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NEW OBSERVATIONS ON *LIOSARCOPHAGA AEGYPTICA* (SALEM, 1935) (DIPTERA, SARCOPHAGIDAE) REARED FROM COLONIES COLLECTED ON THE UNIVERSITY CAMPUS OF LEJONA (VIZCAYA, NORTHERN SPAIN)

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Abstract: New data is presented about *Liosarcophaga aegyptica* (Diptera, Saarcophagidae), completing the work published in a previous paper. Material was collected on the campus of Leioa University (Biscay, northern Spain) by means of selective attraction traps baited with pig kidney placed in different ecosystems over a calendar year. In June 2003 and October 2004 several females were collected and identified as *Liosarcophaga aegyptica*, based on reared males. Some of the specimens reared in November 2005 were infested by a parasitic wasp identified as *Nasonia vitripennis* (Walker, 1836) (Hymenoptera, Pteromalidae). Details of the mouthparts and posterior spiracle morphometry are included, and the morphology of all the preimaginal instars is presented, as well as new data about ovoviviposition and diapause. Specimens of all instars have been preserved for a detailed description of a species whose biology remains little known. Previous records seem to indicate that the distribution of this species of forensic interest is expanding.
Key words: Diptera, Sarcophagidae, *Liosarcophaga aegyptica*, ovoviviposition, development, diapause, morphometry, Vizcaya, España.

Nuevas observaciones sobre *Liosarcophaga aegyptica* (Salem, 1935) (Diptera, Sarcophagidae) procedentes de colonias colectadas en el campus de la Universidad de Lejona (Vizcaya, norte de España)
Resumen: Se aportan nuevos datos y resultados sobre *Liosarcophaga aegyptica* (Diptera, Sarcophagidae) obtenidos en el campus de la Universidad de Leioa (=Lejona, Vizcaya, norte de España). Durante un año completo se realizaron muestreos continuos en diferentes ecosistemas colocando trampas de atracción selectiva cebadas con riñón de cerdo. En Junio 2003 y Octubre 2004 se recogieron nuevos grupos de hembras y se identificaron como *Liosarcophaga aegyptica*, siendo la identificación posible gracias a los machos capturados. Algunos individuos criados en Noviembre del 2005 fueron infestados por un himenóptero parasitoide, identificado como *Nasonia vitripennis* (Walker, 1836) (Hymenoptera, Pteromalidae). En el presente trabajo se amplía la información sobre la morfología de todas las fases larvarias, incluidas la fase I y el huevo, y se aportan nuevos datos sobre la ovoviviposición y la diapausa observadas en esta especie de sarcofágido. También se incluyen resultados morfométricos de las piezas bucales y de los espiráculos posteriores.
Palabras clave: Diptera, Sarcophagidae, *Liosarcophaga aegyptica,* ovoviviposición, desarrollo, diapausa, morfometría, Biscay, Spain.

Introduction

The biology of many species of necrophagous flies remains unknown apart from their direct application in forensic research. As regards to Sarcophagidae species, only adults (Povolný & Verves, 1997; Pape, 1998) and some instars of a few species (*cf.* Sanjean, 1957; Ishijima, 1967) are well documented. Sarcophagidae are hard to identify due to the absence of clear external characteristics in females, and errors in their identification may explain some atypical rearing records in the relevant literature (Dahlem,1991) For many species, only the study of male genitalia results in adequate information for specific identification. Therefore, if no males are collected specific identification may not be possible. We consider the contribution of the description of the life cycle of this species, including all the preimaginal instars, to be of special importance.

Liosarcophaga aegyptica Salem (1935) has recently been recorded for the first time in the Iberian Peninsula (Saloña Bordas & Gonzalez Mora, 2005). We complete the descriptions made in the first paper with new information about the first instar, ovoviviparity and diapause. We expect the complete description of all the instars of the species, identified from males reared from larvae extracted from captured females, will help clarify the taxonomy of this family in the future.

L. aegyptica is a subtropical thermophilous species that has a wide distribution in the Eastern Hemisphere, although that distribution seems to follow a large expansion. It has recently been recorded as an invader in central Europe based on material collected in South Moravia (Povolný & Hula, 2004). Checking the pictures and description of the collection area, we confirm the preference of the species for open fields with artificial dams in the area. A previous synonym has been proposed for *L. salemiana* Lehrer, 1995 and S*arcophaga parkeri* Rohdendorf, 1959 based on an analysis of the male genitalia of both taxa (*cf.* Povolný, 1998; Peris *et al*., 1999).

