Micron 165 (2023) 103397

Contents lists available at ScienceDirect

Micron

journal homepage: www.elsevier.com/locate/micron

Combined stereomicroscope and SEM disentangle the fine morphology of the undescribed larva and puparium of the hoverfly *Milesia crabroniformis* (Fabricius, 1775) (Diptera: Syrphidae)

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ARTICLE INFO

Keywords:
Chestnut tree
Conservation
Chaetotaxy
Immature stage
Larva
Rot hole

ABSTRACT

With over 80 species, *Milesia* Latreille, 1804 is a hoverfly genus (Diptera: Syrphidae) found in all continents except for Australia and the Antarctica. However, little is known about its life cycle and biology. The three *Milesia* species for which early stages are known have saproxylic larvae, suggesting that the larvae of all other *Milesia* species are also saproxylic. The early stages of the three *Milesia* species occurring in Europe are undescribed. *Milesia crabroniformis* (Fabricius, 1775), a mimic of the hornet *Vespa crabro* Linnaeus, 1758, is the largest hoverfly in Europe and is listed as Least Concern in the IUCN European Red List of Hoverflies. We here report the first early stages of *Milesia* ever found in Europe, describing them and their breeding sites. Larvae of *M. crabroniformis* were collected in water-filled tree holes of live chestnut trees (*Castanea sativa* Mill.) in Málaga, Southern Spain in 2020–2021. Various studies based on stereomicroscope and scanning electron microscopy (SEM) techniques have proven useful in diagnosing hoverfly early stages by observation of their fine morphology. Thus, these techniques were also used here to characterize the second (L2) and third (L3) stage larvae of *M. crabroniformis*, as well as the puparium. A Leica M205C binocular stereomicroscope and a Jeol JSM-ITH500HR SEM were used. The head skeleton and chaetotaxy of the L3 larva were described and illustrated. Adjustments to the diagnosis of the larvae of *Milesia* are proposed based on the number of hooks from the primary row of the main group of hooks. The new early stages are compared with those of other *Milesia* hoverflies, as well as with those of the sister group *Spilomyia* Meigen, 1803. The knowledge of the larval biology and breeding sites of saproxylic insects is useful for implementing forest management measures and species' conservation programs.

1. Introduction

Syrphidae is a worldwide family with more than 6000 species and 284 genera, being one of the largest in the Diptera (Brown et al., 2018; Dunn et al., 2020). The Syrphidae are commonly known as syrphids, hoverflies (due to their hovering flight) or flower flies (due to their frequent visits to the flowers) (Dunn et al., 2020). Adult syrphids feed on nectar and pollen as a source of energy and to reach sexual maturity (Omkar and Mishra, 2016; van Rijn and Wäckers, 2016). For this reason, the syrphids are frequent flower visitors and considered to be one of the most important pollinator groups. Within the Syrphidae, most species are comprised in the subfamilies Syrphinae and Eristalinae, with around 3800 and 1800 species, respectively, known worldwide (Rotheray and Gilbert, 2011; Klecka et al., 2018; Doyle et al., 2020).

Unlike adults, the larvae have a broad spectrum of feeding habits. They can be (a) saprophagous, filtering bacteria or detritus found on decomposing plant materials or in liquid breeding sites rich in organic materials (e.g., water pockets in the leaf axis of bromeliads or wet rot-

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https://doi.org/10.1016/j.micron.2022.103397
Received 9 November 2022; Received in revised form 12 December 2022; Accepted 13 December 2022
Available online 16 December 2022

holes in trees) (Rotheray et al., 2006, 2007; Ricarte et al., 2007); (b) phytophagous, feeding on the aerial and subterranean parts (e.g., bulbs) of living plants, as leaf miners or stem borers (Stuke, 2000; Ricarte et al., 2017); (c) predatory, feeding mainly on soft-bodied Hemiptera (Rojo et al., 2003; Reemer and Ståhls, 2013).

Within the Syrphidae, *Milesia* Latreille, 1804 has 80+ species nearly worldwide, with the greatest species diversity occurring in the Oriental Region (Hippa, 1990; Gharali and Reemer, 2014; Speight, 2020; Saab, 2021). *Milesia* species are amongst the largest in size within the syrphids, or even with the whole order of Diptera (Hippa, 1990). Adults can be separated from those of all other syrphids by the structure of the metathoracic spiracle, which has an outer and inner row of branched hair-like structures partially closing the spiracular opening instead of only one row (Hippa, 1990). Batesian mimicry is quite usual in adults, as they mimic wasps as a defence mechanism (Fleenor and Taber, 2009; Penney et al., 2014; Speight, 2020), which is the reason why they can be misidentified by non-experts.

Milesia is a rarely collected genus with a rather-well known adult morphology (Hippa, 1990), but a poorly known morphology and biology of larvae (Rotheray and Gilbert, 1999). The larvae of this genus can be distinguished by the presence of a large group of hooks located at the front of each anterior respiratory process, consisting of 3–4 hooks on a primary row and a variable number of smaller hooks on a second and third row, as well as a second smaller group of hooks located below each of the large groups of hooks (Rotheray, 1993). Currently, the larvae of only three *Milesia* species have been found: *M. virginiensis* (Drury, 1773) in the USA (Maier, 1982; Rotheray, 1993), *M. scutellata* Hull, 1924 in the USA too (Fleenor and Taber, 2009), and *M. tadzhikorum* Peck & Hippa, 1988 in Tajikistan (Krivosheina, 2001). The larvae of all these three species have been found in tree rot holes, what leads to the assumption that the larvae of all *Milesia* species are saproxylic (Snow, 1958; Rotheray, 1993; Rotheray and Gilbert, 1999; Krivosheina, 2001; Fleenor and Taber, 2009). In addition, *Milesia undulata* Snellen van Vollenhoven, 1863, is known to use rot holes as oviposition site too (Iijima, 2016) and *M. crabroniformis* is supposed to breed in rot holes of *Fraxinus angustifolia* Vahl, 1804 in Spain due to the capture of adults with emergence traps in this breeding site (Quinto et al., 2014).

During some part of their life cycle, saproxylic organisms are dependent on decaying materials associated with woody parts of trees (Rotheray et al., 2001). Almost a third of insects species are saproxylic (Ulyshen and Šobotník, 2018; Martínez-Pérez et al., 2021) and this fact addresses the taxonomic and ecological importance of this insect group in their forest ecosystems (Stockland et al., 2012; Williams et al., 2017). Saproxylic have an important role in nutrient recycling and the adults of many species are responsible for the pollination of plants in forest (Schlaghamersky, 2003; Ricarte et al., 2009). Coleoptera and Diptera (e.g., syrphids) are the most diverse taxa within the saproxylic guild (Dajoz and Álvarez, 2001; Micó et al., 2013; Martínez-Pérez et al., 2020). Various research projects and studies have been carried out across Europe to better know and conserve the saproxylic community of insects, mainly Diptera, especially syrphids within them (Rotheray and MacGowan, 2000; Rotheray et al., 2001; Reemer, 2005; Sánchez-Galván et al., 2014; Ramírez-Hernández et al., 2014). Certain management strategies lead to an increase in the diversity of saproxylic syrphids, for example the use of artificial rot holes in trees (Rotheray et al., 2009; Rotheray, 2013), the removal of specific trees allowing the remaining ones to develop for the restoration of habitats, or the non-removal of ill/dead trees (Reemer, 2005).

