A great little ally: revealing the morphology of the immature stages of the aphid pest predator *Sphaerophoria rueppellii* (Wiedemann, 1830) (Diptera: Syrphidae)

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Abstract

Morphology, at both adult and larval stages is crucial for the correct identification of an insect and a better understanding of its biology and behaviour. The lack of morpho-functional information in insects is much more general in the immature stages than in adults, and major insect orders, such as Diptera are no exception. Syrphids (Diptera: Syrphidae) include various genera with aphidophagous larvae playing a key role in the control of pest insects in both natural and agricultural systems. The aphidophagous *Sphaerophoria rueppellii* (Wiedemann, 1830) is a syrphid widely distributed in the Palearctic Region and it is of commercial importance as a biological control agent against aphid pests. However, little is known about the fine morphology of its immature stages because it was described in 1939, when microscopy did not allow detailed studies of certain morphological features. In this work, stereomicroscope and scanning electron microscopy (SEM) were used to get a deeper and more detailed picture of the immature stage morphology of this syrphid. SEM was used to examine in detail the chaetotaxy of three larval instars, the larva/puparium posterior respiratory process (PRP), and the chorionic structure of the egg. We describe for the first time the egg, first and second larval stages, and also give a complete updated description of the third-stage larva and the puparium. The three larval instars vary from each other, especially in the number of sensillae, PRP form, colour, and body size. The thickness of both the egg chorion and puparium integument were also measured. A possible interpretation of the reasons for the variability in the number of sensillae is discussed. Illustrations and full descriptions are provided for the egg, larva, and puparium of *S. rueppellii*, including the head skeleton of the third larval stage.

Keywords: Aphidophagous hoverflies, chaetotaxy, egg, head skeleton, sensilla

Introduction

Syrphidae, commonly known as syrphids, hoverflies, or flower flies, is a Dipteron family consisting of more than 6000 species distributed around the world (Rotheray & Gilbert 2011). The adults feed on pollen and nectar, which they use, respectively, for gonad maturation and for maintaining their vigorous flight activity (Branquart & Hemptine, 2000; Almohamad et al. 2009; Amorós-Jiménez et al. 2012; Van Rijn & Wäckers 2016). Since they feed on pollen, they play an important role as pollinators in agriculture and natural areas, especially where other pollinators are absent (Pérez-Bañón et al. 2007; Campoy et al. 2020; Dunn et al. 2020; Pekas et al. 2020). Syrphid larvae differ from other fly larvae in having the two posterior breathing spiracles fused together, longitudinal grooves on the prothorax dorsally,

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and the anus located on the anterioventral margin of the anal segment (Miranda & Rotheray 2018).

The immature stages of syrphid show great adaptability, as suggested by their different feeding habits: phytophagous (aerial and subterranean parts of plants), saprophagous (bacteria or vegetal detritus feeders), or predatory (e.g., Rotheray et al. 2009; van Zuijen & Nishida 2010; Martínez-Falcón et al. 2012; Amorós-Jiménez et al. 2014; Soubah-Dols et al. 2020).

Over a third of syrphid species worldwide have predatory larvae, mainly feeding on soft-bodied Hemiptera, such as aphids (Aphididae) and psyllids (Psyllidae) (Rojo et al. 2003; Láska et al. 2006), with an even higher preference towards aphids (Almohamad et al. 2009).

For this reason, some syrphid species are regarded as natural controllers of certain insects’ populations in nature, but they are also useful in biological and integrated pest control programmes (Rotheray & Gilbert 2011; Gómez-Polo et al. 2014; Prieto-Ruiz et al. 2019; Dunn et al. 2020; Irvin et al. 2021). One of these syrphid species, Sphaerophoria rueppellii (Wiedmann, 1830), is currently being commercialised as pest control agent (Amorós-Jiménez et al. 2012, 2014).

Adult stage morphology and behaviour, in general, are well known for syrphids, whilst the immature stages of many species remain unknown (Speight 2020). The main reasons for the lack of knowledge of the immature stages are that breeding sites are frequently unknown in nature and/or the difficulty of rearing larvae under laboratory conditions (Rotheray & Gilbert 1988, 1999). Behaviour studies on immature syrphids are usually conducted to analyse different aspects of their natural history, for example: life cycle, lifespan, feeding habits, abiotic requirements (temperature, light, and humidity) for larval development or pupation, feeding preferences, and prey availability; and these studies are often focused on species with potential as biological control agents (Láska et al. 2006; Amorós-Jiménez et al. 2012, 2014). However, historical descriptions of immature stages of many syrphid species lack detail due to the limitations of the observation techniques of samples in the early 20th century. For example, Bhatia (1939), a classic paper on immature stage descriptions of syrphids, deals with several syrphid species of economic importance today, including S. rueppellii and Epiusyrphus balticus (De Geer, 1776). Since the late 20th century, the SEM has been used for the larval description of hoverflies resulting in more detailed and accurate descriptions (Rupp 1989; Pérez-Bañón & Marcos García 1998; Scarparo et al. 2017; López-García et al. 2022). Ideally, the larvae of these species of economic importance should be studied with modern techniques (e.g., SEM) for a better understanding of their morphology and function.