Materials and methods

Females of *L. aegyptica* were isolated from attraction traps placed on our university campus (Leioa, Biscay, northern Spain). Following the design of Hwang and Turner (2005), traps were built and baited with pig kidney. Traps are being set seasonally for necrophagous fly collection in the area. Ending in August 2003, October 2004 and June 2005, some females of this species were collected and their abdomens opened to extract the larvae, which were reared on pig kidney. Adults emerging from the second and third

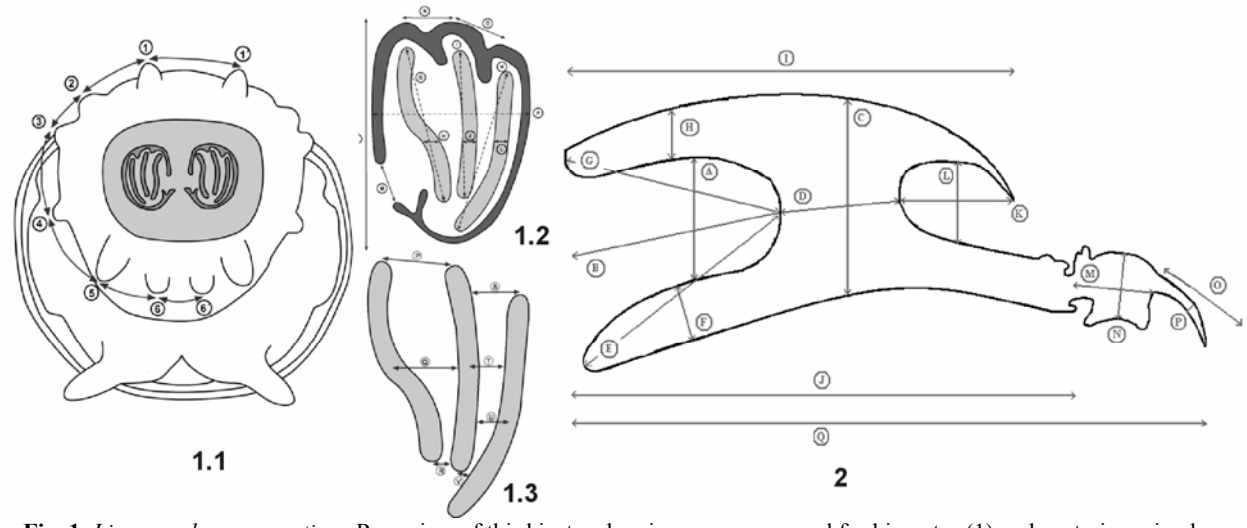


Fig. 1. *Liosarcophaga aegyptica*. Rear view of third instar showing areas measured for biometry (1) and posterior spiracles (2 and 3). **Fig. 2.** *Liosarcophaga aegyptica* mouthparts. Lateral view showing areas measured for biometry

Table I. **Mean temperatures measured daily during the study (ºC), length (L) measured in milimeters (mm) and accumulated degree days on base 10 [ADD (-B10)] estimation for larval instars (L1, L2, L3) postfeeding (Pf), pupae (P) and adult (A)**

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 16	Day 17
Lab Temp. °C	23.5	24.7	25.4	26	26	26	25	27	26
Instar	L1	L2	L3	L3	Pf.	P	P	A	A
L (mm)	0.9-5.1	5.2-7	10-16	18.9-20.5	18-19	9.1-11.2		12.0-14.4	
ADD (-B10)	13.5	28.2	43.6	59.6	75.6	91.6	106.6	123.6	139.6

population were kept alive in cages (25 cm. long by 25 cm. wide by 25 cm. high) for culture under laboratory conditions.

