Bulletin of Insectology **77** *(2): 271-281, 2024*
ISSN 1721-8861 eISSN 2283-0332

First description of the immature stages of the uncommon *Triglyphus* Loew 1840 hoverflies (Diptera Syrphidae)

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Abstract

With 10 species described, *Triglyphus* Loew 1840 is a rarely recorded genus of hoverflies (Diptera Syrphidae) from the Holarctic and Australian Regions. The biology of *Triglyphus* larvae is practically unknown as the only information available is that they prey on aphids in galls on *Artemisia vulgaris* L. (Asteraceae). We here report the first morphological description of the immature stages of a *Triglyphus* species and confirm their breeding site. Adults of *Triglyphus primus* Loew 1840 can be found in forests fringes, gardens, and on open grounds in the Palaearctic Region. Larvae of *T. primus* were collected preying on the aphid *Cryptosiphum artemisiae* Buckton 1879 (Hemiptera Aphididae) in galls on *A. vulgaris* in Denmark, in 2023. The first (L1) and the third (L3) larval instars, the puparium, head skeleton, and posterior respiratory process (PRP) were studied and analysed by using stereomicroscopic and scanning electron microscope (SEM) techniques. The chaetotaxy of the L3 and the types of sensilla are also described and illustrated. A taxonomic diagnosis of the larvae of *Triglyphus* is proposed to distinguish it from larvae of other syrphid genera by using the labrum shape in the head skeleton.

Key words: Denmark, larva, mugwort, Pipizinae, *Triglyphus primus*.

Introduction

The family Syrphidae (Insecta Diptera), commonly known as hoverflies, flower flies, or syrphids, consists of 6674 species with 284 genera distributed worldwide, except in Antarctica (Dunn *et al*., 2020). Syrphids occupy diverse ecological niches, exploiting a very wide variety of trophic resources through their larvae and adults. The adults mainly feed on nectar and pollen for sexual maturation and to maintain their hovering flight. For this reason, they are frequent visitors of flowers and therefore considered important pollinators (Dunn *et al*., 2020). The larvae have a broad food spectrum and include predatory, mycophagous, phytophagous, and saprophagous species (Rotheray and Gilbert, 1999; 2011). Their intimate important participation in ecosystem services, together with a wide range of taxonomic resources for their identification, makes syrphids good bioindicators (Sommaggio, 1999; Montoya *et al*., 2021).

Syrphids are today classified into four subfamilies: Eristalinae, Microdontinae, Syrphinae, and most recently established Pipizinae (Mengual *et al*., 2015; Skevington *et al*., 2019, Wong *et al*., 2023). With over 180 species, Pipizinae are present in all but the Afrotropical Region, and especially diverse in the Holarctic Region (Mengual *et al*., 2015). According to Vujić *et al*. (2013) and Mengual *et al*. (2015), Pipizinae are currently divided into eight genera: *Pipiza* Fallen 1810, *Cryptopipiza* Mutin 1998, *Heringia* Rondani 1856, *Pipizella* Rondani 1856, *Trichopsomyia* Williston 1888, *Triglyphus* Loew 1840, *Neocnemodon* Goffe 1944, and the recently added *Claussenia* Vujic et Stahls 2013. Pipizinae and Syrphinae are considered as sister subfamilies (Ståhls *et al*., 2003; Mengual *et al*., 2015; Wong *et al*., 2023).

The genus *Triglyphus* is a genus with only 10 species (Evenhuis and Pape, 2024), two of which, *Triglyphus escalerai* Gill Collado 1929 and *Triglyphus primus* Loew 1840, are the only species found in Europe (Speight, 2020). According to Thompson and Rotheray (1998) and Vujić *et al*. (2013), *Triglyphus* can be distinguished from other syrphid genera by having long erect hairs on the anterior anepisternum, tergites 2 and 3 are well-developed and subequal in length, tergite 4 is very small and barely visible from above, they are small in size, dark in colour, with a pilose post-pronotum, a flat face lacking a facial knob, a straight vein R_{4+5} and the crossvein R-M is perpendicular, ending before the middle of the discal cell.

T. primus is a Palaearctic species found from the Iberian Peninsula to Korea (Flügel, 2004; Ricarte and Nedeljković, 2020; Speight, 2020), but this distributional range may be incomplete due to the limited information on this species. It is widely distributed in Europe, preferring central and northern European countries (e.g. Ukraine, Finland) (van der Ent *et al*., 2021). Forest fringes, gardens, and on open grounds are the best habitats to encounter *T. primus* (Flügel, 2004; Speight, 2020). This rarely detected small hoverfly can be distinguished from other species of the genus by the black colour of the short pile on the posterior part of the scutum, and the shape of the male genitalia and basoflagellomere (Vujić, 1994; Ricarte and Nedeljković, 2020).

There is no information available on the morphology of the immature stages of *Triglyphus*. Concerning breeding sites, the only data available is that the larva of *T. primus* inhabits leaf galls on *Artemisia absinthium* L. (Asteraceae), and *Artemisia vulgaris* L. preying on the gall-forming aphid, *Cryptosiphum artemisia* Buckton 1879 (Hemiptera Aphididae) (Leclercq, 1944; Sedlag, 1966; Flügel, 2004).

This study aims to provide the first morphological description of the larva and puparium of *Triglyphus* hoverflies. In addition, a taxonomic diagnosis of the larva/puparium is proposed for the genus *Triglyphus*.

Figure 1. *Artemisia vulgaris* with galls caused by *Cryptosiphum artemisiae*. Circle indicates the galls. Photo by Leif Bloss Carstensen.

