.50 $\pm$ 0.97 ^a	26.33 $\pm$ 0.48 ^b	17.50 $\pm$ 0.78 ^c	15.39 $\pm$ 0.32 ^d
ADD larval	178.20	183.96	162.52	154.00	183.06
ADD total	–	358.05	334.39	309.75	349.35

^{abc} Different letters within rows indicate significant differences ($P < 0.05$).

Fig. 8. The mean ($\pm$ SD) length of *S. nudiseta* larvae, measured at 24-h intervals for each temperature regime ($n = 50$). At 12°C, after the sixth day larvae were measured every 72 h ($n = 25$).

Fig. 9. Isomorphen-diagram for *S. nudiseta*, showing the duration of developmental stages from oviposition to eclosion, at 15, 20, and 30°C. ●, egg hatching; ○, pupation; ▼, adults emergence.

northwards. In addition, continuous sampling at the Campus of the University of Alicante shows that abundance of *S. nudiseta* has increased during the last years, frequently being observed in large numbers during warmer months from early summer onwards (Y. Velásquez, personal observation). This species should not be rare in human remains in other areas of southern Europe. Probably, this species has been overlooked previously because it does not appear in most available 'forensic' identification keys and, moreover, may have been confused with other Muscidae (wrongly identified as genus *Muscina* Robineau-Desvoidy, 1830 or *Musca* Linnaeus, 1758).

Separation of the larvae of *S. nudiseta* from other species of Muscidae that are also found during forensic entomology examinations is restricted because of limited information on larval morphology of these flies. The identification of the third instar larvae is relatively easy, thanks to the keys of Ishijima (1967), Skidmore (1985) and recently Velásquez *et al.* (2010). According to these authors, identification of the third instar is based on the presence of the suprabuccal teeth and cutaneous teeth (figs 3E and 5C), as well as on the peculiar S-shaped slits of posterior spiracles (figs 4F and 7C) and dark pigmentation of the peritreme. Providing a key or any information allowing identification of the first and second instars is seriously limited. Tentatively, it could be stated that identification of the first instar of *S. nudiseta* is possible because of the presence of sharply ended cirri (fig. 1B), the smooth surface of the dorsal side of thoracic segments (fig. 1A) and a small triangular anal plate surrounded by well-developed papillae (fig. 6C, E, F).

For the second instar, the most valuable characters are the small triangular anal plate surrounded by well-developed papillae (fig. 2G, H) and F-shaped mouthhooks (figs 4C and 5B) (Grzywacz *et al.*, unpublished data). On the other hand, relatively easy identification of adults is possible based on the uniquely orange flagellomere and tip of abdomen (fig. 11A) together with wing vein M_1 curved forward towards vein R_{4+5} (fig. 11B), not angular (fig. 11C), and lack of the orange or reddish tip of the scutellum (Pont, 1991; Carvalho, 2002; Couri, 2007).

Larval morphology of *S. nudiseta* was described by Siddons & Roy (1942); we have focused here on characters insufficiently described or completely omitted. Results of our study agreed with their descriptions and most differences are due to the different terminology; however, a few contradictions were found, mainly in the cephaloskeleton structures. The parastomal bar of the first instar of *S. nudiseta* in Siddons & Roy's (1942) fig. 5 is shown as simple and not connected with the basal sclerite. During this study, a process diverging from the parastomal bar was found, and parastomal bars are continuous with the basal sclerite (figs 4A and 5A), as has been reported in other families (Courtney *et al.*, 2000; Szpila & Pape, 2005; Szpila *et al.*, 2008b; Szpila, 2010; Szpila & Villet, 2011). We clearly differentiate the epistomal and labial sclerites in the second and third instars, which Siddons & Roy (1942) showed as an integral part of the intermediate sclerite, except for an imprecise outline on the drawing of the third instar in their fig. 15.

Table 2. Summary of the cases from IAFM and IMLA where *S. nudiseta* appeared breeding in human corpses. Postmortem interval estimated by forensic pathologist or from witness testimonial of the last sighting of the individual alive.

Year	Date of discovery	Place	Locality ¹	PMI _{max} (days)	Samples collected
2004	September	Indoor	Madrid	6	LIII
	October	Indoor	Madrid	4	LIII
2006	May	Indoor	Madrid (Valdemoro)	60	EP
	April	Indoor	Alicante	90–120	LIII
2008	September	Indoor	Madrid	9	LIII
	September	Indoor	Ourense	12	P
	October	Indoor	Madrid	20	EP
	October	Indoor	Madrid	6	E
	October	Indoor	Madrid	10	LIII
	October	Indoor	Madrid	18	P
2009	June	Indoor	Madrid	14	EP
2010	January	Indoor	Madrid	60	EP
	January	Indoor	Alicante (Torrevieja)	20–30	LI, LII, LIII
	April	Outdoor	Alicante	30–40	LIII, P, EP
	June	Indoor	Alicante (San Vicente del Raspeig)	7–10	LIII
	October	Indoor	Alicante (Torrevieja)	15–30	LIII
	November	Indoor	Alicante (Torrevieja)	40	LIII, P
2011	October	Indoor	Madrid	10	P
	October	Indoor	Madrid	13	P
	October	Indoor	Madrid	18	LIII
	October	Indoor	Alicante (Torrevieja)	7	L?
	November	Indoor	Alicante (Benidorm)	30–40	LIII, P, EP

¹ Madrid (central Spain); Ourense (northwest Spain); Alicante (southeast Spain)

E, eggs; LI, larva of first; LII, second; LIII, third instar; P, pupa; EP, empty puparia; L?, larva of unknown instar.

