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Morphology of the immature stages of *Cheilosia vernalis* (Fallén, 1817) and an updated description of the larva of *Cheilosia canicularis* (Panzer, 1801) (Diptera: Syrphidae)

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Abstract. With almost 500 species, *Cheilosia* Meigen, 1822 (Diptera: Syrphidae) is the most diverse genus of hoverflies in the Palaearctic Region. The larval morphology and biology of over 40 species of *Cheilosia* are known, but only the description of the immatures stages of 28 species has been done. The known larvae of *Cheilosia* have a wide range of feeding modes, including mycophages, borers in roots and stems, leaf-miners, cambium and sap feeders, and saprophages. In this study, all three larval stages and puparium of *Cheilosia vernalis* (Fallén, 1817) are described. This species was confirmed based on morphological, DNA (i.e., 5′ end region of the Cytochrome *c* oxidase subunit I gene) and geographic evidence. Larvae of *C. vernalis* were found in the stems of *Leucanthemun vulgare* Lam. (Asteraceae), *Matricaria chamomilla* L. (Asteraceae), and *Tripleurospermum inodorum* (L.) Sch.Bip. (Asteraceae) in Denmark, and all the immature stages were studied by stereo microscopy and scanning electron microscopy. The larval head skeleton and chaetotaxy were also described and illustrated. In addition, a clarification to identify larvae of *Cheilosia canicularis* (Panzer, 1801) is provided, according to an updated description of their immature stages. To facilitate the species identification, a key to all known root and stem-boring larvae of *Cheilosia* is provided.

Keywords. Chaetotaxy, Rhingiini, Denmark, head skeleton, larva key, phytophagous.

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Introduction

Over 6300 species comprise the family Syrphidae Latreille, 1802 (Diptera) worldwide, widely known as hoverflies due to the distinctive flight of the adults (Skevington *et al*. 2019; Dunn *et al*. 2020). The hoverflies are considered to be one of the most important pollinator families, because of the frequent flower visiting of the adults to feed on nectar and pollen (Doyle *et al*. 2020). The larvae develop in a totally different ecological niche, with a broad spectrum of feeding habits. They can be saprophagous, mycophagous, phytophagous or predators, with a greater or lesser level of trophic specialisation depending on the species concerned (Rotheray & Gilbert 1999; Stuke 2000). Within Syrphidae, one of the genera with the highest number of species (nearly 500 worldwide), and the largest in the Holarctic Region, is *Cheilosia* Meigen, 1822 (Vujić *et al*. 2019; Barkalov & Ståhls 2022). The genus comprises black to brown syrphids with a shiny thorax and abdomen covered with black, white, yellow or red hairs (Ståhls *et al*. 2008).

With over 40 known immature stages and only 28 of those known immature stages have a partial or full description available (Stuke 2000; Speight 2020), one might imagine that there is a lot of information available to help in their identification. Nevertheless, there is a lot of controversy regarding identification characters and species description of *Cheilosia* immature stages, and a revision is needed to help clarify the existing taxonomic problems. Since the immature stages of *Cheilosia* are complicated to identify, Rotheray (1993) recommended grouping them by feeding mode (mycophagous, phytophagous, or saprophagous). In *Cheilosia*, the phytophages can be separated into three groups: a) leaf-mining; b) boring in roots and stems, or c) cambium and sap feeding (Rotheray & Gilbert 1999; Krivosheina 2019).

Cheilosia vernalis (Fallén, 1817) belongs to the *Cheilosia melanura* Becker, 1894 group, comprising species with the following characteristics: antennae separated by the lunula; hairy eyes (at least on upper half); well-expressed mouth edge; katepisternum with upper and lower hair patches separated by a bare area; scutellar margin with or without black bristles; sternites usually shining; similar male genitalia, with the surstylus short and rounded, the ventral lobe of the gonostylus elongate, widening towards the apex, and the dorsal lobe of the gonostylus short, with both lobes of approximately the same width (Ståhls *et al*. 2008; Ballester-Torres *et al*. 2024). The taxonomy of some species is complicated, as for example *C. vernalis* appears to form a complex of morphologically similar species due to its highly variable morphology and the inconclusive molecular results (Ståhls *et al*. 2008). *Cheilosia reniformis* Hellén, 1930 is known to share intermediate forms with *C. vernalis*, sometimes displaying overlapping morphological features and weak molecular differences (Ståhls *et al*. 2008; Ballester-Torres *et al*. 2024). Even before it was described, the larva of *C. vernalis* was known to be an internal feeder of *Achillea* L., *Cirsium* (L.) Mill., *Matricaria* L., and *Sonchus* L. stems, as well as the involucre of *Tragopogon* L. (Stuke 2000; Speight 2020).