Materials and methods

Examined material and adult/larva identification

Larvae of *T. primus* (n = 7) and one empty puparium were found in the leaf galls of *C. artemisiae* on *A. vulgaris* (figure 1) in the following Danish localities (figure 2): Bjerringbro C (56.3750, 9.6480), Dragsmur, Knebel (56.1641, 10.5291), Finderup Øvelsesterræn (56.4287, 9.2213), Møllebakken, Bjerringbro (56.3637, 9.5989) and Vindum (56.3833, 9.5702). Larvae were collected in August and September 2023 by Leif Bloss Carstensen. The larvae were reared in small plastic containers with several leaves of *A. vulgaris* infested with *C. artemisia*. The containers were stored in a cupboard at environmental temperature. The containers were checked regularly to record changes in the immature development. Five larvae [one of first instar (L1) and four of third instar (L3)] of *T. primus* were preserved in 70% alcohol, and the rest reared for adult identification. Three larvae of *T. primus* pupated, and two adults emerged from these puparia (figure 3). The match between the empty puparium and the larvae was based on the features of the posterior respiratory process (PRP). Adults were identified with Vujić (1994) and Ricarte and Nedeljković (2020). Examined specimens are deposited at the CEUA-CIBIO collection, University of Alicante, Spain.

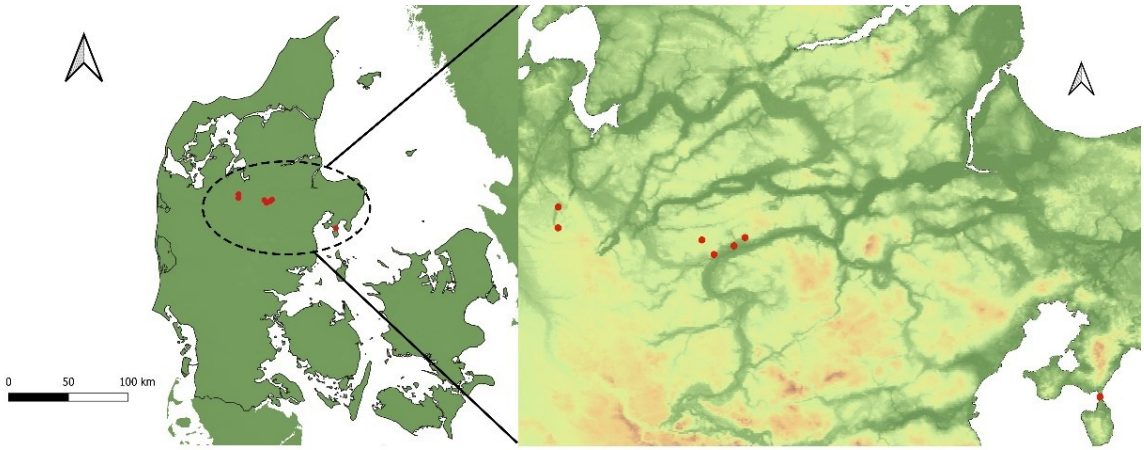

Figure 2. Localities where larvae and puparium of *Triglyphus primus* were found in Denmark.

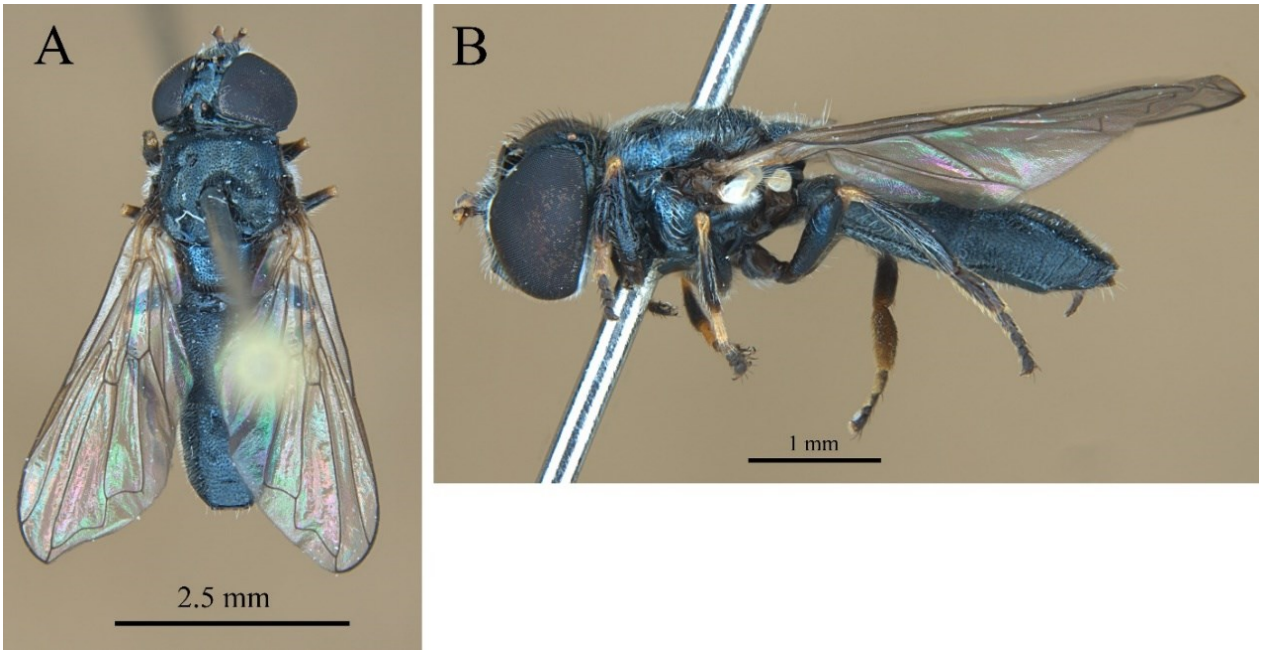

Figure 3. Adult male of *Triglyphus primus* reared from a larva collected in Denmark: (**A**) dorsal view; (**B**) lateral view.

Sample preparation and study

The larvae and puparium were cleaned in an Ultrasonic bath for 5 minutes and brushed to remove any dirt. The head skeleton was removed from the puparium by soaking it in hot 10% KOH, and examined in glycerine. General features of the larva, puparium, and head skeleton were observed under a Leica M205 C binocular stereomicroscope. The asterisks (*) in the section '*Triglyphus* immatures: taxonomic diagnosis' indicate the most distinguishable characters. Measurements of the larval body, PRP, and puparium follow Orengo-Green *et al*. (2024). Photos were produced as stacks of individual images made with a camera (Leica DMC 5400) attached to a binocular stereomicroscope (Leica M205 C). Stacks were made in Leica Application Suite Las X®, v.4.12.0. A map of the distribution of the *T. primus* larvae and puparium collected in Denmark was produced with the software QGis 3.32. For a more detailed description of the sensilla and PRP a scanning electron microscope (SEM) was used. One larva and two puparia were prepared on aluminium stubs with double-sided adhesive carbon tape, they were not gold-coated. To be able to recover the material, the samples were imaged with a Jeol JSM-IT500HR in variable pressure mode.