The syrphid genus Sphaerophoria Le Peletier & Audinet-Serville, 1828 (Syrphidae: Syrphinae), comprises 72 species with predatory larvae widely distributed in the Holarctic, Afrotropical, Oriental, and Australasian Regions (Evenhuis & Pape 2021; Mengual et al. 2021). Of the 41 Palaeartic species, 21 occur in Europe (Thompson 2019; Speight 2020). Sphaerophoria is monophyletic (Mengual et al. 2021) and sister to Allograpta, as shown in phylogenetic studies based on larval morphology (Rotheray & Gilbert 1999) and molecular evidence (Mengual et al. 2008, 2021).

Sphaerophoria rueppellii is one of the three Sphaerophoria species with known immature stages (Bhatia 1939; Scott 1939; Dixon 1960: Rotheray 1987). The third-stage larva (L3) and puparium of this species has been studied with stereomicroscopic techniques (Bhatia 1939). However, some morphological details of the L3/puparium are still unknown or poorly characterised, such as the chaetotaxy. In addition, the egg and the L1/L2 larval stages are undescribed yet. Sphaerophoria rueppellii is the most used syrphid as a biological control agent in Mediterranean crops (Hondelmann & Poehling 2007; Amorós-Jiménez et al. 2014; van Lenteren et al. 2020) and a more detailed description of all immature stages is needed. The study of the number and position of body sensillae (chaetotaxy) is especially important because sensillae are the sensitive structures that inform the larva of external conditions, both biotic and abiotic, in such a way that certain larval behaviours can be understood.

With the purpose of better understanding the morpho-function of the immature stages of S. rueppellii, we describe in detail the egg, posterior respiratory process (PRP), and the three larval stages (L1, L2 and L3), including the L3 head skeleton and the number and relative positions of the sensillae on each thoracic and abdominal segment of the L3 larva.

**Materials and methods**

**Experimental insects**

The eggs, L3, and pupae of S. rueppellii used for the descriptions, were provided by BioNostrum Pest Control Company®. During the study, larvae were fed with the aphid *Rhopalosiphum padi* (Linnaeus, 1758) on barley plants (*Hordeum vulgare* L.). To compare
with *S. rueppellii*, five eggs of *E. balteaus* and five puparia of *Sphaerophoria scripta* (Linnaeus, 1758) were used.

**Preparation, observation, and imaging of the examined material**

Larvae and eggs of all studied syrphid species were fixed and preserved in KAAD liquid (70% alcohol of 95%, 14% glacial acetic acid, 8% toluene, and 8% dioxane). The syrphid larvae and puparia material were cleaned on an Ultrasonic bath for 4 and 12 min, respectively. The larvae were submerged in 10% KOH for a period of 7–10 h for a deeper cleaning (Schneeberg et al. 2017). The head skeleton was removed from an empty puparium after a 40 min bath in KOH solution and it was examined in glycerine. Samples were brushed to remove any dirt before observation and description.

General features of the head skeleton, L3 and puparium were observed under a Leica M205 C binocular stereomicroscope. The length of the L3 (n = 15) and puparium (n = 20) were made dorsally from the tip of the prothorax to the tip of the PRP. The width and height of the larva/puparium were measured at their maxima, always in the abdomen. For the PRP, we measured the width and length of the spiracular plate and additionally its height in lateral view. Photos were produced as stacks of individual images made with a camera (Leica DFC 450) attached to a binocular stereomicroscope (Leica M205 C). Stacks were made in Leica Application Suite LAS®, v.4.12.0. The larva was drawn from a printed photo.