Milesia crabroniformis, *Milesia semiluctifera* (Villers, 1789) and the recently discovered *Milesia cretica* Bot & van Steenis, 2022, are the three *Milesia* species occurring in Europe (Speight, 2020; Bot et al., 2022), with the immature stages of *M. crabroniformis* being the ones found and described in the present work. *Milesia crabroniformis* is distributed from northern France to southern Spain, and around the Mediterranean Basin (Hippa, 1990; Speight, 2020). In addition, there are records from central Europe and unconfirmed sightings in Britain (Speight, 2020). In the Iberian Peninsula, *M. crabroniformis* is present in Gibraltar, Portugal, and Spain. In Spain, it is widespread, with confirmed records in 17 out of the 50 provinces (Ricarte and Marcos-García, 2017). This species is the largest hoverfly in mainland Europe and mimics the hornet *Vespa crabro* Linnaeus, 1758 (Speight, 2020). However, information about immature stages morphology and breeding sites was largely unknown until now. This study aims at expanding knowledge of the biology and lifecycle of this syrphid species and genus. For the first time, the second (L2) and third (L3) larval stages, puparium, head skeleton, and posterior respiratory process (PRP) of a *Milesia* species are described in detail.

2. Materials and methods

2.1. Study area

Larvae of *M. crabroniformis* were found in rot holes filled with water and debris in two standing living sweet chestnut trees (*Castanea sativa* Mill.) located in El Juanar Nature Reserve, at Sierra Blanca (Ojén, Málaga, southern Spain) (Fig. 1) by Javier Quinto in February 2020 (two larvae) and March 2021 (13 larvae). This mountainous Reserve is nestled in the Penibaetic system and occupies an area of around 6500 ha of old-managed mixed mature forest of eucalyptus (*Eucalyptus globulus* Labill., 1800), pine (*Pinus nigra* Arnold and *Pinus radiata* D. Don) and sweet chestnut, with a mean elevation of around 800 m a.s.l. The studied rot holes were basal, directed upwards, water-filled with high content of organic matter at the bottom and presumably result from the traditional coppice management of chestnut orchards in the area, based on the pruning of the main stem of chestnut trees (Fig. 2A, B). Each selected chestnut tree had a single basal rot hole. Seven larvae were collected from one rot hole and the remaining eight from the other.

2.2. Larva rearing

The larvae were reared in plastic boxes containing decaying material from the rot holes and maintained in laboratory conditions following the protocol in Sánchez-Galván et al. (2014). Rearing boxes were checked almost daily to record changes in the immature development.

2.3. Morphological study

Seven larvae were preserved in KAAD (70% alcohol of 95%, 14% glacial acetic acid, 8% toluene, and 8% dioxane) and the rest were reared for adult identification to species. Seven larvae pupated and three adults emerged from these puparia (Fig. 3). Adults were identified using the taxonomic key of Hippa (1990). Four L2 larvae, three L3 larvae and seven puparia were examined.

2.3.1. Stereomicroscopy

The puparium was cleaned in an Ultrasonic bath for 20 min and after that, it was brushed to remove any dirt before observation and description. The head skeleton was removed from an L3 larva by soaking them in hot KOH 10% for 11 h and it was examined in glycerine. General features of the L2, L3, puparium, and head skeleton were observed under a Leica M205C binocular stereomicroscope. The length of the L2, L3, and puparium was measured 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. Larval specimens were measured when turgid. Photos were produced as stacks of individual images made with a camera (Leica DFC 450) attached to a binocular stereomicroscope (Leica M205C). Stacks were made in Leica Application Suite LAS X ®, v.3.0.4.16529. The drawings were made from printed photos. The morphological terminology used for the larva/puparium follows Doležil (1972) and Rotheray (2019). For each body segment, sensillae were numbered in the dorso-ventral direction (Rotheray, 1991). The terminology used for the head skeleton follows Rotheray and Gilbert (2008) and Campoy et al. (2020).

Fig. 1. Location (in red) of Málaga (Southern Spain), where el Juanar Nature Reserve (Ojén) is situated.

Fig. 2. El Juanar Nature Reserve: A. Living chestnut tree with a central tree rot hole; B. Detail from the rot hole of the trunk where larvae of *Milesia crabroniformis* were found.

Fig. 3. Adult male of *Milesia crabroniformis* reared from a larva collected at el Juanar Nature Reserve, Southern Spain.

2.3.2. Scanning electron microscopy (SEM)

For a more detailed description of the prolegs, anterior respiratory process (ARP), and pupal spiracles, scanning electron microscopy was used. The cleaned puparium was dehydrated following the procedure of Kanturski et al. (2015). Two puparia were mounted on aluminium stubs with double-sided adhesive carbon tape and sputter-coated in a Quorum 150 T ES Plus with a 30 nm layer of platinum. The samples were imaged with a Jeol JSM-IT500HR. Examined specimens are deposited at the CEUA-CIBIO collection, University of Alicante, Spain.

3. Results

Examined material. Summarised in Table 1.

3.1. Shared characters between the L2 and L3 larval instars of Milesia crabroniformis

Tear-shaped, with the last segment extended (Fig. 4A, B); short-tailed larva; integument surface covered with a heavier number of setae. Dark brown colour. Head with a pair of well-developed antenna-maxillary organs with the tip divided into two small lobules (Fig. 5). Prothorax and mesothorax not retracted inside the metathorax. Prothorax with a

Table 1
Information about the material examined of *Milesia crabroniformis* in El Juanar Nature Reserve, Málaga, Spain. Seven larvae and eight puparia were studied in total. An asterisk indicates that the larva was preserved for description.

Number of individuals	Collecting date of larvae	Date of pupation	Date of adult emergence	Sex of the adult
1	12/02/2020	Unknown	24/05/2021	Female
1	12/02/2020*	–	–	–
3	26/05/2021*	–	–	–
1	26/05/2021	03/06/2021	–	–
1	26/05/2021	07/06/2021	29/06/2021	Male
4	26/05/2021*	–	–	–
1	26/05/2021	31/05/2021	22/06/2021	Male
1	26/05/2021	02/06/2021	–	–
2	26/05/2021	03/06/2021	–	–

smooth surface with a pair of small anterior respiratory processes (ARP) (Fig. 6A). Cream coloured spicules with black/dark brown tips are located dorsally and laterally, surrounding the mouth (Fig. 6A, B). Smooth ventral surface. Mesothorax with one pair of large hooks dorsally with two large curved hooks in the primary row, three hooks in the secondary row, and none to three small hooks in the third row (Fig. 6B, C, D). Laterally with a smaller pair of small hooks with two curved spines surrounded by smaller ones (Fig. 6C). A pair of prominently developed prolegs in the mesothorax and from the first to the sixth abdominal segment (Fig. 7A). Abdomen with eight segments. The eighth segment (= anal segment) extended with a posterior respiratory process (PRP) and a pair of lappets (Fig. 4A, B). The anal segment covered with a noticeable heavier number of setae (compared to the other abdominal segments) and a sticky substance (unknown origin).