Cheilosia canicularis (Panzer, 1801) belongs to a group of species to which it gives its name, the *canicularis* species group (Stuke & Claußen 2000), originally comprising the following European species: *C. canicularis*, *C. himantopa* (Panzer, 1798) and *C. ortotricha* Vujić & Claußen, 1994. Vujić & Šikoparija (2001) also include the Japanese endemic *C. japonica* (Hervé-Bazin, 1914) and *C. yesonica* Matsumura, 1905 as part of the *C. canicularis* group. Similar to the *C. vernalis* complex mentioned, the *C. canicularis* group is considered taxonomically difficult, due to the morphological, phenotypic and larval development similarities between the species, especially those of *C. canicularis* and *C. himantopa* (Stuke & Claußen 2000; Speight 2020). For instance, the *C. canicularis* third-instar (L3) stage was described by Rotheray (1990), but Stuke & Claußen (2000) considered that the description belonged to a larva of *C. himantopa*, based on phenology, as this was considered one of the few differences between both species (Stuke & Claußen 2000; Vujić & Šikoparija 2001). Later on, Ludoški *et al*. (2008)

proved the validity and delimitation of the three European species in the group based on wing geometric morphometrics.

The aims of the present study are (1) to expand the knowledge of the morphology of the immature stages of *Cheilosia* and (2) to clarify the taxonomic problem in *C. vernalis* and between *C. canicularis* and *C. himantopa*. The first (L1), second (L2), and L3 larval stages and puparium of *C. vernalis* and the pupal spiracles of *C. canicularis* are described in detail. A taxonomic key to all known larvae/puparia of the *Cheilosia* boring in roots and stems is provided for the first time.

Material and methods

Examined material and adult/larva identification

Larvae of *C. vernalis* (n = 8) and larvae (n = 5) and one pupa of *C. canicularis*, were found in *Leucanthemun vulgare* Lam. (Asteraceae) and *Petasites hybridus* (L.) G. Gaertn, B. Mey & Scherb. (Asteraceae) respectively, in several locations of Denmark (Fig. 1) by Leif Bloss Carstensen from June to October 2023. The larvae were reared in several plastic containers with leaves, stems and roots of *L. vulgare* and rhizomes of *P. hybridus* (Fig. 2). The containers were checked daily to record changes in development. Six larvae (four larvae of *C. vernalis* and two larvae of *C. canicularis*) were preserved in alcohol 70% and the rest were reared for adult identification. Four larvae of *C. vernalis* and three larvae of *C. canicularis* pupated, and two adults from each species emerged from these puparia. The match between the larvae and the puparium was based on the features of the posterior respiratory process (PRP). Adults were identified by Iván Ballester-Torres and Antonio Ricarte. Adult and immature material are deposited at the CEUA-CIBIO collection, University of Alicante, Spain.

Sample preparation and study

The procedure of the material preparation follows Orengo-Green *et al*. (2024). For five minutes, the larvae and puparium were cleaned in an ultrasonic bath and a brush was used to remove any dirt remaining. The head skeleton was removed from the puparium in hot 10% KOH for five minutes and observed in glycerine. A Leica M205 C binocular stereo microscope was used to observe general features of the larva, puparium, head skeleton, and PRP. The measurements of the larvae, puparium, and PRP follow Orengo-Green *et al*. (2023). Photos were produced as stacks of individual images made with a

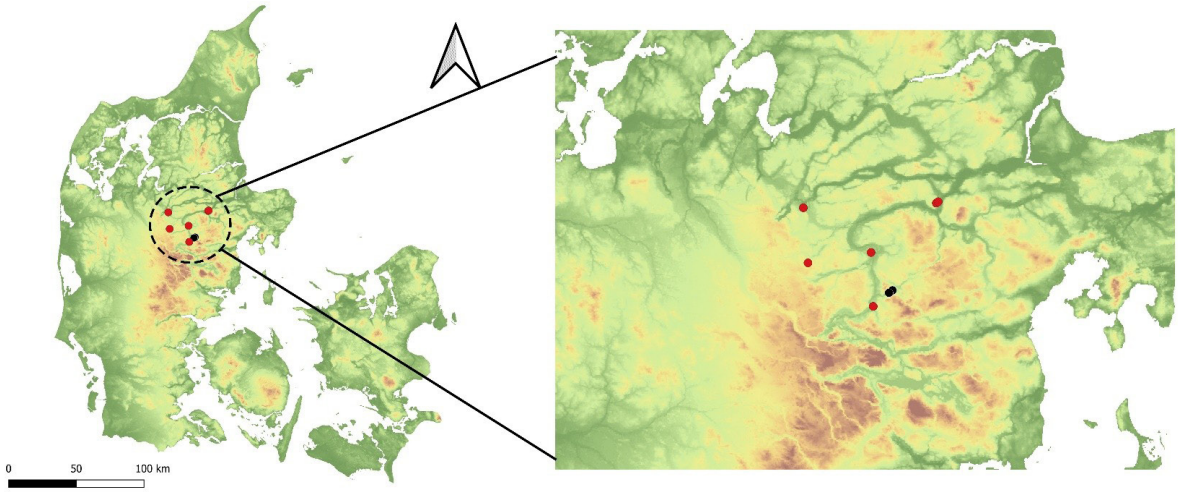

Fig. 1. Localities where *Cheilosia* Meigen, 1822 larvae were found in Denmark. Legend: red circles = *Cheilosia vernalis* (Fallén, 1817); black circles = *Cheilosia canicularis* (Panzer, 1801).