Scanning electron microscopy (SEM) was used for a more detailed description of the egg, L1, L2, L3 and puparium. The egg (n = 8) and larvae were dehydrated following a procedure slightly modified from Kanturski et al. (2015). They were dehydrated using an ethanol/water series of two baths of 70% for 5 min each, followed by one bath of 80%, 90%, and 96% ethanol for 5 min each, and two baths of absolute ethanol for 5 min each. Dehydrated eggs/larvae were taken to a Leica EM CPD300 critical point dryer. For the eggs, L1 (n = 10) and L2 (n = 10), the length and width were measured. Three eggs were cut in half to measure the chorion thickness. The height and width measurement of the papilliform sensillae with/without setae were taken from the L3 larval stage. The puparium material (n = 5) was dehydrated following Kanturski et al. (2015). Three puparia were cut in half to measure the dorsal and lateral integumental thickness. All the samples were mounted on aluminium stubs with double side adhesive carbon tape and sputter-coated in a Quorum 150 T ES Plus with a 30 nm layer. The samples were imaged with a Hitachi SU8010 FE-SEM microscope at 7 and 10 kV accelerating voltage, except for one egg photo that was done at 15 kV.

All examined material is deposited at the CEUCIBIO collection, University of Alicante, Spain.

**Morphological terminology**

The morphological terminology used for the head skeleton follows Hartley (1963) and Rotheray and Gilbert (2008). The egg of *S. rueppellii* has not been described before, so we follow the description that Chandler (1968a) made for the eggs of other *Sphaerophoria* species. The morphological terminology used for the larva follows Láska et al. (2006) and Rotheray (2019). For each body segment, sensillae were numbered in the dorso-ventral direction (Rotheray 1991). A superscript (A2 ...) is used to indicate in which body segment a sensilla is located (e.g., 1A2 – first sensilla of the second abdominal segment). A compilation of abbreviations for zoological features used in this publication is shown in Table I.

**Results**

**Description of the immature stages of Sphaerophoria rueppellii**

**Egg.**

White when recently laid, yellowish darker when older. Oval, with the dorsal tip concave due to the micropyle (Figures 1A, B). The surface with an

| Table I. Abbreviations used for morphological features in *Sphaerophoria rueppellii* immature stages descriptions. |
|---------------------------------------------------------------|
| **Abbreviations** | **Abbreviations** |
|-------------------|-------------------|
| ARP | Anterior respiratory process |
| ES | Ecdysial scar |
| ESO | Extra sensory organs |
| IS | Short inter-spiracular setae |
| LB | Labium |
| LM | Labrum |
| M | Mandible |
| MG | Median groove |
| MS | Mesothorax |
| MT | Metathorax |
| PG | Perispiracular gland |
| PRP | Posterior respiratory process |
| PT | Prothorax |
| TS | Lateral lips |
opaque pattern (Figure 1C). The chorionic sculpturing consists of irregular hexagonal units slightly raised with lateral branches that contact with other units (Figure 1C). For egg size and thickness see Table II and Table III.

Larva.
Shared characters between all three larval instars. Oval in cross-section, tapering anteriorly and truncate posteriorly (Figure 2). Mouthparts typical of predatory syrphid larvae adapted to piercing the prey (Figures 3A, B) (Hartley 1963). Reduced mouth hooks (Figure 4B, C). Prothorax and mesothorax are usually retracted inside the metathorax. Prothorax with a smooth dorsal surface with a pair of ARP (Figure 5B). Smooth ventral surface. Seven abdominal segments plus anal segment (= eight abdominal segment) (Figure 2C). Pairs 1, 2, and 3 of papilliform sensillae with setae (Figure 6B) located on the same fold on the first abdominal segment (Figures 7, 8A); on the second to seventh abdominal segments, pair 1 of sensillae located on the anterior fold and pairs 2 and 3 located on the next fold behind (Figure 8B). PRP apex with a central depression. PRP without dorsal spurs.