3.2. L2 larva of *Milesia crabroniformis*

Length: 7.79–12.11 mm; width: 2.34–3.25 mm ($n = 4$). *PRP:* Light brown from the base to the tip. The surface above the transverse ridge puncture with holes of different size (the surface below the transverse ridge could not be observed). Light brown spiracular plate with four pairs of long interspiracular setae, one pair of perispiracular glands, and three pairs of spiracular holes slightly curved, in 'S' shape. Each long interspiracular seta has four feathery branches. Anal papillae could not be observed.

3.3. L3 larva of *Milesia crabroniformis*

Length: 23.5–28 mm; width: 5.5–0.6 mm; height: 4–5 mm ($n = 3$). *Head skeleton* (Fig. 8): Dorsal cornu highly sclerotized, epipharyngeal plate highly sclerotized connected to the labium through the sclerotized labial rods. The mandible is slightly sclerotized, with a mouth hook located on the anterior part of the mandibular lobe. Labrum translucent, labium sclerotized. Ventral cornu slightly sclerotized, but the pharyngeal ridges are visible. Lateral lips covered with setae. All sensillae observed across the larva were bearing a setae. *Prothorax*: ARP with four straight spiracular openings in a semi-circular position (Fig. 9). Dorsally with four pairs of sensillae, laterally with three pairs of sensillae, and ventrally with three pairs of sensillae (Fig. 10). *Mesothorax:* prolegs with 3 rows of crochets facing backwards and forming a rectangular aggregation (Fig. 11A, B, C). Dorsal side with three pairs of sensillae; the lateral side with two pairs of sensillae, and ventrally with three pairs of sensillae (Fig. 10). *Metathorax*: Dorsally with three pairs of sensillae, laterally with two pairs of sensillae, and ventrally with three pairs of sensillae (Fig. 10). *Abdomen*: prolegs with three rows of crochets pointing backwards (crochets of smaller size in the outer row and larger size in inner row), the crochet rows in the shape of a horseshoe from the first to the six abdominal segments (Fig. 7A, B). Anal segment with bilaterally symmetrical simple unbranched anal papillae with six papillae (Fig. 4C). From the first to the seventh abdominal segment

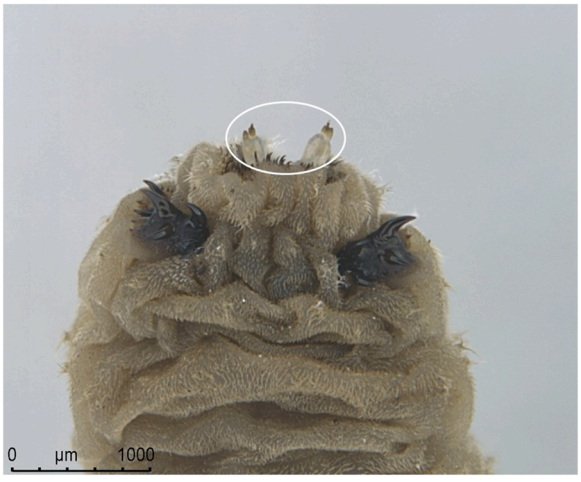

Fig. 5. Larva of *Milesia crabroniformis*: L2, thorax, dorsal view (antenna-maxillary organs encircled).

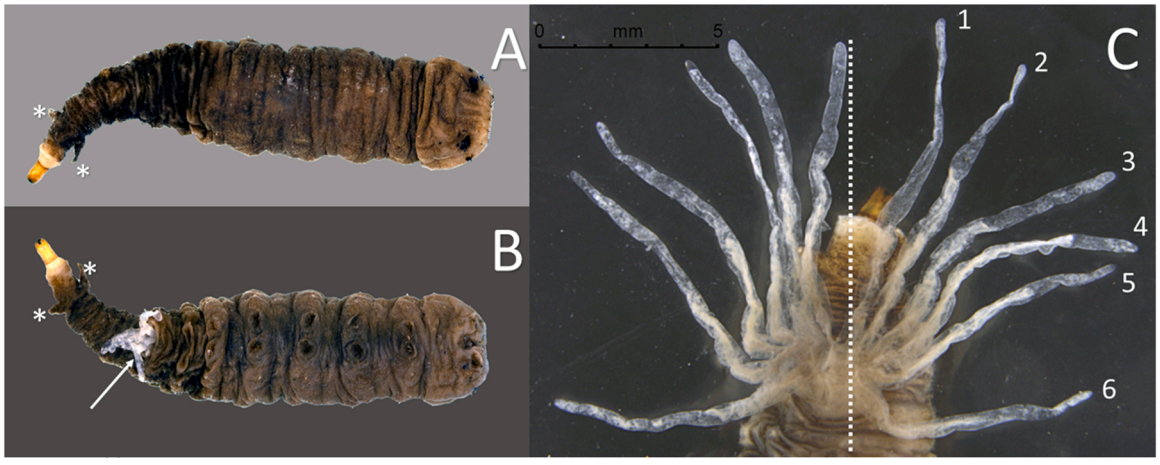

Fig. 4. Third stage larva (L3) of *Milesia crabroniformis*: A. Dorsal view (asterisk indicates the lappets); B. Ventral view (arrow indicates the evaginated anal papillae). C. Simple unbranched anal papillae.