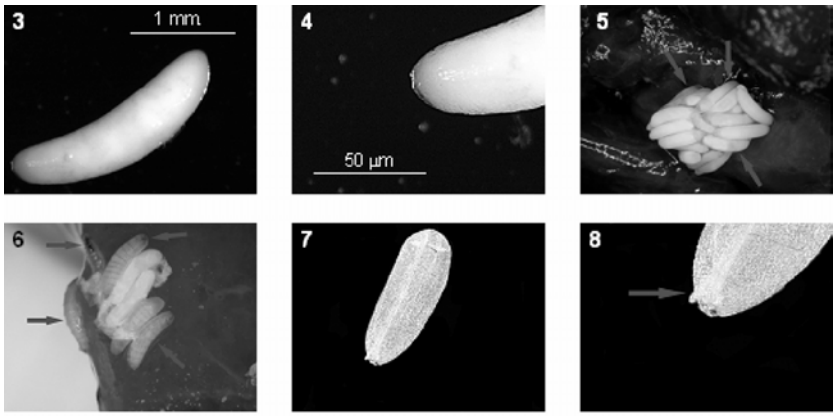
A protein-rich organic source based on a mixture of powdered milk and sugar (50:50) was provided to the adults so that their reproductive system would develop adequately. Pig kidney was used as a rearing substrate. Laboratory mean temperature was measured daily to estimate the Accumulated Degree Days (ADD) (Highley & Haskell 2001); we used a Cable Free Thermo-hygrometer with temperature and humidity-trend indicators (Oregon Scientific). Colonies were checked periodically to monitor instar development times and a sample of the larvae reared were boiled daily in hot water for 1 min. and preserved in 80% ETOH for morphology and biometry (*cf.* Adams & Hall 2003). Larvae were clarified with lactic acid for observation under the microscope. Microscopes (Nikon SMZU and Eclipse E600) were used as technical support for morphometry. Figs. 1 and 2 report measurements made for biometrical analysis. All measurements are expressed in μm.

Results

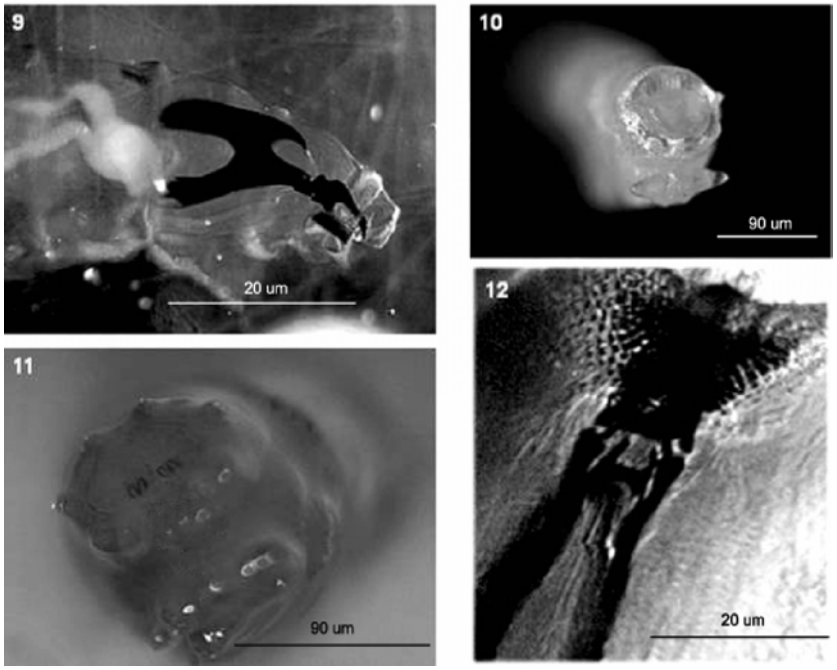
Females of *L. aegyptica* collected from baited traps were used for this research, reared in cages and bred. Larvae were extracted directly from the females' abdomens and fed with pig kidney to maintain a colony of this species. No regulation of environmental temperature, humidity or exposure to daylight was possible during the experiment. Nevertheless, the temperature in the laboratory does not fluctuate widely over the year: it is heated in winter along with other University premises. In October 2004, when the second population of this species was reared, the temperature oscillated between 19 and 27 ºC. No cooling process occurs but no adults emerged from puparia until March 2005. The light-darkness cycle (L:D) was probably the inductor of this delay in development.