Leica DMC 5400 camera attached to a binocular stereo microscope (Leica M205 C). Stacks were made in Leica Application Suite Las X®, ver. 4.12.0 (Leica 2024). The drawing of the head skeleton was made from printed photos and the dark colour indicates a heavy level of sclerotization. A scanning electron microscope (SEM) was used for a more detailed description of the PRP and the pupal spiracles. One puparium of *C. vernalis* and one puparium of *C. canicularis* were prepared on aluminium stubs with double-sided adhesive carbon tape. To be able to recover the material, the samples were imaged with a Jeol JSM-IT500HR in variable pressure mode. A map of the distribution of the collected *C. vernalis* and *C. canicularis* in Demark was produced with the software QGIS ver. 3.32 (QGIS 2024).

Morphological terminology

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

Molecular study

To test our hypotheses, given that morphological data is not always clear enough to guarantee reliable identification of the species, a molecular approach was carried out. DNA was extracted from four adult (vouchers CEUA_S124–125, CEUA_S127–128) and a puparium (voucher CEUA_S435) of *C. vernalis*, as well as from a *Cheilosia* (voucher CEUA_S436) that happened to belong to the same species after matching with sequences available from the internet. DNA was extracted

Fig. 2. Third larval stage of *Cheilosia canicularis* (Panzer, 1801) found in rhizome of *Petasites hybridus* (L.) G.Gaertn., B.Mey. & Scherb. (Photo by Leif Bloss Carstensen).

using the NZY Tissue gDNA Isolation kit, following the manufacturer's protocol. PCR amplification of the 5′end region of the Cytochrome *c* oxidase subunit I gene (COI-5′) was performed using the primers Heb-F (5′-GGTCAACAAATCATAAAGATATTGG-3′) (Folmer *et al*. 1994) and 780-R (5′-CCAAAAAATCARAATARRTGYTG-3′) (Gibson *et al*. 2011). Amplifications were carried out in a total volume of 25 µL containing 1 × of Buffer reaction, 0.4 mM of dNTPs, 0.2 µM of each primer, 0.65–2 mM of $MgCl_2$ and 1–2 units of DNA polymerase. Thermocycler conditions followed those used by Aguado-Aranda *et al*. (2023) for COI-5′. PCR products were visualized in a 2% agarose gel. All products were purified and sequenced at Macrogen (Macrogen Inc., Seoul, Republic of Korea). The editing of the resulting sequences was conducted with the software Sequencher ver. 5.4.6. (Ann Arbor, MI, USA). Then, COI-5′ sequences of each species of the *C. melanura* group available at GenBank and BOLD were downloaded (see Supp. file 1). The alignment was performed manually and checked with the program Aliview ver. 1.25 (Larsson 2014). The final matrix had a length of 604 bp. The Neighbor-Joining (NJ) and Maximum Likelihood (ML) analyses were conducted in MEGA7 (Kumar *et al*. 2016) with 1000 replications using the Maximum Composite Likelihood model and the General Time Reversible model with gamma distribution (+G) and invariant sites (+I) proposed by the corrected Akaike information criterion (AICc), respectively. The resulting trees were rooted based on a *Ferdinandea cuprea* (Scopoli, 1763) sequence.

Results

Taxonomy

Class Insecta Linnaeus, 1758
Order Diptera Linnaeus, 1758
Family Syrphidae Latreille, 1802
Subfamily Eristalinae Newman, 1834
Tribe Rhingiini Meigen, 1822
Genus *Cheilosia* Meigen, 1822

Cheilosia vernalis (Fallén, 1817) immature stages

Description

First stage larva (L1) (Fig. 3)

Length: 2.4 mm; width: 0.75 mm; height: 0.68 mm (n = 1). The 8th abdominal segment (anal segment) with two pairs of lappets. Oval in cross-section and flat ventrally with one pair of poorly developed crochet-less locomotory organs in the mesothorax and in the 1st and 7th. Head with well-developed antenno-maxillary organs. Anterior part larger than the posterior part. *Colour*: yellowish. *PRP*: light yellowish. Wrinkled surface above, smooth surface below transverse ridge.

Second stage larva (L2) (Fig. 4)

Length: 4–4.34 mm; width: 1.32–1.5 mm; height: 1.32 mm (n = 2). *Colour*: light brown. *Head skeleton* (Fig. 5). One pair of heavily sclerotized mouth-hooks. A pair of small accessory teeth located behind the mouth-hooks. Labrum, mandibular apodeme, mandibular lobe and tentorial bar heavily sclerotized. Mandibular apodeme and mandibular lobe fused. Tentorial arm not sclerotized. Dorsal cornu almost two thirds of the length of the ventral cornu. *PRP*: fused and shiny yellowish-brown, with a noticeable transverse ridge. Surface above transverse ridge with wrinkles, surface below smooth. Length above transverse ridge: 0.18–0.28 mm; length below transverse ridge: 0.04–0.07 mm; width at the transverse ridge: 0.30–0.38 mm.