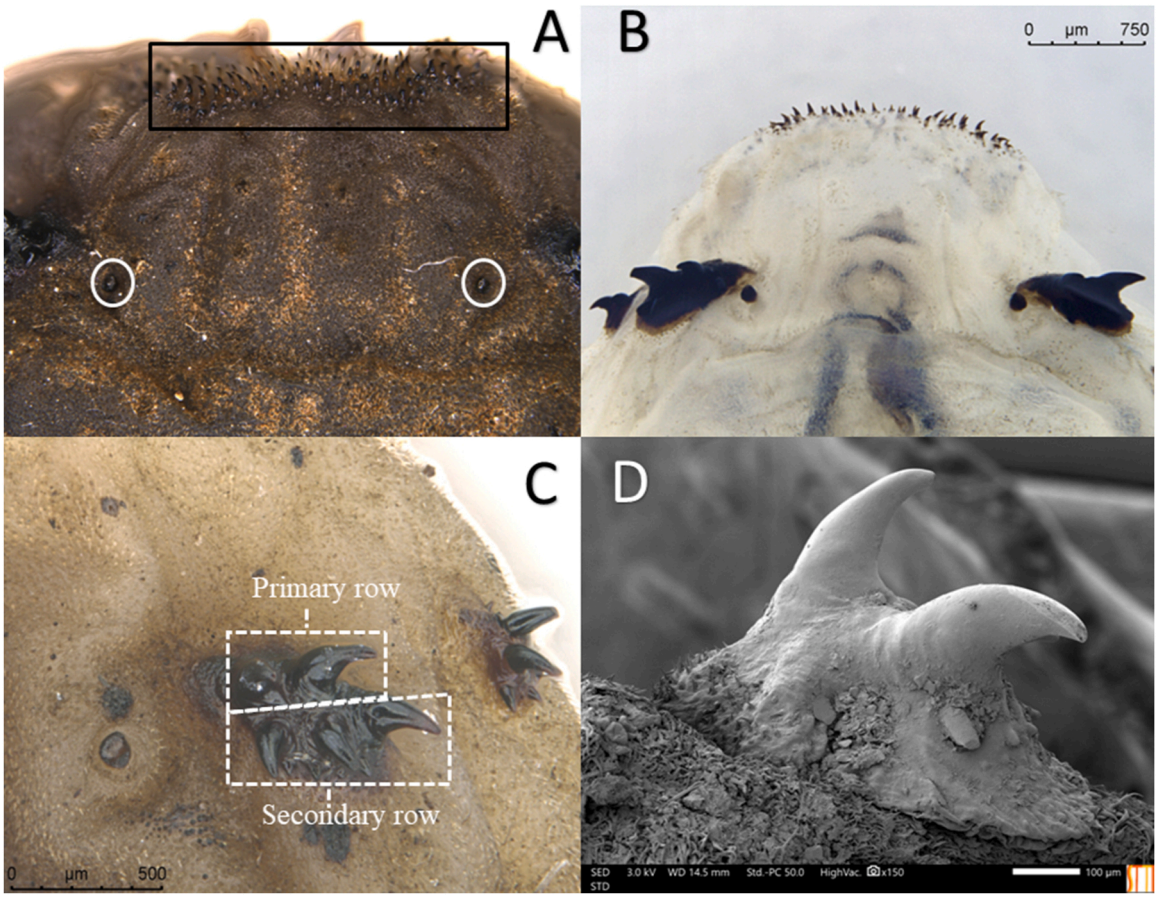

Fig. 6. Third stage larva (L3) of *Milesia crabroniformis*: A. Prothorax, dorsal view (anterior respiratory process encircled; group of spicules indicated with a rectangle); B. Thorax, dorsal view (the white colour is due to heating in KOH); C. Detail of the large group of hooks under stereoscopic microscope; D. Large hooks under SEM microscope.

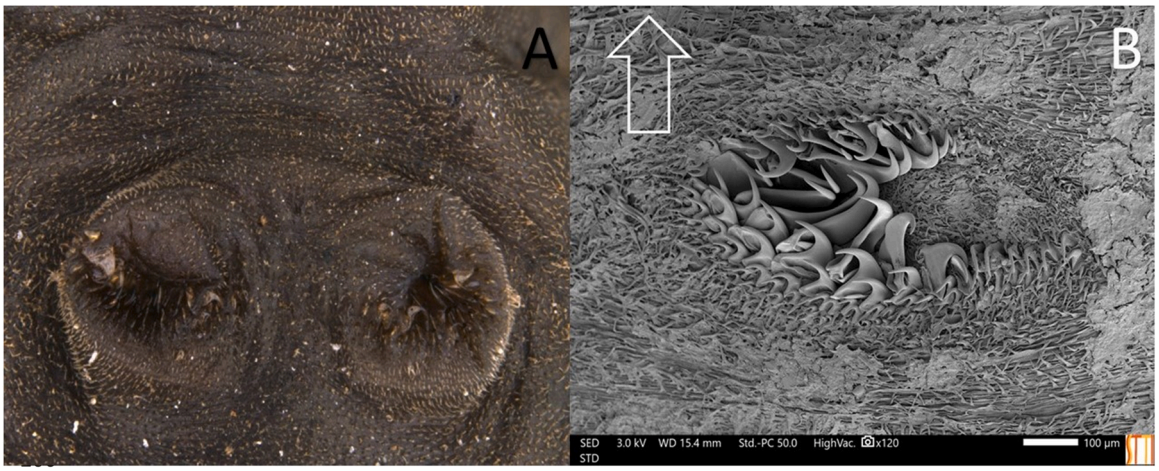

Fig. 7. *Milesia crabroniformis* prolegs: A. L3 prolegs with crochets; B. Puparium prolegs with crochets of abdominal segments (arrow indicates the anterior part of the puparium).

dorsally with three pairs of sensillae, laterally with six pairs of sensillae, and ventrally with two pairs of sensillae (Fig. 10). Anal segment with two pairs of sensillae located in dorsal base of the PRP, two pairs of sensillae at the ventral base of the PRP, one pair of sensillae at base of the lappet, one pair of sensilla at the tip of the lappet, and ventrally with three pairs of sensillae (Fig. 10). *PRP*: Light brown, darker towards the apex, with a distinct transverse ridge (Fig. 12A, B). Width at the level of the transverse ridge: 0.63–0.71 mm ($n = 3$); length above the transverse ridge: 0.64–0.78 mm ($n = 3$); length below transverse ridge: 0.25–0.44 mm ($n = 3$). The surface above the transverse ridge punctured with holes of varied size; the surface below the transverse ridge smooth. Dark brown spiracular plate with four pairs of long interspiracular setae, one pair of perispiracular glands, and three pairs of spiracular openings slightly curved, in 'S' shape (Fig. 12C, D). Each long interspiracular seta has four feathery branches. (Fig. 13).

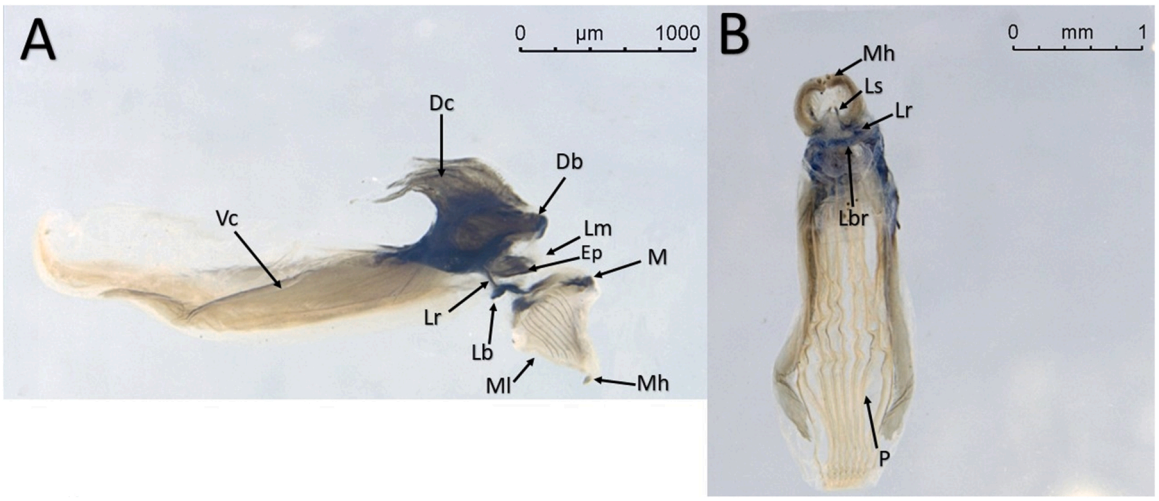

Fig. 8. Head skeleton of *Milesia crabroniformis*: A. Lateral view; B. Ventral view. Legend: Db, dorsal bridge; Dc, dorsal cornu; Ep, epipharyngeal plate; Lb, labium; Lbr, labial bridge; Lm, labrum; Lr, labial rods; Ld, labial sclerite; M, mandible; Mh, mouth hook, Ml, mandibular lobes; P, pharyngeal ridges.