A further population collected in June 2005 was maintained and reared for ADD estimations (table I). Mean temperature was recorded daily and is detailed in Table I. The adults that emerged were held in rearing chambers at 26 ± 3 ºC, with an environmental humidity of about 60 - 65% and a L:D cycle close to 12:12. No diapause was observed in preimaginal instars in summer 2003 (1st population reared) or in spring 2005 except at the end of October, when pupae went into diapause at the end of the month. In one of these colonies all the specimens were infested by a parasitic wasp (Figs. 22 and 23) identified as *Nasonia vitripennis* (Hymenoptera, Pteromalidae), which emerged four weeks later (25 November 2005). The remaining colonies that did not seem to be infested by the wasp went into diapause for the whole winter, as did those reared the year before.

Adults emerged from the first generation developed as detailed (Table I). Females began to lay eggs instead of larvae, resting directly on the substrate or on the container during oviposition. The leading specialists in this family agree that Sarcophagidae species usually eject their first instar larvae directly onto the food source. They thus deposit just few specimens but in adequate conditions to ensure the successful development of the descendents. Nevertheless, females of this species kept in the rearing chamber usually rested on the container or on the kidney itself, laying small groups of 10-20 eggs. Oviposition has been reported sporadically for other species of this family (*i.e.* Lopes & Leite, 1989; Grassberger & Reiter, 2002; Sukontason *et al*., 2005) and is clearly unusual but not impossible for the species of this family. The deposition of fully incubated eggs may be due to the optimal breeding conditions in the laboratory as an adequate source of protein in the diet



Figs. 3-8. *L. aeg*yptica, photographs of the egg and emergence of first instar larvae. **3.** Lateral view of the whole egg. **4**. Rough surface of the edge of the egg. **5**. Egg mass, some embryos are beginning to be differentiated and can be seen by transparency (arrows). **6**. Emergence of larvae (arrows). **7**. Egg capsule after the emergence of the larva. **8**. Micropillum.



Figs. 9-12. First instar larvae of L. aegyptica. **9**. Cephalopharyngeal skeleton, lateral view. **10**. Posterior spiracles with anal tubercles, posterior slits can be appreciated by transparency. **11**. Posterior spiracles at the bottom of the posterior cavity delimited with dorsal and ventral tubercles. **12** Hypopharyngeal sclerite, frontal view.

may induce oviposition in Sarcophagidae, (*cf.* Pape, 1998) or to the stress of laboratory conditions. Although oviposition was observed during the rearing process, most of the eggs were not viable and did not complete their development, deteriorating in a few days. Just for those where the embryo was beginning to be differentiated by transparency through the egg cover only, emergence occurred after a short time (30 – 60 min.) and they developed successfully. Success in development was extremely variable for each oviposition. About 10 to 50% of the eggs laid in groups developed without problems. After emergence some larvae died, probably due to the exudations of the fresh kidney, as they became glued to the sticky surface (Sanjean, 1957). To avoid this problem, the kidney surface was regularly drained with tissue paper.

For other necrophagous species such as greenbottle flies (Luciliinae, Calliphoridae), migratory larvae should be placed in bigger containers with adequate substrate to monitor the migration and pupation process. Focusing on *L. aegyptica*, we found that no specific conditions in substrate are necessary for pupation. They pupated on the organic substrate itself (food remains) and on paper, in soil or in vermiculite, with no developmental problems. Therefore, puparia of this species may be found on a cadaver, or in clothes. Similar results have been observed for *Calliphora vicina* Robineau-Desvoidy, 1830 reared simultaneously with them (paper in process).

Preimaginal instars

Povolný (1987) and Povolný & Verves (1997), give a detailed description of adults in the reviews of the family. Therefore, only preimaginal instars are described here. As a complement to anatomical description, a table is included with the morphometry of mouthparts and posterior spiracles. All the larval instars and the puparium have single pointed spines and posterior spiracles sunk in a posterior cavity.

Egg (Fig. 3-8)

Length 1.62 – 2.12 mm; width 0.41 – 0.47 mm.

The egg body is elliptical with a cylindrical shape due to the curvature of its edges. Although the egg surface seems to be completely smooth, it is in fact rough, as we can observe in Fig. 4.

Fig. 5 shows a mass group of about 20 eggs where some embryos can be seen by transparency through the egg envelope. In Fig. 6 we can observe three recently emerged larvae that are beginning to abandon the egg mass and feed on exudation.