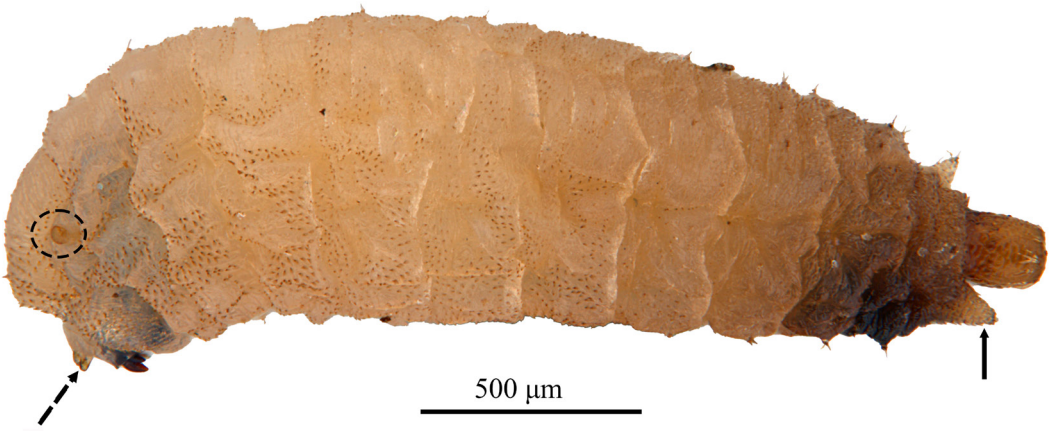

Fig. 3. First stage larva (L1) of *Cheilosia vernalis* (Fallén, 1817) (CEUA-CIBIO), lateral view. Dash circle indicates anterior respiratory process; dash arrow indicates antenno-maxillary organs; complete arrow indicates lappet.

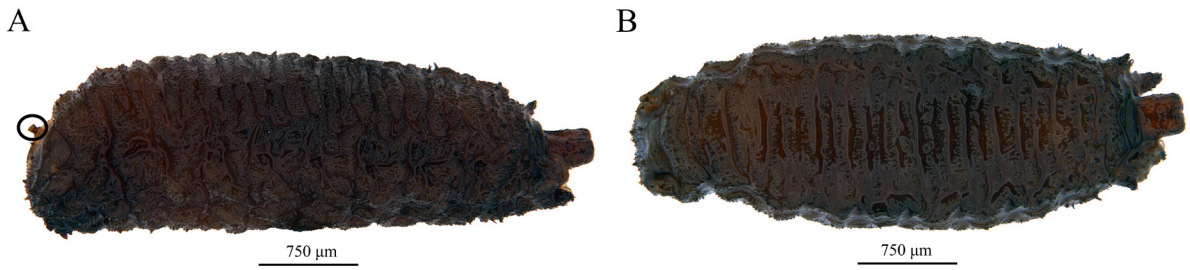

Fig. 4. Second stage larva (L2) of *Cheilosia vernalis* (Fallén, 1817) (CEUA-CIBIO). **A**. Lateral view. **B**. Dorsal view. Circle indicates the anterior respiratory process.

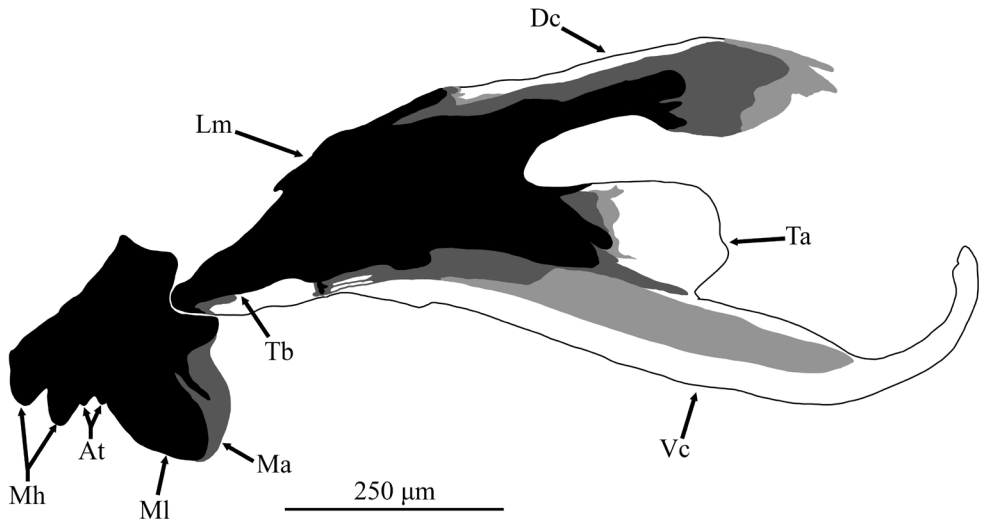

Fig. 5. Head skeleton of a second stage larva (L2) of *Cheilosia vernalis* (Fallén, 1817) (CEUA-CIBIO), lateral view (drawing). Abbreviations: At = accessory tooth; Dc = dorsal cornu; Lm = labrum; Ma = mandibular apodeme; Mh = mouth-hooks; Ml = mandibular lobe; Ta = tentorial arm; Tb = tentorial bar; Vc = ventral cornu.