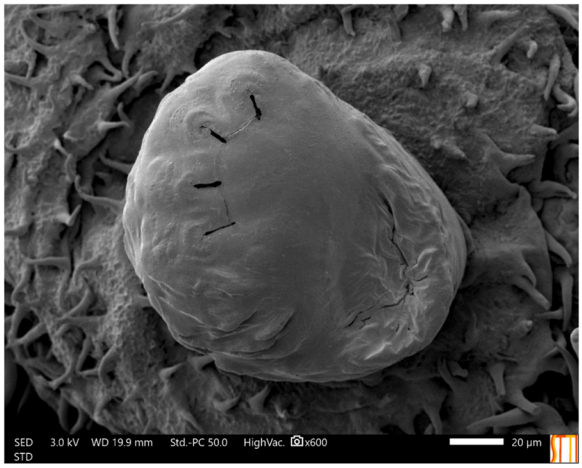

Fig. 9. Anterior respiratory process of a *Milesia crabroniformis* puparium.

3.4. Puparium of Milesia crabroniformis

Length: 13.76–15.90 mm; Height: 4.74–6.97 mm; Width: 4.82–7.89 mm ($n = 7$). Teardrop-shaped with anterior part wider and flat ventrally (Figs. 13 A, B). Surface of the studied puparia covered with debris and sticky substance. *Pupal spiracles*: dark brown/black cylindrical tapering apically and inclined backwards (Fig. 14A). Surface of the anterior side with spikes (Fig. 14B) and posterior side with light brown circular tubercles (Fig. 14C). Each tubercle has 5–6 spiracular openings (Fig. 14D).

4. Discussion

The adult morphology of *M. crabroniformis* is well illustrated in Hippa (1990). Nevertheless, there is very little information available about the larval biology of this species and no information about the early stage morphology. The puparium of *M. crabroniformis* was succinctly described by Matile and Leclercq (1992), but Ricarte et al. (2007) and Speight (2020) stated that it was actually the puparium of *Mallota cimbiciformis* (Fallén, 1817). *Mallota cimbiciformis* can be easily separated from *Milesia* since it has retractile anterior spiracles and a long 'tail' (see figure 17 in Rotheray, 1993). In this work, we present for the first time the description of the L2, L3, head skeleton, and puparium of

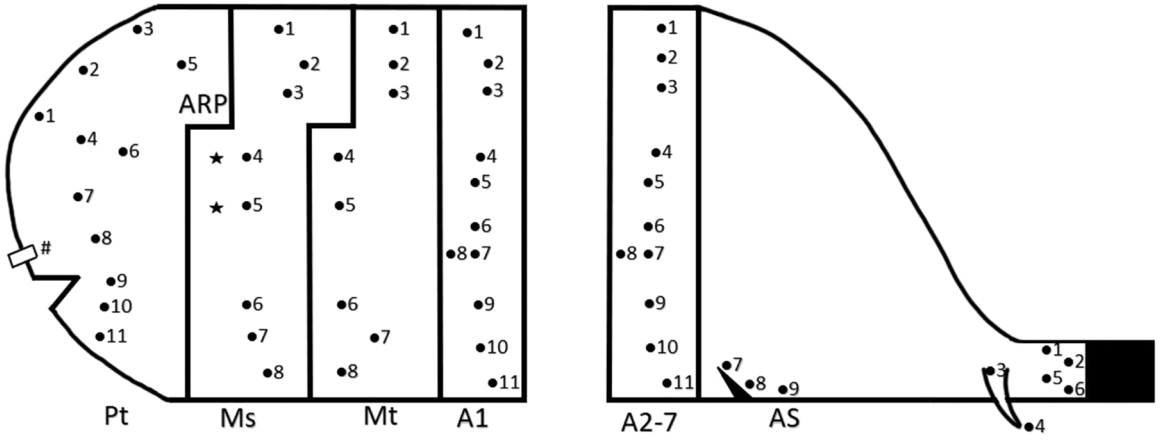

Fig. 10. Third stage larva of *Milesia crabroniformis* showing the number and relative positions of the body sensillae. Legend: P, prothorax; Ms, mesothorax; Mt, metathorax; A1, A2–7, abdominal segments; AS, anal segment; ARP, anterior respiratory process; PRP, posterior respiratory process. #, antenno-maxillary organs; •, sensilla with setae; ★, hooks.