When larvae emergence is completed we can observe the real texture of the egg: it is entirely rough, and the micropillum is much easier to distinguish, as we can see in Figs. 7 and 8. The micropillum is centrally located. The envelope of the egg is transparent and it is difficult to find it on the substrate after larval emergence.

Larvae

1st instar (L1) Fig. 9-12. Table II.

Length 3.27 – 4 mm; width 0.9 – 1.05 mm.

The cephalopharyngeal skeleton is deeply pigmented. Dorsal and ventral cornu of similar length, with the dorsal cornu being as broad as or broader than the ventral one Dahlem (1991). In the first instar the characteristic windows of the cornu are not developed and their surface is totally smooth, with no invagination.

The sinus is curved, but this curvature seems to be bigger than in other species. It is due to the gap between the two cornu, being it bigger. In other species such as *Sarcophaga scoparia*, the dorsal cornu goes down and the sinus is smaller (Sanjean, 1957). The most characteristic feature is the shape of the dorsal arch, which is a prolongation of the dorsal cornu that finishes in a head that looks like a hook, called the anterodorsal process. This structure reaches almost to the hypostomal sclerite.

The parastomal sclerite is not defined in this instar. Mouth hook with a long and slender hook curved at the edge and with the anteroventral angle well defined (Fig. 9). Hypopharyngeal sclerite is H–shaped as observed in a ventral view (Fig. 12).

Posterior spiracles V shaped with the two branches almost parallel and very close together. They are sunk in a posterior cavity. The slit and the prolongations are nearly vertically orientated (Figs. 10 and 11). The peritreme is not yet developed. Six dorsal tubercles around the posterior cavity, and six ventral tubercles behind this cavity can be observed. Anal tubercles are very well developed and conical.

Body segments are surrounded by several rows of single-pointed spines. Anterior rows are complete in all segments except the last one. Posterior row of spines absent in first segments (one to five), incomplete in sixth to eighth segments, covering 1/3 of lateral surface in sixth segment, ½ in seventh and 2/3 in eighth one; posterior rows complete in segments nine to eleven. Twelfth segment has only the anterior rows of spines complete.

2nd instar (L2) Fig. 13-16. Table II.

Length 5.2 - 7 mm; width 0.9 – 1 mm.

The cephalic region of the second instar is tapered in its anterior area. Anterior spiracles with 14-16 lobes well defined and sited in the posterior margin of the prothorax. Openings arranged in a single row.

The cephalopharyngeal region is heavily pigmented. Cephalopharyngeal skeleton with a long and thin parastomal bar slightly curved upwards. Anterodorsal process extended further than posterior margin of mandibular hooks describing a profound anterior sinus. Dental sclerite ovoid with a central hollow. Windows from dorsal and ventral cornu opened distally. Broad tentorial phragma. Dorsal cornu longer than ventral one.

Posterior spiracles long and ovoid inside a profound hexagonal shaped atrium, surrounded by rows of long, thin, single-pointed spines and two marginal, fleshy tubercles. The peritreme is incomplete and does not encircle the two slits completely. This distance between peritremes is shorter than their diameter.

Body segment rows of single-pointed spines in similar disposition as in previous instar, with the anterior rows complete in all segments except the last one in the upper area. No posterior rows of spines in first segments (one to five), incomplete in sixth to eighth segments, covering 1/3 of lateral surface in sixth segment, ½ in seventh and 2/3 in eighth one; posterior rows complete in ninth to eleventh segments. Twelfth segment has only the anterior rows of spines complete.

3rd instar (L3) Fig. 17-18. Table II, III.

Length 18.98 – 20.54 mm; width 3.82 – 4.23 mm.

Anterior spiracles with 14-15 well defined lobes. Openings arranged in a single row.