Morphological terminology

The terminology used for the larva and puparium descriptions follows Rotheray (2019). For each body segment, sensilla were numbered in the dorso-ventral direction (Rotheray and Gilbert, 1999). The terminology used for the head skeleton follows Rotheray and Gilbert (1999) and Rotheray (2019).

Results

Triglyphus primus immature stages

L1 larva (figure 4):

D e s c r i p t i o n

Length: 3 mm; width: 1.2 mm; height: 1.21 (n = 1).

Colour: whitish when alive, yellowish when dead and preserved.

PRP: barely visible, not fused.

L3 larva (figure 5):

D e s c r i p t i o n

Length: 4-6.07 mm; width: 1.67-1.97; height: 1-1.60 mm (n = 3). 8th abdominal segment (anal segment) with one pair of lappets. Oval in cross-section and flat ventrally with one pair of poorly developed crochet-less locomotory organs on the mesothorax and further pairs on the 1st-7th abdominal segments (figure 6). Head with well-developed antenno-maxillary organs (figure 7). A pair of anterior respiratory processes (ARP) are slightly sclerotized, cylindrical in shape (figure 7).

Colour: greenish when alive, dark brown when dead and preserved.

PRP (figure 8): width at the level of the transverse ridge: 0.139-0.203 mm; length above the transverse ridge: 0.27-0.34 mm; length below the transverse ridge: 0.3-0.368 mm. Yellowish/brownish with a noticeable transverse ridge when viewed dorsally. The transverse ridge is present as a constriction between the basal and polar part of the PRP (figure 8). The surface below the transverse ridge has large nodules; the surface above the transverse ridge has smaller nodules and is smooth when it reaches the spiracular plate. The diamond-shaped spiracular plate has four pairs of inter-spiracular setae, one

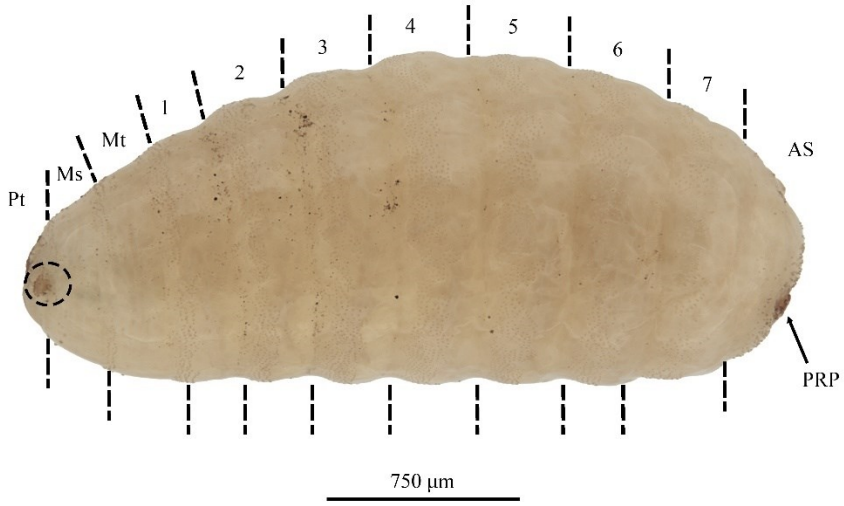

Figure 4. *Triglyphus primus* first instar larva (L1), lateral view with indication of boundaries between segments. AS, anal segment, Ms, mesothorax; Mt, metathorax; PRP, posterior respiratory process; Pt, prothorax; 1-7 indicate the abdominal segments. Circle indicates the anterior respiratory process (ARP).

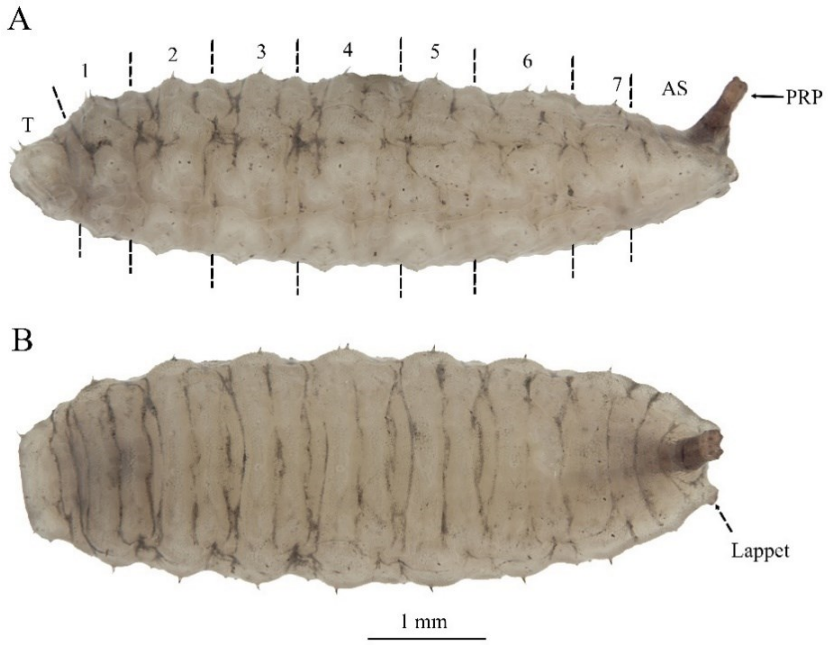

Figure 5. *Triglyphus primus* third instar larva (L3): (**A**) lateral view with indication of boundaries between segments; (**B**) dorsal view. AS, anal segment; PRP, posterior respiratory process; T, thorax; 1-7 indicate the abdominal segments.

pair of perispiracular glands, and three pairs of spiracular openings on top of a sclerotized carina (figure 9). The spiracular plate is separated by a median groove. The equidistant spiracular openings extend over the side of the spiracular plate. The ecdysial scar has a round form. Each inter-spiracular seta is almost half as long as a spiracular opening.