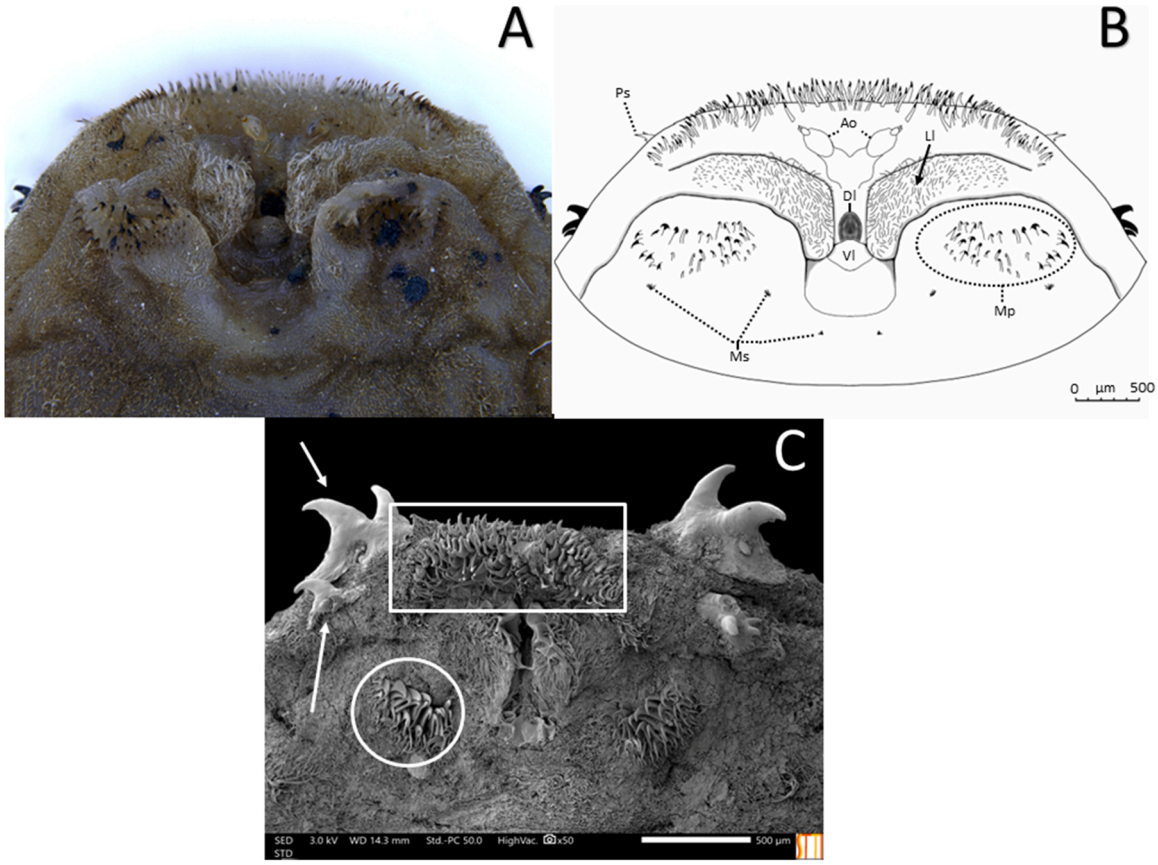

Fig. 11. Third stage larva (L3) and puparium of *Milesia crabroniformis*: A. Thorax, ventral view, stereoscopic microscope; B. Thorax, ventral view, drawing; C. Detachable thoracic plate (arrow indicates a group of hooks; group of spicules indicated with a rectangle; prolegs of the mesothorax encircled). Legend: Ao, antenna-maxillary organs; Dl, dorsal lip; Ll, lateral lip; Mp, mesothoracic prolegs with crochets; Ms, mesothoracic sensillae; Ps, protoracic sensillae; Vl, ventral lip.

M. crabroniformis and give the most comprehensive overview of the immature stages of a *Milesia* species. Currently, there are not descriptions of *Milesia* early stages as detailed as those presented here.

Rotheray (1993) provided a diagnosis of the *Milesia* larva based on one of the Nearctic species, *M. virginiensis*, whilst Krivosheina (2001) described the puparium of the Palaearctic *M. tadzhikorum*. The habitus of *M. crabroniformis* larva is coincident with that of *M. virginiensis* sensu Rotheray (1993), as both are short-tailed, similar size, have two thoracic groups of hooks, well-developed prolegs with crochets and a pair of lappets on the apex of the anal segment. Thus, the larvae of the European species of *Milesia* do not differ in their gross morphology from those of the Nearctic species (Rotheray, 1993). However, a difference can be found in the number of hooks, since there are 3–4 hooks on the primary row in *M. virginiensis*, whilst there are 2 hooks on the primary row in *M. crabroniformis*. To find more differences between the larvae of these two species, it is necessary to compare actual specimens, since the description of the larva of *M. virginiensis* is succinct and there were not larvae of this species available to us.

Information about the overall appearance of the immature stages of *Milesia* species is fairly scarce. Krivosheina (2001) found some larvae of *Milesia* in rot holes of euphrates poplar tree (*Populus eufratica* Olivier, 1807) and reared them to get adults of *M. tadzhikorum*. According to Krivosheina (2001), the puparium of *M. tadzhikorum* has a group of hooks consisting of two large hooks and one small hook. The results of Krivosheina (2001) differ from ours since the large group of hooks of *M. crabroniformis* has five large hooks, not two. Some similarities can be found between these two species, for example, their PRPs have three pairs of curved spiracular openings, their anal segments have one pair of lateral lappets, and their prolegs with crochets are located in the mesothorax and from the first to sixth abdominal segments. The pupal spiracles are brown, cylindrical and with tubercles in both species. Finally, whilst the anterior and posterior sides of the pupal spiracles of *M. crabroniformis* are of strongly contrasting surface ornamentation (Fig. 14), the structure of the pupal spiracles of *M. tadzhikorum* is unknown (Krivosheina, 2001). As a result of our study and that of Krivosheina (2001), the diagnosis of the larva of *Milesia* is redefined by the number of hooks from the primary row of the main group of hooks, 3–4 according to Rotheray and Gilbert (1999) and 2–4 from the present study.

Milesia crabroniformis lay eggs on rot holes of chestnut trees and narrow-leafed ash *F. angustifolia* Vahl, 1804 (Quinto et al., 2014), filled with water and debris, a fact that coincided with the other known *Milesia* species (Snow, 1958; Krivosheina, 2001; Fleenor and Taber, 2009; Iijima, 2016). According to Snow (1958), the larva and puparium of *M. virginiensis* were found on a man-made stump hole of a sweet gum tree (*Liquidambar styraciflua* L., 1753); the egg and larva of *M. scutellata* on rot holes in fire-gutted pines (Fleenor and Taber, 2009), the larva of *M. tadzhikorum* in a stump hole of euphrates poplar tree (Krivosheina, 2001). *Milesia undulata* was observed laying eggs/searching oviposition sites in rot holes of Japanese chestnut trees, *Castanea crenata* Siebold & Zucc., 1846 (Iijima, 2016). Many of these stump holes remained saturated with water and debris long enough for the *Milesia* to complete their life cycle, which can last up to two years according to our results. Regarding *M. crabroniformis*, adult behaviour can be guessed from the information available on the other species of *Milesia*. Males of both *M. scutellata* and *M. virginiensis* show heavy territoriality around trophic resources and hilltops, or during the breeding season (Maier and Waldbauer, 1979; Fleenor and Taber, 2009), suggesting that males of *M. crabroniformis* may also be territorial.