Third stage larva (L3) (Fig. 6)

Length: 6.72 mm; width: 2.15 mm; height: 2.02 (n = 1). The 8th abdominal segment (= anal segment) with two pairs of lappets. Oval in cross-section and flat ventrally, with one pair of poorly developed crochet-less locomotory organs in the mesothorax and in the 1st and 7th abdominal segments. Head with well-developed antenno-maxillary organs. *Colour*: dark brown. *ARP*: cylindrical, tapering with a fan-shaped tip with seven spiracular openings. *PRP* (Fig. 7): shiny light brown with a slightly noticeable transverse ridge. Length above the transverse ridge: 0.25 mm; length below transverse ridge: 0.13 mm; width at the level of the transverse ridge: 0.32 mm. Surface above the transverse ridge with wrinkles and punctures; surface below the transverse ridge with smooth surface. Spiracular plate (Fig. 7B) with four pairs of long interspiracular setae, one pair of perispiracular glands, four pairs of irregular spiracular openings, and small groove at the center of the spiracular plate. A pair of ecdysial scars located in the middle of the spiracular plate. *Chaetotaxy* (Fig. 8): all observed sensilla bearing a seta. *Prothorax*: eight pairs of sensilla. *Mesothorax*: dorsally with three pairs of sensilla, laterally with three pairs of sensilla, and ventrally with two pairs of sensilla. *Metathorax*: dorsally with three pairs of sensilla, laterally with three pairs of sensilla, and ventrally with two pairs of sensilla. *Abdomen*: 1st to 6th abdominal segments: dorsally with three pairs, laterally with five pairs and ventrally with three pairs of sensilla. 7th abdominal segment: dorsally with three pairs, laterally with five pairs and ventrally with three pairs around the anus. The anal segment with nine pairs of sensilla observable.

Fig. 6. Third stage larva (L3) of *Cheilosia vernalis* (Fallén, 1817) (CEUA-CIBIO). **A**. Lateral view. **B**. Dorsal view.

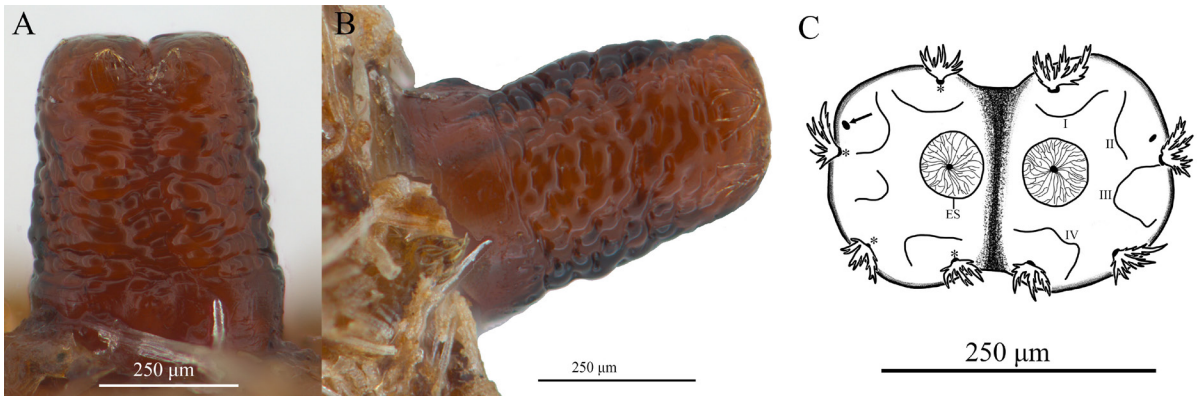

Fig. 7. Posterior respiratory process of a third stage larva (L3) of *Cheilosia vernalis* (Fallén, 1817) (CEUA-CIBIO). **A**. Dorsal view, stereo microscope image. **B**. Lateral view, stereo microscope image. **C**. Polar view, drawing. Arrow indicates the perispiracular gland; I, II, III, IV = spiracular openings; ES = ecdysial scars; asterisk (*) indicates the interespiracular setae.

Puparium (Fig. 9)

Length: 5.44–6.44 mm; width: 2.19–2.85 mm; height: 1.98–2.54 mm (n = 4). *Colour*: light brown. *PRP*: same as the L3 larva. Length above the transverse ridge: 0.14–0.15 mm; length below the transverse ridge: 0.32–0.44 mm; width at the level of the transverse ridge: 0.37–0.39 mm. *Pupal spiracles* (Fig. 10):