Chaetotaxy (figure 10): Prothorax: all observed sensilla are without setae. Dorsally with two pairs, laterally and ventrally with two pairs of sensilla. Mesothorax: dorsally with three pairs of sensilla that are shark-fin-shaped (figure 11A), laterally with two pairs of sensilla without setae, and ventrally with three pairs of sensilla without setae. Metathorax: dorsally with three pairs of sensilla that are shark-fin-shaped, laterally with two pairs of sensilla without setae, and ventrally with three pairs of sensilla without setae. Abdomen: 1st to 7th segments: dorsally with three pairs of sensilla that are shark-fin-shaped; laterally with one pair of sensilla that is shark-fin-shaped and four pairs of sensilla without setae; ventrally with three pairs of sensilla without seta. Anal segment with six pairs of sensilla without setae and one pair of a set of three sensilla with one seta on each (figure 11B).

Head skeleton (figure 13): see the description of the puparium.

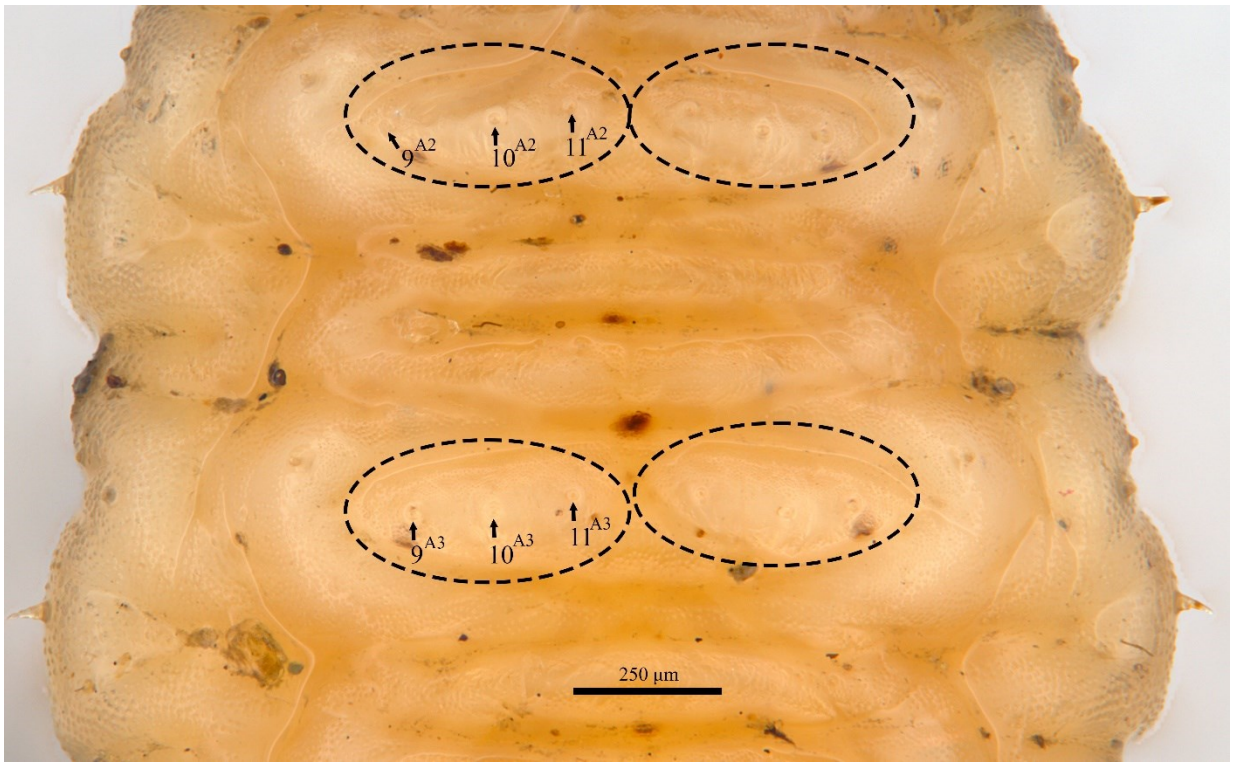

Figure 6. Ventral view of the 2nd and 3rd abdominal segments. Circles indicate the position of the locomotory organs. Arrows indicate the sensilla on the locomotory organs. The superscript indicates the abdominal segment.

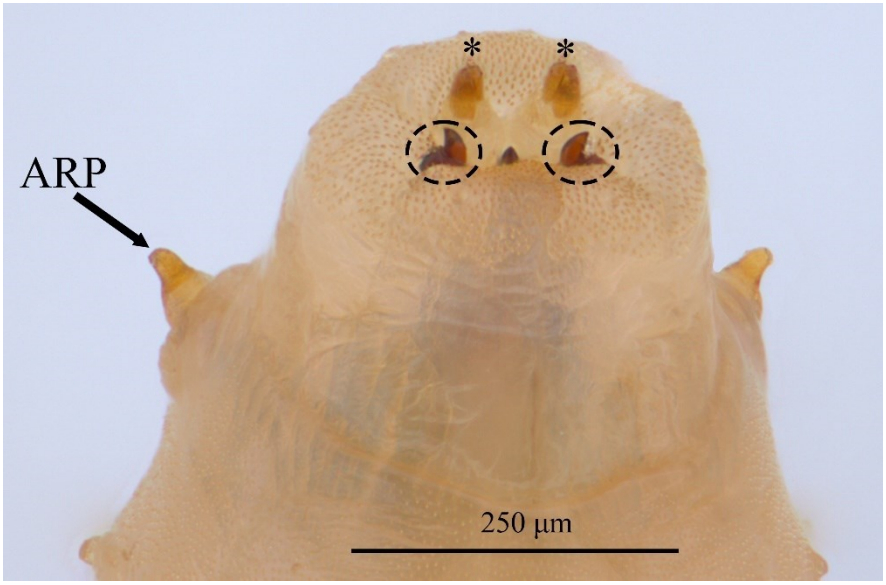

Figure 7. Ventral view of the prothorax. Circles indicate the hook shaped lateral lips; * indicates the antenno-maxillary organs; ARP, anterior respiratory process.