L1 larva. Colour: Live larva whitish transparent. Size: See Table III. Thorax: On this instar, the sensillae are not visible or are very difficult to see under a stereomicroscope. Prothorax with 10 pairs of sensilla barely visible at the stereomicroscope. Positioned as follow: four pairs dorsally, three pairs laterally, and three pairs ventrally. Mesothorax with ten pairs of sensillae in the following order: one pair of sensillae with setae dorsally; six pairs of sensillae
without setae laterally, and three pairs ventrally. Metathorax with eleven pairs of sensillae arranged in the following way: two pairs of sensillae with setae dorsally, six pairs of sensillae without setae laterally, and three pairs ventrally (Table II). Abdomen: Abdominal segments with 13 pairs of papilliform sensillae and eight pairs in the anal segment (Table II). Dorsally with three pairs of papilliform sensillae with setae, laterally with three pairs of papilliform sensillae with seta, two pairs of papilliform sensillae without setae, and two pairs of ESO. Ventrally with five pairs of papilliform sensilla without setae. The anal segment with eight papilliform sensillae without seta. PRP with an obvious separation between the two spiracular tubes compared to the L3 larva (Figure 9A). PRP: PRP divided by a V-shaped MG as deep as the PRP height. The carinae and the ES are located on an elevated zone on the PRP. A triangular spike appears on the surface of the ES and the carinae. One straight orifice on top of each of the three undeveloped carinae. Under SEM, one IS and one PG are observed (Figure 9A).
L2 larva. Colour: Live larva yellowish white. Size: See Table III. Thorax: Prothorax with eleven pairs of sensillae barely visible under a stereomicroscope. Sensilla distributed as follows: four pairs dorsally, four pairs laterally, and three pairs ventrally. Mesothorax with 13 pairs of papilliform sensillae without setae, arranged in the following order: three pairs dorsally, five pairs laterally, and four ventrally. Metathorax with 13 pairs of papilliform sensillae without seta and one pair of ESO, arranged in the following way: three pairs of sensillae dorsally, six pairs of sensillae with an ESO between sensillae 9th and 10th laterally, and ventrally with four pairs (Table II). Abdomen: Abdominal segments with 13 pairs of papilliform sensillae and 10 pairs in the anal segment (Table II). Dorsally with three pairs of papilliform sensillae with setae, five pairs of sensilla without setae laterally, and five pairs ventrally. The anal segment with 10 papilliform sensillae without setae. PRP with an obvious separation between the tubes compared to L3 larva (Figure 9B). PRP: PRP divided by a V-shaped MG as deep as the PRP height. An ES can be present near the first carina. The straight orifices on top of each of the three developed carinae extend to the lateral side of the PRP. Under a stereomicroscope, four sensillae and a gland can be barely observed, in the following distribution: four IS, and one PG (Figure 9B).

L3 larva. Colour: Live larva from yellowish-green to light green, with one white line on either dorsal side (Figure 2A). Size: See Table III. Head: Labrum elongated, sclerotized, and fused in the front to form a sharply pointed end. The labrum is located at the uppermost part of the head skeleton. The labium is also elongated, sclerotized, and with a sharp pointed end, but smaller in size compared to the labrum. Labium can be found under the labrum, and between them, there is a pair of mandibles. Lateral margins of the mouth with a pair of lateral lips (Figures 4B, C). The surface around the mouthparts has small spines (Figure 4A). Head with a pair of well-developed antennomaxillary organs (Figure 4D). Thorax: Prothorax with 10 pairs of sensillae barely visible at the stereomicroscope (Figures 5A, 7). Positioned as follow: four pairs dorsally, three pairs laterally, and three pairs ventrally. Mesothorax with eight pairs of papilliform sensillae without setae and one pair of ESO arranged as indicated in Figure 7: three pairs dorsally (Figure 5B), two pairs laterally, and three pairs ventrally; ESO sensillae between sensillae sixth and eighth (Figure 5C). Metathorax with one pair of papilliform sensillae with setae, eight papilliform sensillae without setae, and one pair of ESO sensillae (Figure 7). Sensilla distributed as follows: one pair of papilliform sensilla with setae and two pairs of papilliform sensillae without setae in the dorsal surface of the metathorax (Figure 5D), three...
Figure 5. Thorax of the third instar larva of *Sphaerophoria rueppellii*. A. Dorsal surface of prothorax (Pt); B. Dorsal surface of mesothorax (Ms) (ARP, anterior respiratory process); C. Ventral surface of mesothorax (Ms) (circle, extra sensory organ); D. Dorsal surface of metathorax (Mt). Arrow indicates the head direction.

Figure 6. Abdomen of the third instar larva of *Sphaerophoria rueppellii*. A. Abdominal papilliform sensilla without setae; B. Abdominal papilliform sensilla with setae (BH, base height; BW, base width; SH, seta height; SW, seta width); C. Ventral surface of the second abdominal segment, with sensilla 8–12 (circle, locomotory organs) D. Dorsal view of the anal segment (rectangle, locomotory organs). Arrow indicates the head direction.
Table II. Numbers of sensilla in each larval instar and body segments of *Sphaerophoria rueppellii*.