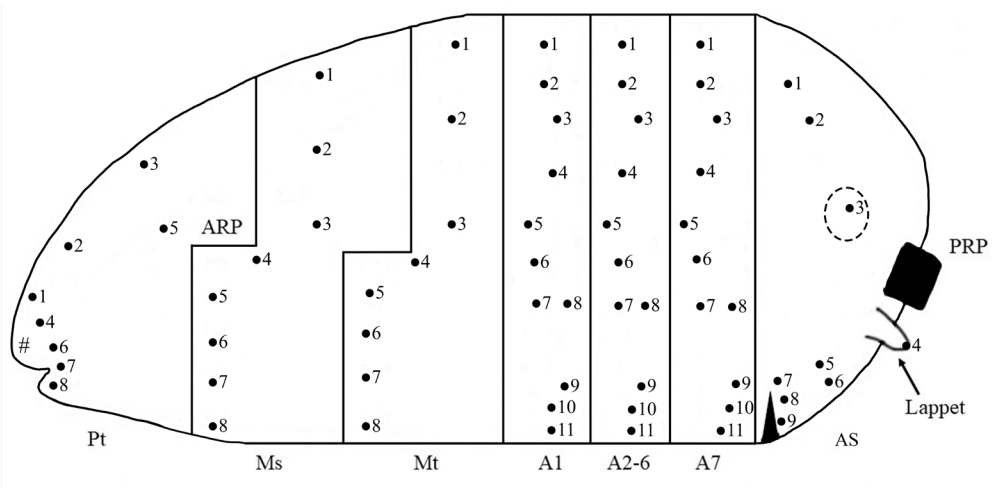

Fig. 8. Chaetotaxy map of *Cheilosia vernalis* (Fallén, 1817) (CEUA-CIBIO) showing the number and relative positions of the body sensilla. Abbreviations: A1–A7 = abdominal segments; ARP = anterior respiratory process; AS = anal segment; Ms = mesothorax; Mt = metathorax; Pt = prothorax; PRP = posterior respiratory process; # = antenno-maxillary organs; • = sensillum with seta. Dashed circle indicates lappet position.

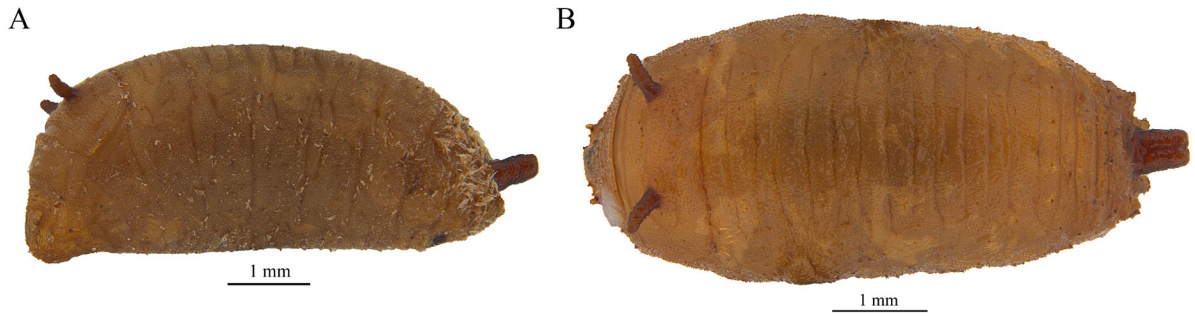

Fig. 9. Puparium of *Cheilosia vernalis* (Fallén, 1817) (CEUA-CIBIO). **A**. Lateral view. **B**. Dorsal view.

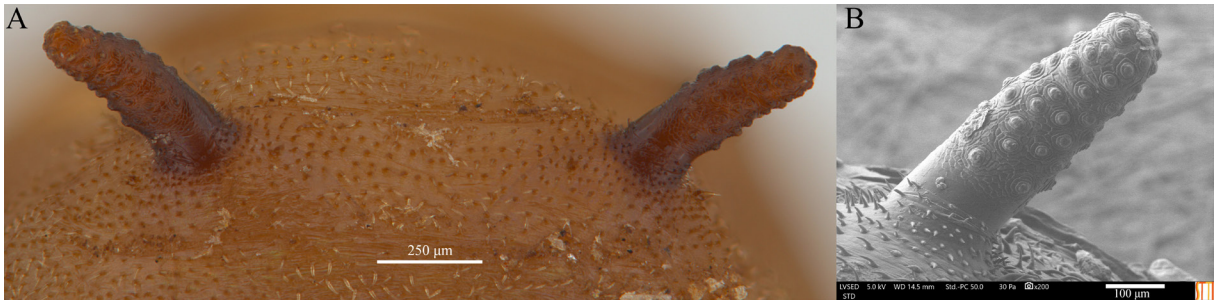

Fig. 10. Pupal spiracles of *Cheilosia vernalis* (Fallén, 1817) (CEUA-CIBIO). **A**. Anterior side, stereo microscope. **B**. Anterior side, SEM image.

length: 0.45 mm; width: 0.17 mm; space between the tips of the pupal spiracles: 1.9 mm. Dark-brown colour; cylindrical, tapering with a rounded tip; surface covered with tubercles except at the base. Smooth surface at the base. Tubercles with five to eight opening holes. *PRP*: same as the L3. Length above the transverse ridge: 0.27 mm; length below the transverse ridge: 0.13 mm. *Chaetotaxy*: same as the L3.

Intraspecific variation within Cheilosia vernalis immature stages

One adult of *C. vernalis* emerged from a puparium that showed some morphological variation in the colour of the pupal spiracles, exhibiting a light-yellow hue (Fig. 11A), and the surface of the PRP, almost completely smooth and with a constriction (Fig. 11B–C). After a molecular study analysing sequences from Danish immature stages of *C. vernalis* with the morphology stated in the above description, the Danish puparium with different pupal spiracles and PRP, as well as sequence from adults of *C. vernalis*