Puparium (figure 12):

D e s c r i p t i o n

Length: 3.36-4.4 mm; width: 1.94-2.1 mm; height: 1.6-1.84 mm (n = 4). Teardrop shape with the anterior part wider and flat ventrally.

Colour: light cream.

Head skeleton (figure 13): labrum and labium elongated and sclerotized. Labrum fused in the front with a sharp curved pointed end. Labium fused in the front with a pointed end. Square-shaped mandibles. Tentorial bar sclerotized. Dorsal cornu heavily sclerotized. Ventral cornu slightly sclerotized and almost twice the length of dorsal cornu. There is a pair of heavily sclerotized hook-shaped lateral lips at the lateral part of the head skeleton (figure 7).

PRP: carinae more sclerotized than those of the L3.

Chaetotaxy (figure 10): same as the L3 larva.

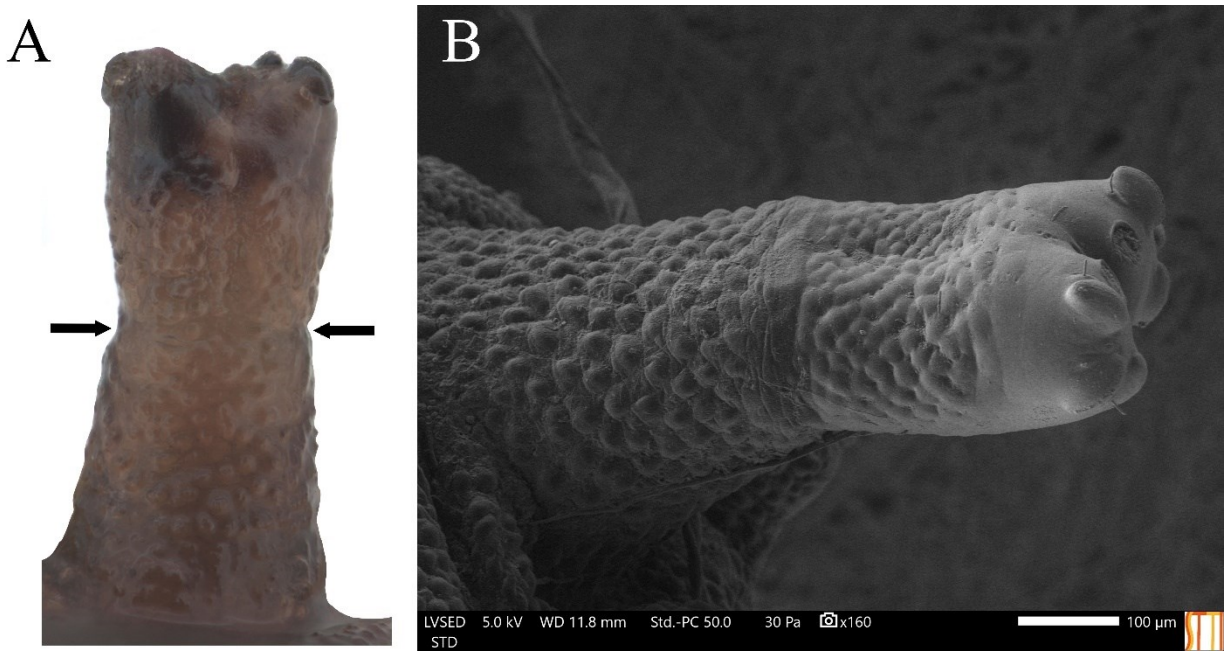

Figure 8. Posterior respiratory process of a third instar larva (L3) of *Triglyphus primus*: (**A**) dorsal view; (**B**) lateral view (photo SEM). Arrows indicate the constriction.

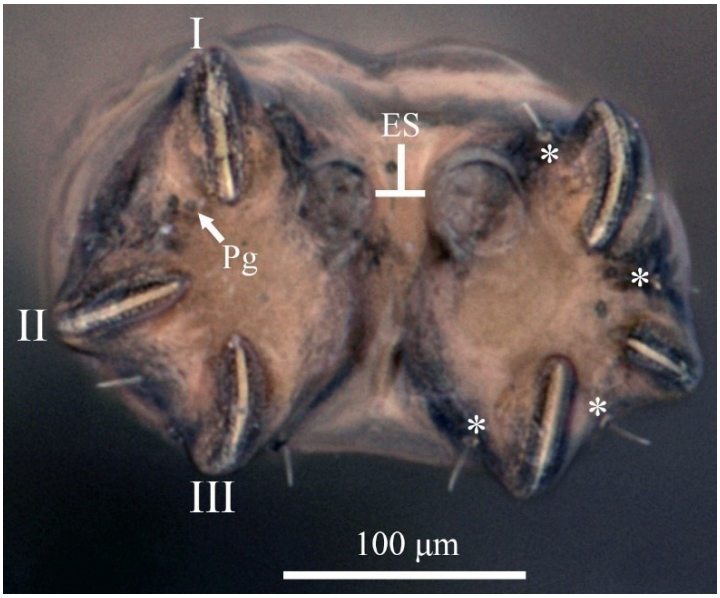

Figure 9. Posterior respiratory process of the puparium of *Triglyphus primus*, polar view. ES, ecdysial scars; Pg, perispiracular gland; I, II, III, spiracular openings; * indicates the inter-spiracular setae.

Triglyphus immature stages: taxonomic diagnosis

Mesothorax and 1st-7th abdominal segments with under-developed crochet-less locomotory organs; PRP: in dorsal view with a constriction in the transverse ridge (figure 8A); length below the transverse ridge greater than the length above the transverse ridge*; spiracular plate diamond-shaped; spiracular openings located on top of a sclerotized carina; equidistant spiracular openings extending over the side of the spiracular plate*; inter-spiracular setae almost half as long as spiracular opening. Head skeleton: labrum sclerotized and with a sharply curved pointed end (figure 13)*. Square-shaped mandibles*. Ventral cornu almost twice the length of the dorsal cornu. Hook-shaped lateral lips*.