|               | L1       | L2       | L3       | L3 according to Bhatia (1939) |
|---------------|----------|----------|----------|--------------------------------|
| **Prothorax** |          |          |          |                                |
| Dorsal        | 4 •      | 4 •      | 4 •      | 3 •                            |
| Lateral       | 3 •      | 4 •      | 3 •      | -                              |
| Ventral       | 3 •      | 3 •      | 3 •      | -                              |
| **Mesothorax**|          |          |          |                                |
| Dorsal        | 1 Δ      | 3 •      | 3 •      | -                              |
| Lateral       | 6 •      | 5 •      | 2 •      | 3 •                            |
| Ventral       | 3 •      | 4 •      | 3 • + ESO| 4 •                            |
| **Metathorax**|          |          |          |                                |
| Dorsal        | 2 Δ      | 3 •      | 1 Δ + 2 •| 1 Δ                            |
| Lateral       | 6 •      | 6 • + ESO| 3 •      | 3 •                            |
| Ventral       | 3 •      | 4 •      | 3 • + ESO| -                              |
| **Abdominal segments 1 – 7** |          |          |          |                                |
| Dorsal        | 3 Δ      | 3 Δ      | 3 Δ      | 2 Δ                            |
| Lateral       | 3 Δ + 2 •+ 2 | 5 •      | 4 •      | 5 •                            |
| Ventral       | 5 •      | 5 •      | 5 •      | -                              |
| **Anal segment** | 8 •      | 10 •     | 9 •      | 1 •                            |

ESO, extra sensory organ; Δ, sensilla with setae; *, sensilla without setae.

Table III. Size of the immature stages and structures.

|                                            | Mean (mm) | Mean (µm) | Range (mm) | Range (µm) |
|--------------------------------------------|-----------|-----------|------------|------------|
| L3 larva (n = 15)                          | Length    | 6.57      | –          | 5.08-8.71  |
|                                            | Height    | 1.58      | –          | 1.36-2.11  |
|                                            | Width     | 1.71      | –          | 1.49-2.05  |
|                                            | Length    | 3.08      | –          | 1.10-4.37  |
| L2 larva (n = 10)                          | Height    | 0.897     | –          | 0.767-1.1  |
|                                            | Width     | 0.952     | –          | 0.863-1.2  |
|                                            | Length    | 0.734     | –          | 0.640-0.820|
|                                            | Height    | 0.280     | –          | 0.230-0.317|
|                                            | Width     | 0.296     | –          | 0.247-0.327|
| Papilliform sensilla without setae (n = 7) | Height    | –         | 19.41      | –          |
|                                            | Width     | –         | 18.47      | –          |
| Papilliform sensilla with setae (n = 7)    | Height    | –         | 10.12      | –          |
|                                            | Width     | –         | 6.74       | –          |
| Papilliform sensilla base (n = 7)          | Height    | –         | 12.41      | –          |
|                                            | Width     | –         | 22.67      | –          |
| L3 PRP (n = 15) (Figure 9G)                | Height    | 0.326     | –          | 0.172-0.404|
| L3 spiracular plate (n = 15) (Figure 9D)   | Length    | 0.194     | –          | 0.135-0.252|
|                                            | Width     | 0.284     | –          | 0.250-0.316|
|                                            | Length    | 4.73      | –          | 4.09-5.09  |
| Puparium (n = 20)                          | Height    | 1.75      | –          | 1.61-1.84  |
|                                            | Width     | 1.83      | –          | 1.69-1.95  |
| Puparium integumental thickness (n = 3)    | Dorsal    | –         | 14.4       | –          |
|                                            | Lateral   | –         | 7.28       | –          |
|                                            | Length    | 0.779     | –          | 0.658-0.835|
| Egg (n = 8)                                | Width     | 0.295     | –          | 0.279-0.323|
|                                            | Chorion thickness | 3.05 | – | 1.54-4.25 |

n, numbers of individuals used for measurement.

Pairs of sensillae papilliform without setae laterally, and ventrally with three pairs of papilliform sensillae without setae; ESO between sensillae seventh and eighth (Table II). Abdomen: Abdominal segments with 12 pairs of papilliform sensillae and anal segment with nine pairs (Figures 7, 8C) (Table II). Dorsal side with three pairs of papilliform sensillae with setae. Laterally with four pairs of papilliform sensillae without setae (Figure 6A).
The last five pairs of papilliform sensillae without setae are located on the locomotory ventral projections. Two poorly developed crochet-less locomotory projections at each segment (Figure 6C). In the anal segment, the locomotory projections are more prominent than in the other segments, forming a grasping bar (Figure 6D). The anal segment has nine pairs of papilliform sensilla without setae (Figures 6 D; 7). For the size of the papilliform sensillae, with and without setae see Table III. Posterior respiratory process (PRP): Light
brown and shiny (Figure 9C). PRP divided by a V-shaped MG about as deep as 1/4 of the height. PRP with a smooth surface. The straight orifices on top of each of the three developed carinae extend to the lateral side of the PRP (Figure 9D). Each carinae is separated by a 90° angle. An ES can be present near to the first carinae. Under a stereomicroscope four sensiliæ and a gland can be barely observed, in the following arrangement: one PG and four IS (Figures 9E, F). Size see Table III (Figures 9D, G).
Puparium. Teardrop-shaped, with anterior part wider and truncated, and flat ventrally (Figures 10A, B). Colour varies depending on the stages of development of the pupa, from light green to dark brown shortly before the adult emergence. The position and the size of the sensilla are the same as in the larva. Integumental thickness varies across the body, the dorsal part being the thickest (Figure 10C) (Table III). The PRP carinae are darker in colour than the tegument of the larva (Figure 10D). For size see Table III.