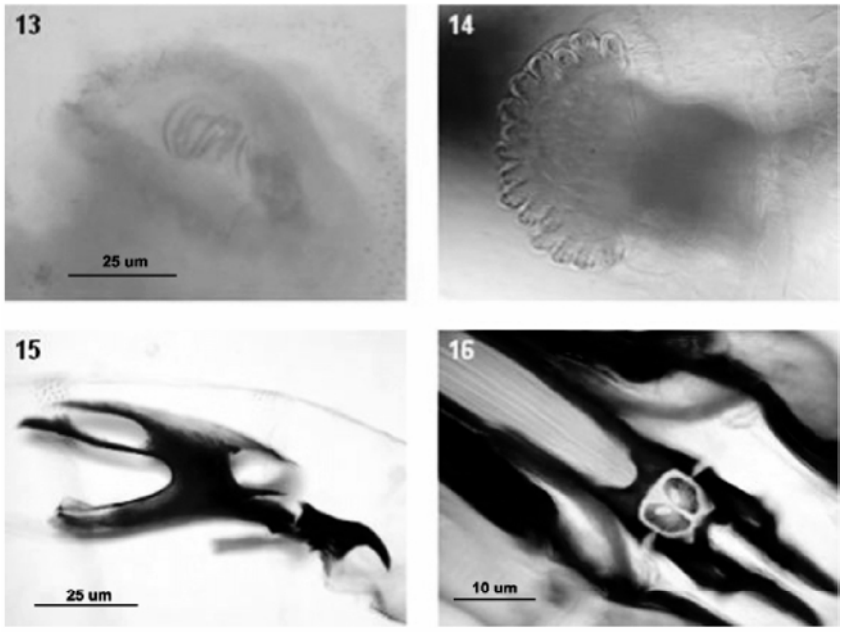
The cephalopharyngeal skeleton preserves the same characteristics of the previous instar with a stronger chitinization that allows the aforementioned characteristics to be observed more clearly.

Single-pointed spines, in a similar disposition to previous instars, with the anterior rows being complete in all segments except the last one in its upper area. No posterior rows of spines in first segments (one to four), incomplete in fifth and sixth segments, covering ½ of lateral surface in fifth segment, 2/3 in sixth one; posterior rows complete in seventh to eleventh segments. No dorsal spines in twelfth segment, with presence in the lateral and ventral area. Anal tubercles are completely covered with thin spines.

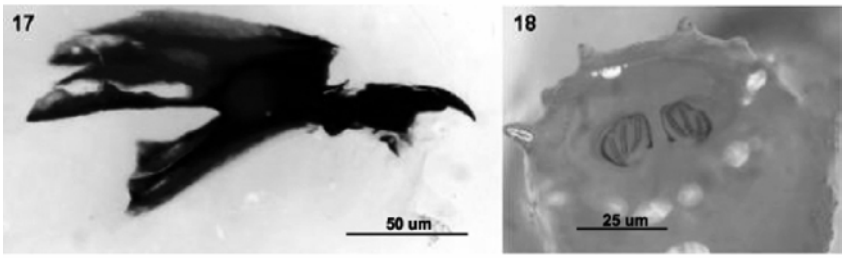
Posterior spiracles slits are long, slender, slightly curved at the bottom and convergent towards the ventro-lateral opening of the peritreme. An incomplete, thin peritreme encircles the slits. Inner projections between slits have no sclerotised ventral arc. Spiracles at the bottom of a posterior cavity with a rectangular shape, surrounded by twelve tubercles laterally placed, three dorsal and three ventral at each side. Distance between central tubercles is greater than distance between lateral ones. Inner and outer dorsal tubercles are very similar in size, but the middle dorsal tubercle is smaller than the others. Entirely the opposite is the case with the ventral tubercles, with the inner and outer tubercles being of similar size, but both smaller than the middle one. Anal tubercles are well developed, bigger than other tubercles and covered with many rows of long, slender spines.

Table II. Biometry of mouthparts in larval instars
(See Fig. 2 for explanation of measurements)