As a result of our study, the larva of *Triglyphus* can be added to the taxonomic key of syrphid larvae of Thompson and Rotheray (1998) in step 58, modified as follows:

58. Larva covered with dome-shaped papillae; larva dark brown, whitish or greenish 59
- Larva smooth, without such papillae; larvae whitish . . . . . *Trichopsomyia* Williston 1888
59. Colour: dark brown larva . . . . *Heringia* Rondani 1856
- Colour: greenish larva . . . . . *Triglyphus* Loew 1840

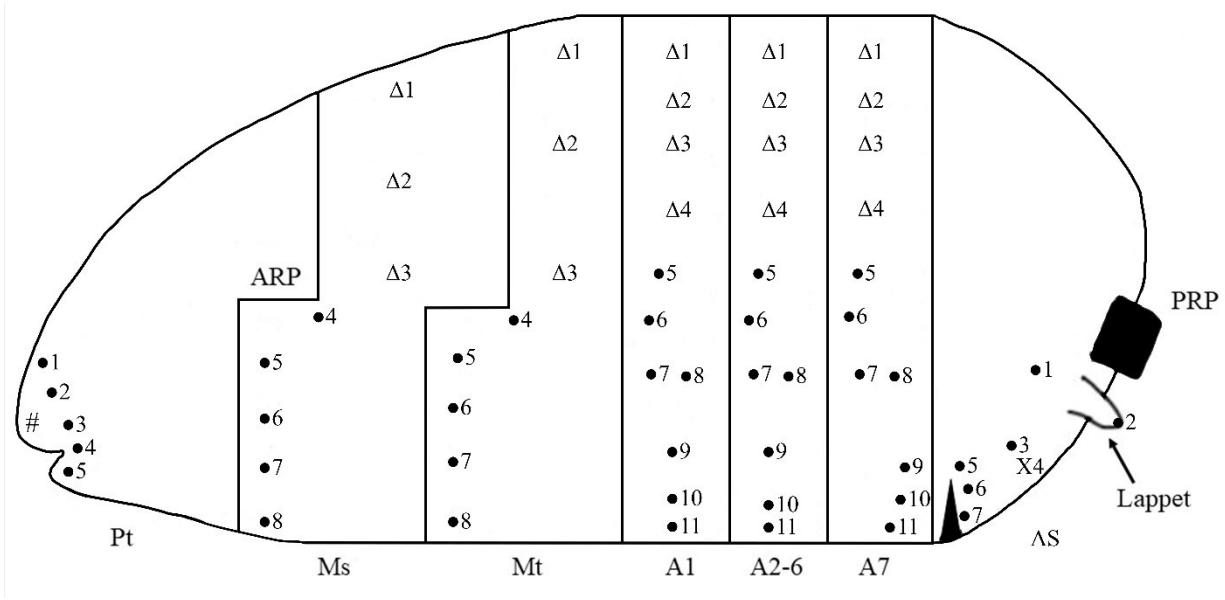

Figure 10. Third instar larva (L3) of *Triglyphus primus* showing the number and relative position of the body sensilla: P, prothorax; Ms, mesothorax; Mt, metathorax; A1, A2-7, abdominal segment; AS, anal segment; ARP, anterior respiratory process; PRP, posterior respiratory process. Symbols: Δ, sensillum with seta; •, sensillum without seta; X, set of three sensilla with seta; #, antenno-maxillary organs.

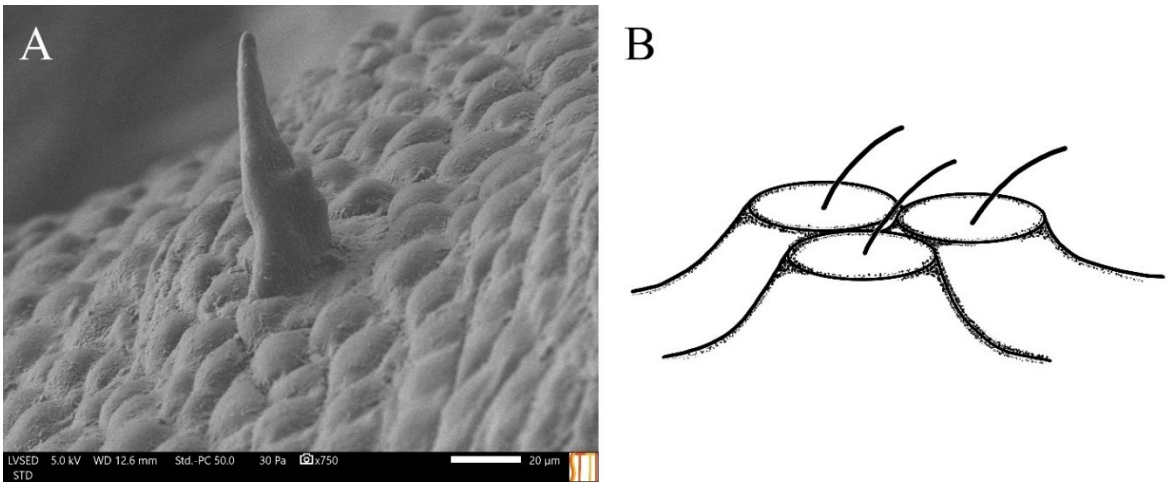

Figure 11. *Triglyphus primus* types of sensillum: (**A**) shark-fin-shaped sensillum, dorsal side of an abdominal segment (SEM); (**B**) set of three sensilla with a seta each, ventral side of the anal segment.

Discussion

The subfamily Pipizinae needs further revision to separate the sister groups of each genus belonging to the subfamily (Vujić *et al*., 2013; Mengual *et al*., 2015). This study of *Triglyphus* larval morphology may help to better define the systematic relationships amongst the genera inside the Pipizinae when the rest of the immature stages (*Claussenia*, *Cryptopipiza*, *Heringia*, *Neocnemodon*, *Pipiza*, *Pipizella*, and *Trichopsomyia*) are better known. In this work, we bring for the first time the diagnosis of the immature stages of *Triglyphus* to the general knowledge of hoverflies. The most distinctive features are the shape of the PRP and the sharply curved pointed end of the labrum.