The early stages of *Milesia* and its sister group *Spilomyia* (Moran et al.,

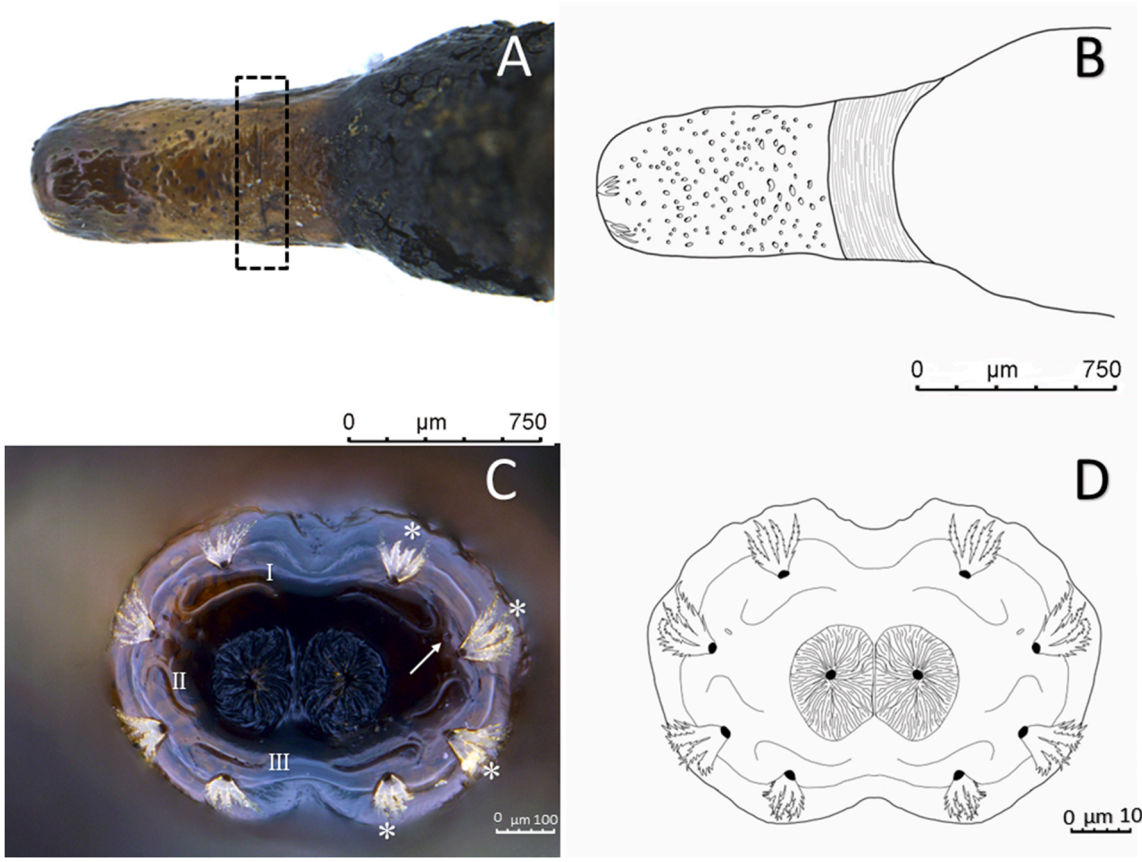

Fig. 12. Posterior respiratory process of a third stage (L3) larva and puparium of *Milesia crabroniformis*: A. Lateral view, photo (transverse ridge indicated with a rectangle); B. Lateral view, drawing; C. Polar view, photo (long interspiracular setae indicated with an asterisk; perispiracular gland indicated with an arrow; I, II, and III, spiracular holes); D. Polar view, drawing.

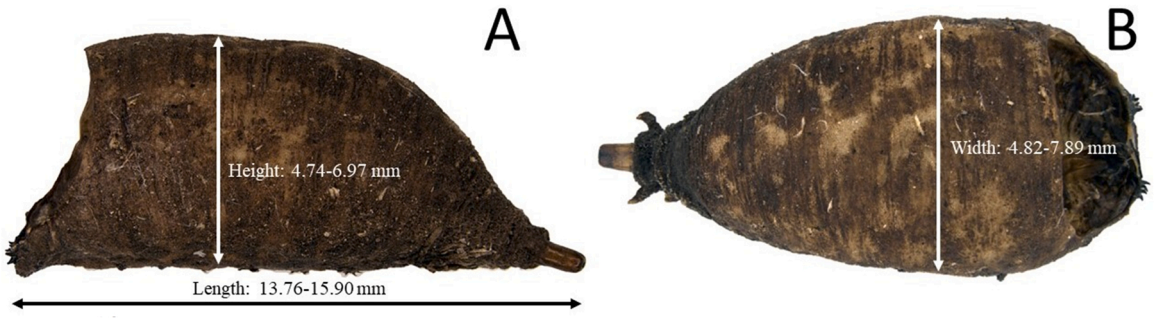

Fig. 13. Puparium of *Milesia craboniformis*: A. Lateral view; B. Dorsal view. (n = 7).

2021) are poorly known. Regarding *Spilomyia*, early stages are only known for *Spilomyia longicornis* Loew, 1872 and *Spilomyia digitata* (Rondani, 1865). Rotheray et al. (2006) observed a long seta located at the centre of each tubercle of the pupal spiracles of *S. digitata*, which contrasts with the absence of setae in the tubercles of *M. crabroniformis*. This could be a valid character to separate both genera, but further studies including species of both genera are required to confirm its validity at the genus level. Nonetheless, the number of pairs of hook groups are still a valid character to distinguish both genera, since *Milesia* has two pairs of hook groups and *Spilomyia* has four pairs (Thompson and Rotheray, 1998).

The larvae of *Milesia* and those of many other saproxylic syrphid genera have large filtering mouthparts that are highly specialized in filtering bacterial aggregates suspended in the water of tree holes (Rotheray and Gilbert, 1999). The mouthparts remain relatively uniform across saproxylic larvae, not as articulated and complex as in the entomophagous species (Rotheray and Gilbert, 1999). The head skeleton of *M. crabroniformis* shows distinctive features of a saproxylic larvae such as mandibular hook reduction, mandibles elongated and thin, pharyngeal ridges, and lateral lips covered by setae (Hartley, 1963; Rotheray and Gilbert, 1999, 2008). Such features evidence that *M. crabroniformis* filter the fluid media contained in chestnut rot holes to feed, in the same way as in other saproxylic larva, such as those of *Meromacrus yucatense* Ricarte et al., 2020 from rot holes of ceiba stump (Ricarte et al., 2020). In general, the sizes of the head skeleton vary according to syrphid species, with the feeding mode being the reason for the different sizes (Hartley, 1963). Thus, differences in the size of the head skeleton of *Milesia* are expected to be found when new larvae of this genus are discovered and described. According to Hartley (1963), the head skeleton between saproxylic larvae varies in the size proportion of the