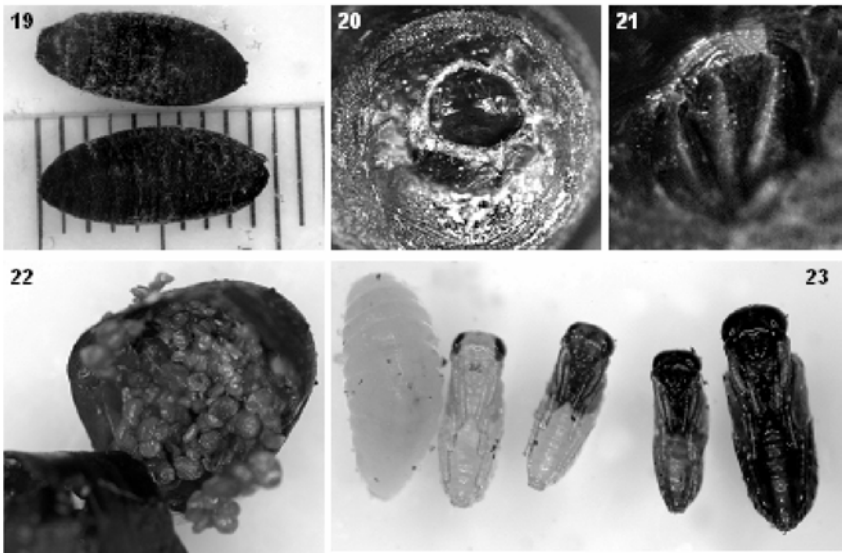
	L1	L2	L3
A	12 - 13 um	9 um	18 - 22 um
B	21 - 23 um	40 um	75 - 76 um
C	19 - 20 um	28 um	36 - 37 um
D	19 - 20 um	19 um	27 - 28 um
E	23 - 25 um	32 um	52 - 55 um
F	5 um	8 um	9 - 12 um
G	23 - 26 um	39 um	78 um
H	5 - 7 um	11 um	25 - 26 um
I	42 - 50 um	71 um	124 um
J	44 - 53 um	73 um	129 - 133 um
K	11 - 15 um	17 um	24 um
L	7 um	9 um	16 um
M	7 - 9 um	13 um	19 um
N	7 - 8 um	9 um	12 - 14 um
O	12 - 13 um	13 um	22 - 24 um
P	2 um	5 um	7 - 8 um



Figs. 13-16. Second instar larvae. **13.** View of posterior spiracles sunk in the posterior cavity. **14.** Anterior spiracles with 16 lobes in a single row. **15.** Cephalopharyngeal skeleton in lateral view, with developed windows. **16.** Cephalopharyngeal skeleton in frontal view to show in detail the sub-hypostomal sclerite.



Figs. 17-18. Third instar larvae. **17.** Cephalopharyngeal skeleton. **18.** Posterior spiracles with tubercles.



Figs. 19-23. *L. aegyptica*. **19.** Puparia completely darkened showing a brown, nearly black colour. **20.** Posterior cavity with spiracles and the row of single-pointed spines of the last segment. **21.** Posterior spiracle in detail. **22-23.** *Nasonia vitripennis* (Hymenoptera, Pteromalidae). **22.** Parasitic wasps infesting a pupa. **23.** Different instars of the parasitic wasp.

Puparium

Figs. 19-21

Length 10.56 – 11.28 mm; width 4.10 – 4.50 mm.

The puparium is ivory white at the beginning of pupation and attains a dark reddish colour in two-three hours, going almost black (Fig. 19). The surface is smooth with no outstanding morphological features. Complete rows of single-pointed spines delimit each segment (Fig. 20). The posterior end is truncated, with an invagination preserved where the posterior spiracles are located. In the posterior angle the invagination is deep and semispheric, extensive and slightly broader than it is high. The edge is armed with small thorny projections. Neither keels nor clefts are observed that interrupt the edge of the invagination. Posterior spiracles are fine and extended, slightly arched, without protuberant rims (Fig. 21).

Some pupae were infested by *Nasonia vitripennis* as we can observe in figures 22 and 23.

Discussion

Sarcophagidae are said to be exclusively larviparous, Aldrich (1916); Dahlem (1991*)* among others. Nevertheless, Pape (1998) mentions the ability of well fed Sarcophagidae to lay eggs. Likewise, Lopes & Leite (1989); Grassberger & Reiter (2002); Sukontason et al. (2005) show the oviposition ability of *Liopygia argyrostoma* and *Liosarcophaga dux* respectively. We report this ability for another Sarcophagid fly reared under laboratory conditions. Although it occurred under controlled conditions, with females fed *ad libitum*, we cannot deny that this may happen under natural conditions in the field due to the specific condition of a corpse as a saturated source for protein resources. Therefore, we consider it extremely important to describe eggs and larvae thoroughly, to offer enough information to check this potential in future research in the field due to the forensic interest of the species.