It is known that the immature stages of the Pipizinae subfamily are predators (Rojo *et al*., 2003). The fact that each genus of this subfamily seems to prey on different types of aphids, coccids, or psyllids is an important information that can be used to distinguish them. According to Thompson and Rotheray (1998), the genus *Pipiza* prey on ground-layer aphids and galling aphids; *Heringia* on gall-inducing aphids in trees, *Pipizella* on root aphids in ant nests, and *Trichopsomyia* on psyllid gall on rushes. The genus *Neocnemodon* preys on aphid eggs, aphids, and coccids (Delucchi *et al*., 1957; Evenhuis, 1959). Leclercq (1944) and Sedlag (1966) mentioned that *Triglyphus* larvae feed on aphid galls on *A. vulgaris*. In addition, Sedlag (1966) mentions that many larvae of *Triglyphus* were found at *Artemisia* galls, suggesting that it was a common syrphid. In this work we confirm what they mentioned.

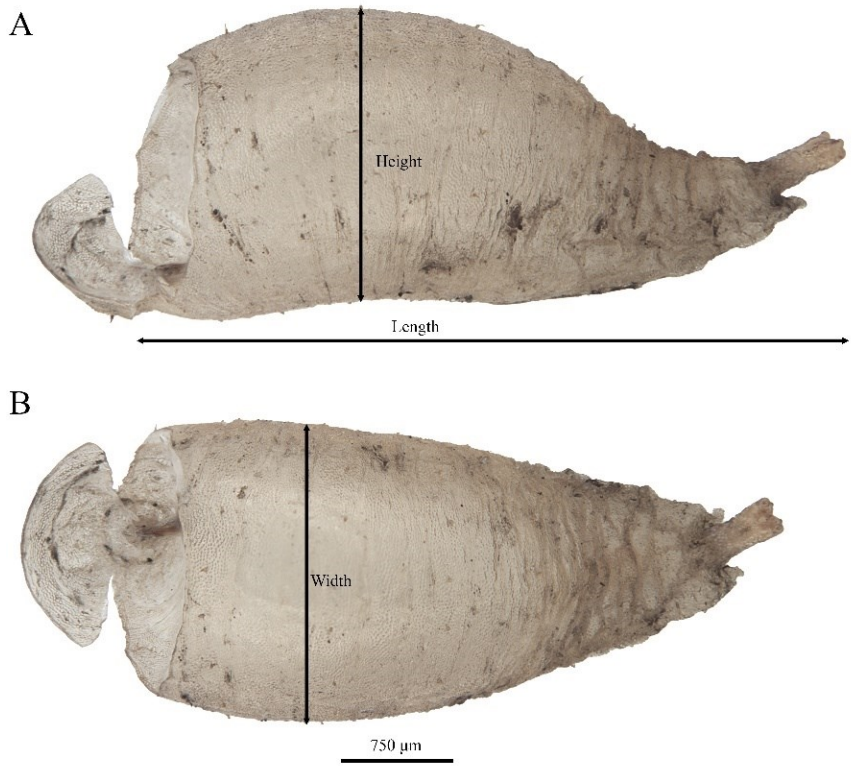

Figure 12. Puparium of *Triglyphus primus*: (**A**) lateral view; (**B**) dorsal view.

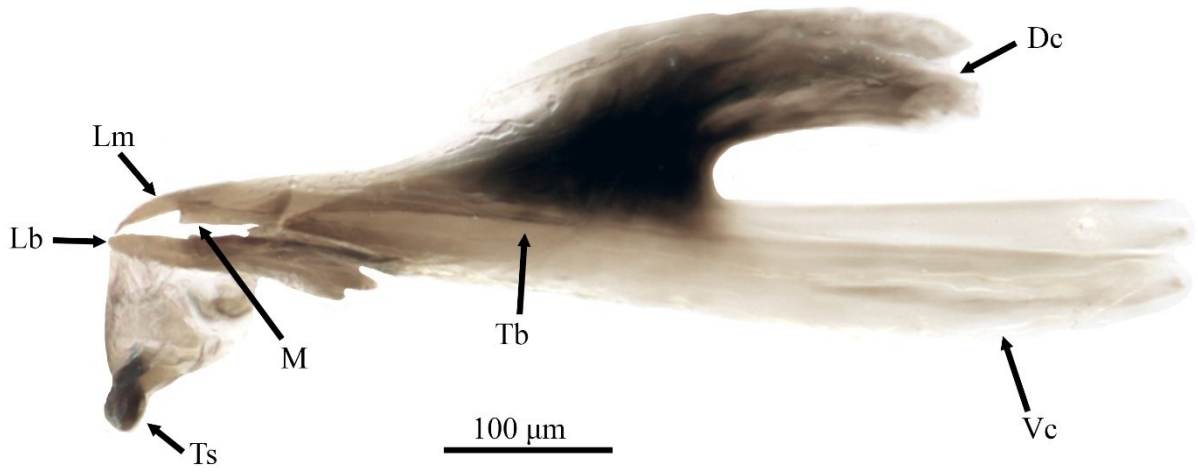

Figure 13. Lateral view of head skeleton of *Triglyphus primus*. Dc, dorsal cornu; Lb, labium; Lm, labrum; M, mandibles; Tb, tentorial bar; Ts, lateral lips; Vc, ventral cornu.