Discussion
The third-stage larva of *S. rueppelli* was described as *Sphaerophoria flavicauda* (Zetterstedt, 1843) by Bhatia (1939). The same author provided a rough drawing of the puparium of this species, as well as indicating its size and colour. We describe here for the first time the egg, the L1, and L2 larvae, the puparium, and the larval chaetotaxy for all three stages. Moreover, the L3 and puparium are redescribed in detail from SEM pictures and the L3 of *S. rueppelli* is compared with those of other congeneric species.

We found that the egg of *S. rueppelli* has a similar choriomic sculpturing to that in *S. scripta* and *S. interrupta* (Fabricius, 1805) (as *S. menthastri* in Chandler 1968a), as expected from congeneric species. However, the SEM analysis of the egg chorion of *S. rueppelli* shows micro-sculpture differences between *S. scripta* and *S. interrupta*. According to Chandler (1968a), the chorionic sculpturing of *S. scripta* has a sharp demarcation between its dorsal and ventral parts, whilst in *S. interrupta* there is a transition zone of at least four rows of polygonal units. In *S. rueppelli*, no transition/sharp demarcation has been observed between the dorsal and ventral sculpturing on the egg chorion. The egg micro-sculpture of *S. rueppelli* is also different from that of other syrphines also used as pest control agents (e.g., *E. balteatus*). The surface of the chorionic sculpturing of *E. balteatus* is porous (Figure 1E) (Chandler 1968a), unlike those of *S. rueppelli*. In addition, the chorionic sculptures around the micropyle of *E. balteatus* are unbranched (Figure 1D), but in *S. rueppelli* are branched (Figure 1B). The size of the egg of *E. balteatus* is larger than in *S. scripta*, *S. interrupta* (Chandler 1968a) and *S. rueppelli*. A variable thickness of the egg chorion in syrphids might relate to a syrphid/prey adaptation (Dziock 2005). For example, a hardened egg chorion can be devoted to the protection against an attack by aphids’ (Mizuno et al. 1997).

The non-telescopic PRP found in *Sphaerophoria* is shared with all Syrphinae (Hartley 1961), indicating that they do not live in aquatic or semi-aquatic conditions, as the ‘long-tailed’ larvae of saprophagous genera

Figure 10. Puparium of *Sphaerophoria rueppelli* A. Dorsal view; B. Lateral view; C. Integumental dorsal thickness; D. Dark carinae (arrow, dark carinae).
such as Eristalis (Rotheray 1993). The larvae of the genus Sphaerophoria differ from those of other Syrphinae genera because the PRP is longer than wide and does not have the presence of dorsal spurs (Láska et al. 2013), and our results are in accordance with this. These dorsal spurs can be found on species, such as Scaeva selenitica (Meigen, 1822) (Láska et al. 2006) or Dioprosopa clavata (Fabricius 1794) (Lillo et al. 2021). In Sphaerophoria, the arrangement of the carinae in the spiracular plate differs when compared with that in genera such as Scaeva and Simosyrphus but is similar to Semiscaeva and Dioprosopa (Lillo et al. 2021). According to Lillo et al. (2021), the carinae II and III on the genera Scaeva and Simosyrphus are parallel, compared to those in Sphaerophoria that form approximately a 90° angle between them. The numbers of IS and PG found in the Sphaerophoria PRP agree with Rotheray (2019). The function of the PG is to secrete a hydrophobic material that protects the PRP from wetting and inundation (Kelin 1944; Rotheray 2019).

The identification of Sphaerophoria larvae at the species level is complicated. For example, Scott (1939) stated that S. scripta and S. rueppellii could be separated only by size (length is smaller than 5 mm in S. rueppellii but greater than 5 mm in S. scripta). Nevertheless, the development and size of the larvae are influenced by food and the different developmental stage (L1, L2, and L3) (Růžička 1975). Another difference is the darkness of the pale lines located on the dorsum of the larva (Bhatia 1939; Scott 1939). However, this character also varies according to the type of prey and amount of food. Other aphidophagous syrphids, such as Scaeva, also have very conspicuous white longitudinal stripes in the body (Láska et al. 2006). Rotheray (1987) mentions that the larva of S. interrupta can be distinguished from other Sphaerophoria larvae by the PRP, which is only as long as its basal width. This apparent contradiction between Rotheray (1987) and Láska et al. (2013) is probably due to the fact that Rotheray (1987) measured the width of the PRP basally, but Láska et al. (2013) measured it at the level of the spiracular plate (apically) which is usually somewhat narrower than the PRP basally.