L. aegyptica is a thermophilous species with a subtropical distribution Povolný & Hula (2004) reported the expansion of this species after collecting several specimens at the Black Sea and in Hungary. We have recently confirmed the expansion of the species through south-western Europe, collecting it for the first time in the Iberian Peninsula (Saloña Bordas & González Mora, 2005), but in a cooler area - the Basque Country - with weather and vegetation closer to the countries of Central Europe than to those of the Mediterranean coast. Nevertheless, the coast has temperate winters with a mean annual temperature of 14 ºC (Castro et al., 2005). Although the coast has mild weather and Mediterranean vegetation, some females were collected in autumn (2 October 2004), with cold nights and warm days. This species has been also collected from traps in summer (2003, 2005) and reared on pig viscera as we consider it of forensic interest as a potential coloniser of carcasses in the Basque Country. The campus shows habitat conditions very similar to those shown by Povolný & Hula (2004) in Southern Moravia; an open field of a deforested rural area with a pond a few meters from the place where the flies were collected. It was quite easy to rear them under laboratory conditions, and we will focus future efforts on adjusting the establishment of development times under more controlled conditions. Although the laboratory never reached freezing conditions, an induction to diapause occurred during pupariation in specimens reared in autumn, probably due to the shorter daylight hours typical during autumn to spring. Therefore, we estimate that the duration of daylight and darkness, known as L:D cycles should be taken into consideration in future development time estimations. Not only temperature and humidity but L:D cycles seem to have an important influence on the development of this species and they cannot be obviated when post-mortem interval (PMI) estimations are drawn up, or misinterpretations of the data may lead to errors.

We offer these preliminary results with first pictures and descriptions of all the preimaginal instars, including eggs, as well as development times obtained under laboratory conditions. We observed no need to offer a specific substrate for pupation, as they often pupated on the organic substrate itself with no specific preferences for soil, tissue paper or absolute absence of substrate for burial. As they were collected with traps baited with animal viscera we expect that this species may be reported in the future on cadavers. Similar results have been observed with *Calliphora vicina* reared simultaneously with them. Taking into consideration that no diapause occurred during spring to summer cultures we estimate that the daylight cycle with shorter days at the beginning of autumn was the main factor that induced this species into diapause.

Acknowledgments

We wish to thank especially to Dr. Gonzalez Mora (Madrid, Spain) for the first identifications of *Liosarcophaga aegyptica,* and to Dr. Nash (Belfast, Ireland) for the identification of the parasitic wasp (*Nasonia vitripennis*), as well as to Dr. Haskell (Rensselaer, Indiana, U.S.A.) for all the knowledge and experience shared during the last summers that have contributed to an important enrichment of the text.

Table II. Biometry of posterior spiracles for third instar (L3) (in µm). (See Fig. 1 and 2 for explanation of measurement codes)

Code/ Samples	05/10/ 2005	08/10/ 2004	09/10/ 2004	Mean	St. Dev.
a	91	86	89	88.7	2.52
b	63	61	63	62.3	1.15
c	56	53	53	54	1.73
d	35	35	38	36	1.73
e	15	13	15	14.3	1.15
f	12	11	11	11.3	0.58
g	10	8	11	9.7	1.53
h	1	1	1	1	0
i	10	8	11	9.7	1.53
j	1	1	1	1	0
k	9	7	10	8.7	1.53
l	1	1	1	1	0
m	3	3	3	3	0
n	4	4	4	4	0
o	5	4	6	5	1
p	4	4	4	4	0
q	3	2.5	3	2.8	0.29
r	1	0.5	0.5	0.7	0.29
s	3	2	2	2.3	0.58
t	2	2	2	2	0
u	2	1.5	1.5	1.7	0.29
v	1	0.5	0.5	0.7	0.29
from 1 to 1´	32	30	30	30.7	1.15
from 1 to 2	18	15	17	16.7	1.53
from 2 to 3	18	16	14	16	2
from 3 to 4	30	26	26	27.3	2.31
from 4 to 5	25	17	16	19.3	4.93
from 5 to 6	10	20	20	16.7	5.77
from 6 to 6´	14	15	15	14.7	0.58

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