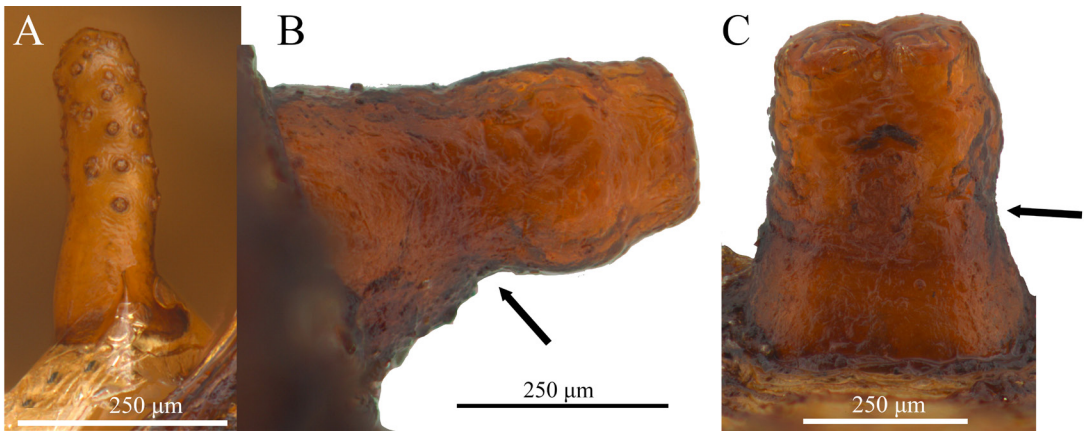

Fig. 11. Morphological variation of *Cheilosia vernalis* (Fallén, 1817) (CEUA-CIBIO). **A**. Pupal spiracle. **B**. Posterior respiratory process, lateral view. **C**. Posterior respiratory process, dorsal view. Arrow indicates the constriction.

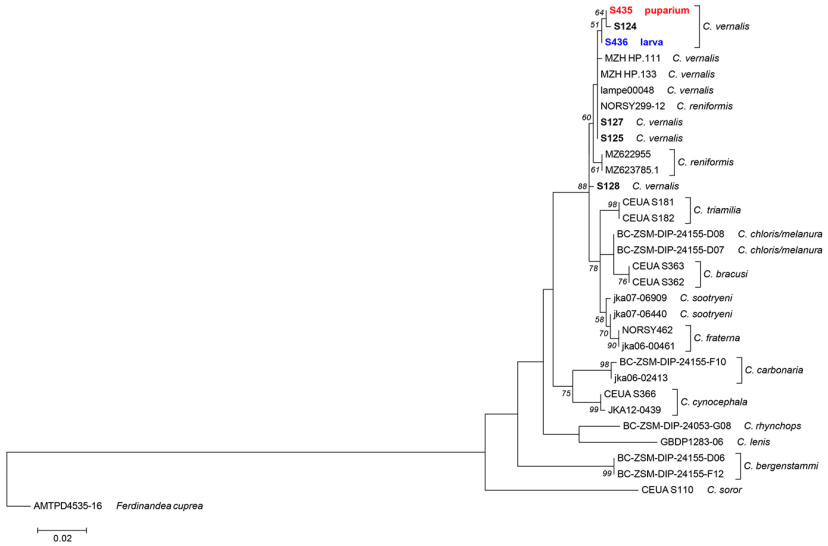

Fig. 12. Maximum Likelihood tree of *Cheilosia melanura* species group based on COI-5′. DNA vouchers of the specimens analysed in this work are highlighted in bold. Bootstrap values >50 are shown near nodes. Branch lengths are measured in the number of substitutions per site.

from different Spanish localities, we concluded that the puparium with a different morphology belonged to the clade of *C. vernalis*/*C. reniformis* (Fig. 12). As the presence of *C. reniformis* has not been confirmed in Denmark, but *C. vernalis* is widespread in this country, and given the morphological similarity between these two species, we conclude that the odd puparium belongs to the species *C. vernalis* too.

Additions to the description of Cheilosia canicularis (Panzer, 1801) in Rotheray (1990)

A picture of the L3 larva (Fig. 13), the puparium (Fig. 14), and the description of the pupal spiracles of *C. canicularis* are now provided for the first time. Pupal spiracles (Fig. 15). Length: 0.4–0.57 mm; width: 0.20–0.26 mm; space between the tips of the pupal spiracles: 2.33–2.92 mm (n = 2). Yellow/light-orange colour; cylindrical shape. Surface covered with tubercles organized in lines; tubercles with four to seven spiracular openings. In addition, a picture comparing the PRP of *C. canicularis* and *C. himantopa* is provided (Fig. 16).

Fig. 13. Third stage larva of *Cheilosia canicularis* (Panzer, 1801) (CEUA-CIBIO), lateral view.

Fig. 14. Puparium of *Cheilosia canicularis* (Panzer, 1801) (CEUA-CIBIO), dorsal view.