The sister group of Sphaerophoria is Allograpta Osten Sacken, 1875 (Mengual et al. 2008, 2021). The L3 of Allograpta exotica (Wiedemann, 1830) share various features with that of Sphaerophoria (Rotheray & Gilbert 1988, 1999; Arcaya 2012). However, and according to Arcaya (2012), A. exotica can be distinguished from S. rueppellii by the degree of development of the PRP protuberances where the inter-spiracular setae are inserted. The results obtained in our work differ from those of Arcaya (2012) since these protuberances in S. rueppellii are as developed as those in A. exotica (see figure 33 in Arcaya 2012). In addition, we observed a difference in the number of setae with sensillae between these two species, with A. exotica having six pairs and S. rueppellii three pairs. Another difference was found in the total number of sensillae, which is higher in S. rueppellii (120) than in A. exotica (113) (Table II).

After the SEM examination of S. rueppellii larvae, we determined that the number of sensillae is in fact different from the number stated by Bhatia (1939) (Table II). The number of pairs of sensillae in the abdomen and anal segment of S. rueppellii is in accordance with the general number of sensillae found in the Syrphidae (Hartley 1961). However, Rotheray and Gilbert (1999) reported up to eleven pairs of sensillae on each abdominal segment in this Diptera family. The number of sensillae found on each segment of the thorax in S. rueppellii is smaller than the number indicated by Hartley (1961) and Rotheray and Gilbert (1999). However, Hartley (1961) mentions that the number of sensillae varies according to the genus. When comparing the number of sensillae with species of other genera such as Platycheirus chalconota (Philippi, 1865), a difference can be observed. Platycheirus chalconota has eleven pairs of sensillae on the prothorax, eight pairs on the mesothorax, nine pairs on the metathorax, eleven pairs in the abdomen, and eight pairs of sensillae on the anal segment (López-García et al. 2022). In contrast, S. rueppellii has 10 pairs on the prothorax, eight pairs on the mesothorax, nine pairs on the metathorax, 12 pairs in the abdomen, and nine pairs on the anal segment. This difference can be also observed in other species, such as S. selenitica which has a lower number of sensillae in the abdominal segments compared to S. rueppellii, but similar to P. chalconota (Láska et al. 2006; López-García et al. 2022).

Our results show that the number of sensillae vary according to larval instar, with L2 having the highest number in total (137), and L3 the lowest in total (120) (Table II). The higher number of sensillae in L2 might be related to the fact that the L2 larva needs to feed and move more than L3 to meet its developmental requirements (Amorós-Jiménez et al. 2012). These results agree with Bargen et al. (1998), who mention that the L2 responds better to contact stimuli than to odours. This could be another reason why the L2 has a higher number of mechanoreceptor sensillae. In contrast, the L3 is closer to pupation and its trophic and locomotory requirements may be reduced. However, other studies of predatory syrphids, such as A. exotica (Arcaya et al. 2017) or E. balteatus (Putra & Yasuda, 2006) found that the L2 eats less, no more than, the L3. When comparing L2 with L1 of S. rueppellii, the L2 still has a higher number of sensilla than L1 (130)
This might be due to the fact that *S. rueppellii* is an aphidozoetic species, meaning that females lay eggs in or close to the aphid colony in a plant, and not on an aphid-free plant (Chandler 1968b; Dunn et al. 2020), resulting in the L1 quickly and easily locating their prey.

The abdomen is the tagma with the highest numbers of sensillae per segment, in all three larval stages, especially in the lateral area (Table II). This might be related to the fact that the lateral margins have the most contact with the surrounding environment, thus helping them to find prey or avoid potential competitors or possible predators and parasitoids. The sensillae located ventrally are frequently covered with a wet and sticky secretion, while the larva is in motion, which gives rise to the formation of a larval track over the plant surface (pers. obs.). This may indicate that the majority of ventral sensillae are mechanoreceptors since this secretion might negatively affect the chemical recognition of these sensillae. The function of the sticky secretion of the larval tracks, is to facilitate the fixation and the mobility of the larva on different substrates, to prevent the arrival of other predators that feed on aphids, or deterrence conspecific oviposition (Almohamad et al. 2010a, 2010b; Almohamad & Hance 2013). Meanwhile, the anal segment is the location with the least sensillae in the three larval stages. A possible interpretation for this is that the sensillae of this last segment are particularly used by the larva to identify appropriate substrate for pupation (generally with low humidity), since this is where the grasping bar is located (Rotheray & Gilbert 2011). All these results need to be studied and analysed further for verification.