The morphology of the immature stages within the subfamily Pipizinae is very similar and difficult to differentiate. The characters commonly used to differentiate them are the colour of the larva, the type of surface of the integument, and the size of the inter-spiracular setae (Rotheray, 1993). Thanks to the body colour, larvae of different pipizine genera can be distinguished: in *Heringia*, dark brown, in *Pipiza*, green/dark brown, and *Trichopsomyia* pale colour pattern (Dušek and Láska, 1960; Rotheray, 1993). The rest of the genera share the same whitish or greenish colouration. The type of surface of the integument is a good character to distinguish genera within the subfamily. According to Rotheray (1993), three types of surfaces can be found in Pipizinae: a) body surface coated in setae (*Pipiza*), b) body surface coated in thick rounded setae (*Pipizella*), and c) body surface coated in dome-shaped papillae (*Heringia*, *Trichopsomyia*, and *Triglyphus*). Unfortunately, the type of surface of *Neocnemodon* is unknown because Delucchi *et al*. (1957) do not mention it. Another character that must be

taken with caution is the length of the inter-spiracular setae because the seta may break off if not handled properly, resulting in an incorrect identification.

The sister group of *Triglyphus* is not well defined because it shows a polytomy between *Cryptopipiza*, *Heringia*, *Pipizella*, *Neocnemodon*, and *Trichopsomyia* (Rotheray and Gilbert 1999; Vujić *et al*., 2013). Of the possible sister groups mentioned above, only the *Cryptopipiza* has no immature described. According to our knowledge, *Heringia* has the description of the immature stages of three species, *Heringia calcarata* (Loew 1866), *Heringia heringi* (Zetterstedt 1843), and *Heringia salax* (Loew 1866) (described as *Pipiza radicum*) (Heiss, 1938; Dušek and Láska, 1959; 1960; Bergh and Short, 2008), *Pipizella* one species, *Pipizella viduata* (L. 1758) (described as *Pipizella varipes*) (Dixon, 1960; Rotheray *et al*., 1996), *Trichopsomyia* three species, *Trichopsomyia joratensis* (Goeldlin de Tiefenau 1997), *Trichopsomyia ochrozona* (Stackelberg 1952), and *Trichopsomyia viduata* (L. 1758) (described as *Trichopsomyia flavitarsis*) (Rotheray, 1997; van Steenis *et al*., 2018), and *Neocnemodon* with five species, *Neocnemodon coxalis* (Curran 1921) (described as *Cnemodon coxalis*), *Neocnemodon latitarsis* (Egger 1865), *Neocnemodon pisticoides* (Williston 1887) (described as *Pipiza pisticoides*), *Neocnemodon rita* (Curran 1921) (described as *Cnemodon rita*), and *Neocnemodon vitripennis* (Meigen 1822) (described as *Cnemodon dreyfusiae*) (Heiss, 1938; Delucchi *et al*., 1957; Brown and Clark, 1960; Dušek and Láska, 1960; Mitchell, 1962).

With little information available on the immature stages of Pipizinae, it can be observed that *Triglyphus* is more closely related to *Heringia* than to the other genera of this subfamily. Both have the same type of surface of the integument, and the PRP, the size of the larvae and the length of the inter-spiracular setae are almost identical. Rotheray (1993) mentions that the PRP of *Heringia* is 2-6× longer than broad, and that of *Triglyphus* is less than 2× longer than broad. This character, however, is not useful to distinguish these two genera, since the length of the PRP of *T. primus* presented in this work is almost 4× longer than broad and thus within the range mentioned by Rotheray (1993) for *Heringia*. More effort is needed in the study of immature stages of these Pipizinae species to be able to properly obtain the knowledge required to correctly define the sister groups.

The most important feature proposed for the identification of *Triglyphus* is the abrupt curvature of the labrum. None of the head skeletons described in the Pipizinae have this abrupt curvature, but *Triglyphus* does have the square-shaped mandible typical of this subfamily (Rotheray and Gilbert, 1999). One possible explanation for the presence of curvature in the labrum may be to facilitate the opening of the galls to reach the aphid inside. However, this needs to be further studied for confirmation.

The "common mugwort", *A. vulgaris*, is an important perennial native weed in Europe, considered an invasive species in North America (Aulakh, 2020). *A. vulgaris* can spread by seeds and a rhizome system, allowing it to colonize gardens, waste areas, roadsides, and agricultural zones very quickly (Barney and DiTommaso, 2003; Weston *et al*., 2005). Barney and DiTommaso (2003) suggest that it could be considered a threat to the diversity of the native flora because *A. vulgaris* produces allelochemicals inhibiting the survival and growth of other plant species. In addition, *A. vulgaris* is one of the ten most important weeds in the greenhouses of the USA (Aulakh, 2020). The management of *A. vulgaris* involves mowing and herbicides (e.g. glyphosate or picloram), the latter being the most used and the most effective (Bradley and Hagood, 2002; Koepke-Hill *et al*., 2011). At least three species of aphids are known to feed on *A. vulgaris*: *Macrosiphoniella artemisiae* (Boyer de Fonscolombe 1841), *Brachycaudus cardui* L. 1758, and *C. artemisiae* (Basky, 2016). According to Basky (2016), *C. artemisiae* is considered a rare gall-forming aphid. This is a possible explanation why *T. primus* is rarely found, because even if other aphids are present on *A. vulgaris*, it seems to prefer *C. artemisiae*. However, it is not specific to this aphid: Láska and Starý (1980) mentioned that *T. primus* prey on *Therioaphis riehmi* (Borner 1949), the "sweetclover aphid" on *Melilotus albus* Medik. (Fabaceae) causing only occasional damage (Manglitz and Hill, 1964).

With the information provided in this work, it is hoped that more immature stages of other *Triglyphus* species can be located to know more about the life cycle of this uncommon genus. More work needs to be done to find and describe the egg and the L2 to be able to complete the characterization of all immature stages of *T. primus*.

Acknowledgements

Thanks to Somporn Phanla-or Carstensen for helping Leif Bloss Carstensen find adults and the immature stages. Thanks to Iván Ballester-Torres for doing the QGIS map, and to Francis Gilbert for providing some hard-to-find publications. Student grant of José J. Orengo-Green is funded by the University of Alicante (UAFPU2019B-57). Thanks to the "Vicerrectorado de Investigación" of the University of Alicante for the funds granted to use the Technical Research Services within their "I+D+I" programme (UAUSTI22-25).

The authors declare that they have no known competing financial interest of personal relationship that could have appeared to influence the work reported in this paper.

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Received June 10, 2024. Accepted October 10, 2024.