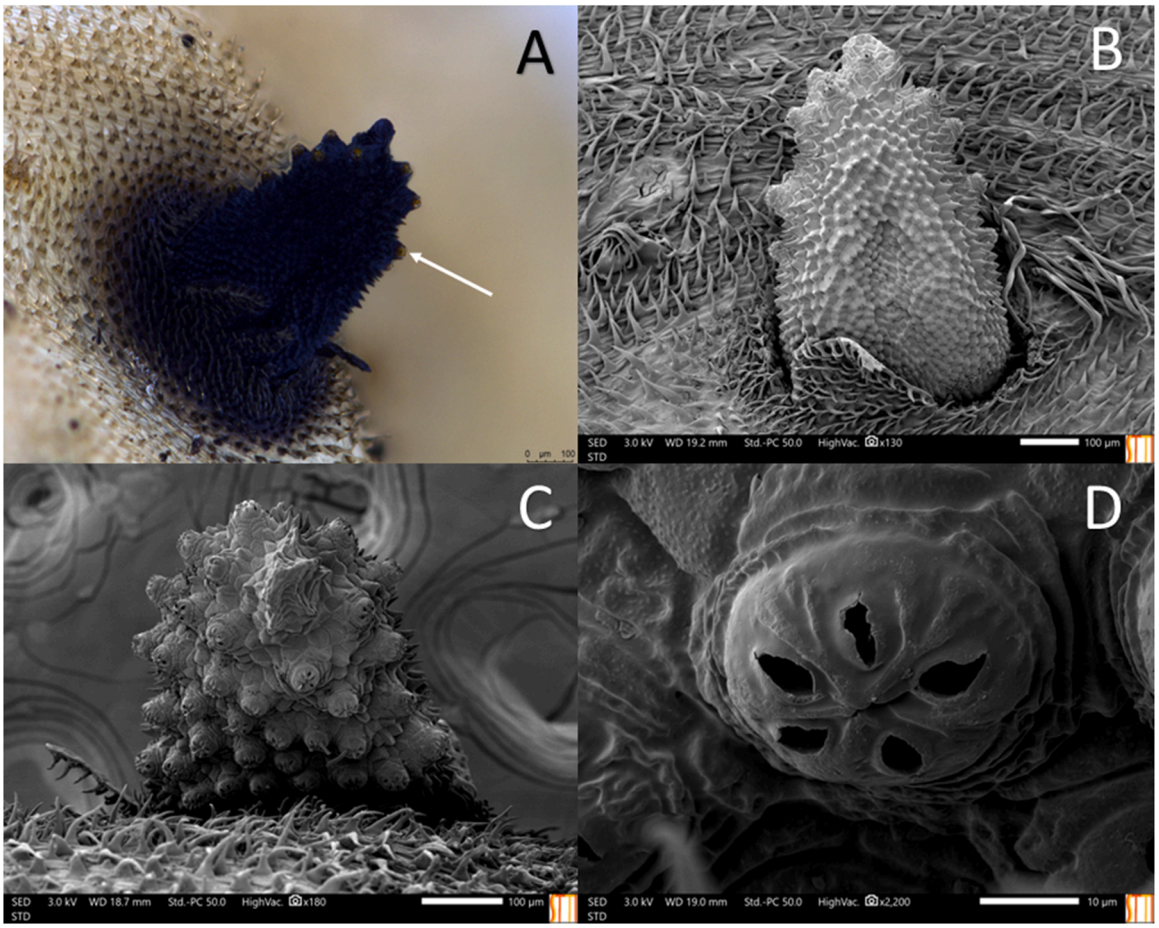

Fig. 14. Pupal spiracles of *Milesia crabroniformis*: A. Lateral view under stereomicroscope (arrow indicates a tubercle); B. Anterior side under SEM microscope; C. Posterior side under SEM microscope; D. Spiracular openings on the tubercles.

sclerites and the degree of sclerotization. The saproxylic larvae of syrphids are immersed in fluids or very wet decaying matter and are mainly found in three constantly changing habitats due to the fall of debris: wet decaying vegetation, wet decaying heartwood, and decaying tree sap (Rotheray and Gilbert, 1999). For this reason, saproxylic larvae have developed prolegs with crochets and hooks around the thorax (Rotheray and Gilbert, 1999). The crochets are used to grip the substrate, preventing the larva of being carried away by the movement of water or floating to the surface (Grieg, 1989; Rotheray and Gilbert, 1999). The crochets found in the mesothorax of saproxylic larvae are larger and of different orientation compared to the rest of crochets (Rotheray and Gilbert, 1999). In our study, we found that *M. crabroniformis* has prolegs with crochets on the mesothorax and from the first to sixth abdominal segment, with those located on the mesothorax being much larger, consistently with Rotheray and Gilbert (1999). The thorax during the locomotion through the debris is protected by the hooks located on the dorsal and lateral sides and it is quite usual to observe scars in the thorax (Rotheray and Gilbert, 1999). This could be the possible function of the pair of hooks observed in the dorsal and lateral parts of the thorax of the *M. crabroniformis* larva.

The existence of the evaginated organs (Fig. 4C) in *Milesia* is remarkable, since these structures appear to have taxonomic importance but are often neglected from taxonomic studies of larvae (Hartley, 1961; Rotheray, 1993). In the literature these structures are known by different names such as retractile processes (Dunavan, 1929), anal papillae (Doležil, 1972) or rectal gills (Rotheray, 1993). The anal papillae are only reported for Eristalinae syrphids (Hartley, 1961; Doležil, 1972; Rotheray, 1988) and we report them for the first time in the genus *Milesia*. The function of the anal papillae is uncertain, as different works propose different theories, for example, Dunavan (1929) considered these organs related with the respiration, Rotheray (1993) with the salt regulation and Rotheray (1998) with locomotion. Thus, further studies are necessary to clarify the role of the anal papillae. According to Rotheray (1988), the anus produce a watery fluid that creates an adhesive effect when the anal papillae are pressed against the substrates during the locomotion. This could be the origin of the sticky substances found in some of the larvae here studied (see Section *3.1*).

Through this study we improve the knowledge about this saproxylic genus of syrphids, *Milesia*. Many species of saproxylic hoverflies are endangered in Europe (Vujić et al., 2022), with deforestation, fires, human activities and climate change among the threats affecting *Milesia* (Pennards, 2021a; b). Other specific threats that may affect *M. crabroniformis* populations are, for example, the European-introduced chestnut gall wasp (*Dryocosmus kariphilus* Yasamatsu, 1951) (Pujade--Villar et al., 2013; Quinto et al., 2021), that impact on the leaf cover of chestnuts leading to higher exposure to the sun of the rot holes where *Milesia* larvae develop; use of pesticides (e.g. Bernal et al., 2010); compositional changes of tree rot-holes by air pollution (e.g. Hallmann and Jongejans, 2021; Barendregt et al., 2022); abandonment of traditional forest management (Micó et al., 2022); and extermination in mistake for the invasive *Vespa velutina* Lepeletier, 1836 (e.g. Rortais et al., 2010; Monceau et al., 2014), which is a species subjected to control.

Further efforts in the study of *Milesia* are necessary to complete our knowledge of its biology and conservation. For example, the egg and first stage larva of *M. crabroniformis* are still unknown, as well as the entire set of early stages of *M. semiluctifera* and *M. cretica*. In addition, a wider survey of their breeding sites is necessary to understand their tree

preferences and other requirements.

Funding

This work belongs to the 'Fauna Ibérica' Project (PGC2018-095851-A-C65) of the Spanish Ministry of Science, Innovation and Universities. Antonio Ricarte's position (UATALENTO17-18) at the University of Alicante is funded by the "Vicerrectorado de Investigación y Transferencia del Conocimiento". Student grant of José Orengo's was funded by the University of Alicante (UAFPU2019B-57). Javier Quinto's position were funded by Andalusian Regional Government (PP.PEI. IDF2019.001) and the Spanish Ministry of Science and Innovation (PID2020-115140RB-I00). This work is part of a PhD thesis of the first author.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

No data was used for the research described in the article.

Acknowledgement

We thank Eva Wong, Juan Ramón Boyero and Martín Aguirrebengoa for their help in the fieldwork and in the daily supervision of rearing boxes. In addition, we thank Graham E. Rotheray and Stephen Hewitt for comments to improve the manuscript and English revision.

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