The presence of the ESO or “micro-sensilla” is poorly described and understood in cyclorrhaphan larvae. For this reason, we were unable to characterise them, their position and scale being the only information to give. In this case, the ESO can be observed on the L3 larva under a stereomicroscope and SEM, but on the L1 and L2 larvae, the SEM photo is necessary for precise identification. Currently, this ESO is not a useful source of taxonomic diagnostic characteristics, although very important functionally (Graham Rotheray, pers. com).

*Sphaerophoria rueppellii* has a sclerotized head skeleton with distinctive features of predacious syrphid larvae (Hartley 1961, 1963; Rotheray & Lyszkowski 2015; Rotheray 2016; Prieto-Ruiz et al. 2019; Lillo et al. 2021). In this sense, the labrum remains elongated and sharply pointed, and the distal ends of the labial sclerites are fused forming a sharp point in the ventral lip (Hartley, 1962). The mandibles are modified as a pair of lateral styles with a weak connection with the slightest sclerotized tentorial bars (Hartley, 1963). These characters also allow morphological differentiation from the head skeleton of saprophagous syrphids (as in *Merodon* or in some *Eumerus* species), which have heavily sclerotized mandibular hooks, a sclerotized tentorial bar, and labrum not prominent (Ricarte et al. 2017; Soubra-Dols et al. 2020).

The larval antennomaxillary organs consist of the ancestral antennae and maxillae, located dorsally in the prothorax (Rotheray & Gilbert 2011). When the sensillae register a tactile stimulus, they send a signal to the antennomaxillary organs, causing them to probe to locate the origin of the stimulus. This enables the larva to establish the nature and location of possible prey, once determined, it will then initiate prey capture (Rotheray & Gilbert 1999). The saliva that comes out of the mouth keeps the prey stuck to the surface of the prothorax. This will create contact with the antennomaxillary organs that causes the larva to retract the prothorax and mesothorax under the metathorax to protect itself from attacks by the prey and prevent it from escaping (Rotheray & Gilbert 1999). It is important to describe the antennomaxillary organs and their sensillae, as it very probably influences the predatory behaviour of each species. The locomotory projections are a very important part of the aphidophagous larvae because they hunt their prey on plants (Rotheray & Gilbert 1999). For this reason, the larvae have developed a grasping bar to prevent slippage, a ventral smooth surface that helps create a better attachment to the surface, and an extra fold on the ventral surface for better maneuverability (Rotheray & Gilbert 1999). The locomotory projections of *S. rueppellii* are not so prominent compared (Bhatia 1939) to other species, for example, of *Scaeva* (Láška et al. 2006).

The information provided by Bhatia (1939), Scott (1939), and Rotheray (1987) to identify the *Sphaerophoria* puparia is very vague. According to Scott (1939), *S. rueppellii* has a characteristic elevation of the posteroventral line, while *S. scripta* does not have it. This elevation was seen in both species during our study, meaning that the substrate on which it pupates affects this character of the pupa. The only difference in the puparium that we found in our study, was on the surface of the PRP, which is smooth in *S. rueppellii* but wrinkled in *S. scripta* (Figure 9H). The puparium of *S. interrupta* is inflated dorsally, uniformly pale brown, and without markings (Rotheray 1987), as in *S. rueppellii*.

In our study, we show the importance of undertaking a morphological revision of immature stages using SEM to describe all the setae present. This study with the help of the SEM enhances knowledge of the immature stages of the genus *Sphaerophoria* and takes the first step towards a detailed comparison with other immature stages in the future. This will help to
distinguish between other predatory syrphid larvae that are not currently used as biological control agents, as _S. rueppellii_ is the most abundant _Sphaerophoria_ species inside Mediterranean greenhouses (Pineda & Marcos García 2008). The identification of _Sphaerophoria_ larvae inside greenhouses will inform decisions on the need to carry out, or not, the release of biological control agents. Overall, there is still a lot of work to be done to be able to develop a taxonomic key to the immature stages of _Sphaerophoria_. One possible subject for further study is to understand the functioning of the sensillae described in this paper.

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No potential conflict of interest was reported by the author(s).

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Immature stages of Sphaerophoria rupepellii 639

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