Fig. 10. Pupae of *S. nudiseta* embedded in the hair of the deceased. (A) Lateral and (B) frontal view, and (C) detail of the pupae.

Fig. 11. (A) Adult female of *S. nudiseta* with orange flagellomere (fl) and tip of abdomen (abd); (B) wing of *S. nudiseta*, dorsal view; and (C) wing of *Musca domestica*, dorsal view. M₁, R₄₊₅, wing veins.

The cirri of the first instar were described by Siddons & Roy (1942) as “the anterior margin of the first (oral) groove”. Similarly to Siddons & Roy (1942) and Skidmore (1985), we did not observe any presence of the suprabuccal teeth in the first instar although these structures have been found among first instars of other Muscidae, e.g. in *Muscina* (Skidmore, 1985), *Philornis downsi* Dodge & Aitken, 1968 (Fessl *et al.*, 2006) (as cuticular teeth) and *Musca domestica* (Linnaeus, 1758) (Skidmore, 1985; Szpila & Pape, 2008). For *S. nudiseta*, there is no doubt that suprabuccal teeth, present in the second and third instars, are separate structures because there are no signs of any connection with mouthhooks. In other families, sclerotized structures present on the facial mask have been recognized unambiguously (Szpila & Pape, 2005; Szpila *et al.*, 2008b; Szpila, 2010; Szpila & Villet, 2011) or with some reserves (Grzywacz *et al.*, 2012) as integral parts of the mouthhooks.

Based on drawings of fig. 7 in Siddons & Roy (1942), one could consider the presence of a parastomal bar in the second instar larva of *S. nudiseta* as Skidmore (1985) did. He has indicated the occurrence of a distinct parastomal bar (as a parastomal sclerite), but the presence of such a feature would be unusual and primitive because of the general absence of the parastomal bar in the second and third instars of Muscidae (Skidmore, 1985). According to our observations, a longitudinal incision is present on the dorsal surface of the lateral arms of intermediate sclerite, forming a bar-like feature that does not reach the basal sclerite (fig. 7B). In our opinion, Siddons & Roy (1942) have wrongly recognized this bar-like feature as separated from the intermediate sclerite and connected with the basal sclerite, causing mistakes in Skidmore’s interpretation of literature data.

Regarding development, information about the growth of this species is limited. According to the description of Skidmore (1985), the range of the length of the third instar is 7.0–19.5 mm, similar to our findings. Compared with the study of Kumara *et al.* (2009), the maximum length of larvae at 28°C was 14.9 mm, considerably lower than our values between 25 and 30°C, but they measured five larvae randomly instead of the largest as in the current study. On the other hand, we observed an increase in developmental periods with lower temperatures. Our findings were similar to those reported by Rabinovich (1970) at 20°C and 28°C, but higher than those found in Asia (Siddons & Roy, 1942; Kumara *et al.*, 2009) and slower than Krüger *et al.* (2002). Differences between development times may be due to different methods used in each study, e.g. the use of

fluctuating temperatures (Siddons & Roy, 1942; Kumara *et al.*, 2009) or the use of a carbohydrate diet instead of a protein diet (Krüger *et al.*, 2002). Moreover, it has been proposed that some species do not exhibit the same growth pattern in different geographical areas (Greenberg, 1991; Grassberger & Reiter, 2001; Donovan *et al.*, 2006; Martínez-Sánchez *et al.*, 2007). In any case, generation of development data for local populations is imperative in order to permit the proper use of this species of tropical origin in forensic investigations in Europe.

Studies related to low temperatures for this species do not exist. Although in trials at 12°C, eggs were reared at high temperatures (23–25°C), larvae were successfully reared at 12°C. We do not know what the development of *S. nudiseta* would be like at low temperatures in natural conditions in the Mediterranean region; but, the fact that adults have been captured in the field at 12°C (Martínez-Sánchez, 2003), larvae have been collected breeding in winter and a minimum development threshold estimated of 3°C and 7.3°C for larval and total development, respectively, suggest that this species of tropical origin could have adapted to the lower temperatures that occur in these latitudes in colder months.

The entomological evidence from reviewed cases suggests that the presence of *S. nudiseta* in human remains is strongly determined by ecological conditions, and its abundance and arrival time varied throughout the year. The high frequency of this species in cases dated from autumn and the heavy infestation observed in this season agree with a phenology study with wind oriented traps (WOT traps) performed in Alicante in 1997, in which this species was present throughout the year with the highest abundance in autumn (Martínez-Sánchez, 2003). Possibly the highest values of humidity produced by autumn rainfall favour the abundance and the arrival time of *S. nudiseta*. Apparently, this species is able to reach bodies not only inside buildings but also outdoors. Until now, it had not been reported in outdoor remains; nevertheless, in that case, the body was wrapped by a blanket, so it is possible that the wrapping creates a particular microhabitat that allowed oviposition and development of this species outdoors.

Abundance of *S. nudiseta* on the corpse can give us some conclusions about the season of death, because its activity is dependent on the warm and wet months. Nevertheless, it seems that the main forensic value of this species is the estimation of minimum time of death when the death occurs during autumn, when this species seem to be one of the first colonizers and is more abundant or, in those cases, when pioneer species do not have access to the body or their maggots migrate away from the remains.

The recent records of *S. nudiseta* from Spain, Portugal and Italy are evidence of range expansion of this species in southern Europe as occurred in the past with other forensically important flies such as *Ch. albiceps* (Grassberger *et al.*, 2003; Verves, 2004; Gosselin & Braet, 2008; Szpila *et al.*, 2008a). Therefore, we should like to draw forensic entomologists’ attention to the possible presence of this species in entomological material collected from experimental and real cases and its potential use as a forensic indicator.

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