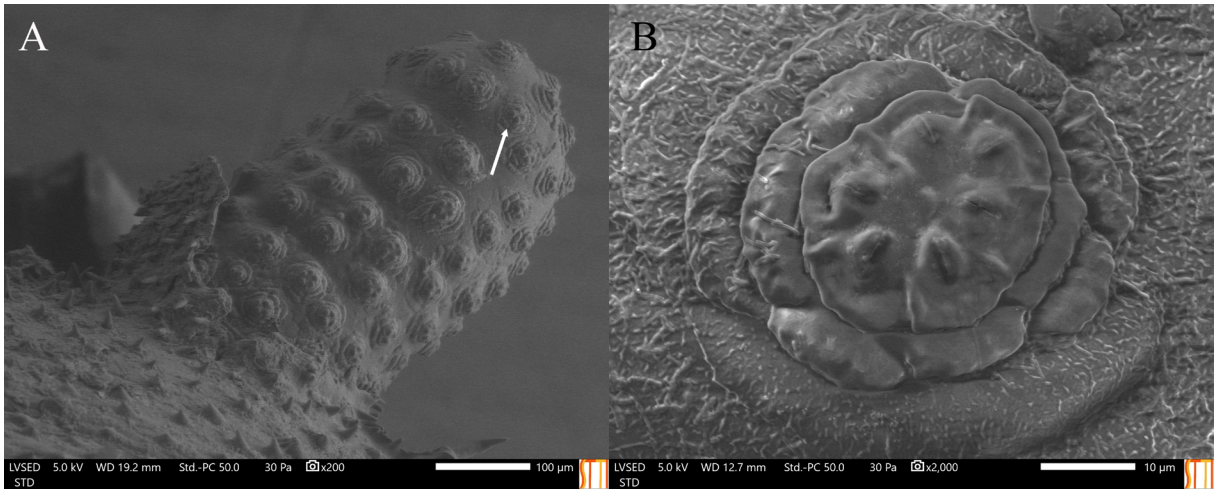

Fig. 15. Pupal spiracle of *Cheilosia canicularis* (Panzer, 1801) (CEUA-CIBIO). **A**. Posterior side. **B**. Close up of tubercle. Arrow indicates tubercle.

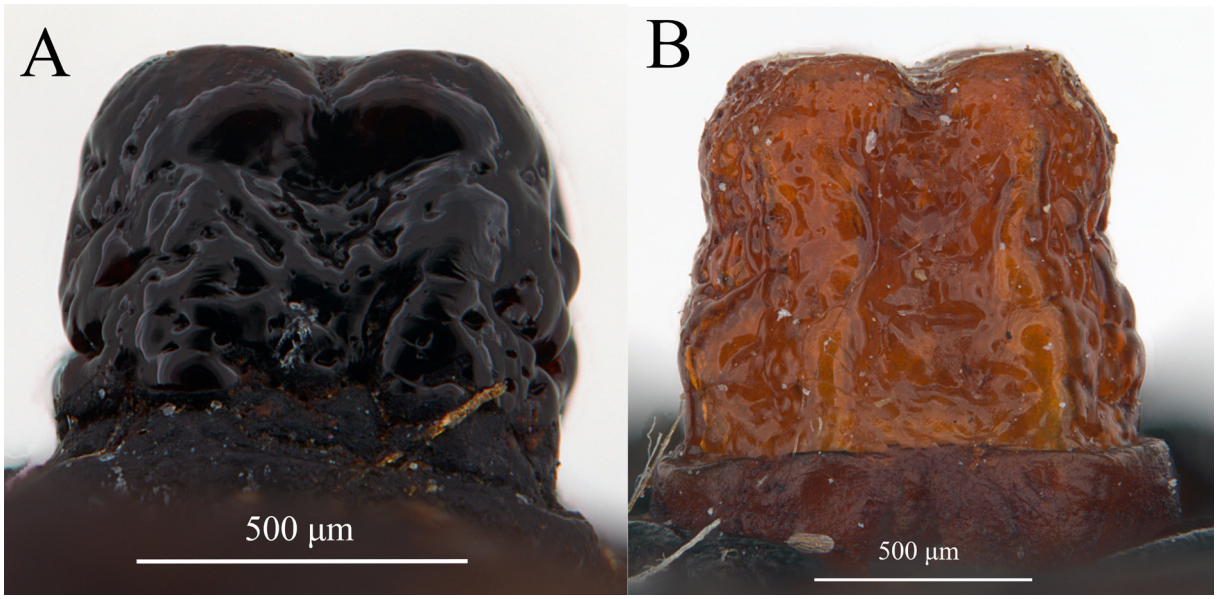

Fig. 16. Posterior respiratory process of *Cheilosia* Meigen, 1822 puparium. **A**. *Cheilosia canicularis* (Panzer, 1801) (CEUA-CIBIO), dorsal view. **B**. *Cheilosia himantopa* (Panzer, 1798) (CEUA-CIBIO), dorsal view.

Taxonomic key to the immature stages of the Cheilosia boring in roots and stems

A key to all known larvae/puparia of *Cheilosia* boring in roots and stems with a description is provided to facilitate identification of the species. This key was elaborated by the examination of *C. canicularis* and *C. vernalis* together with the descriptions by Smith (1979), Rotheray (1988, 1990, 1991, 1999), Brunel & Cadou (1990), Schmid (1999), Stuke (2000) and Stuke & Carstensen (2000, 2002). It must be considered that Stuke (2000) described *C. himantopa* as *C. canicularis*, and vice versa (see Discussion).

1. PRP with a projection in the middle of the spiracular plate (see Rotheray 1988: fig. 2) ..... ***C. albipila*** Meigen, 1838
– PRP without projection in the middle of the spiracular plate 2

2. PRP: spiracular plate with spiny projections in the margin 3
– PRP: spiracular plate without marginal spiny projections 5

3. PRP: spiracular plate with four pairs of spiny projections in the margin (see Rotheray 1991: fig. 10) ..... ***C. albitarsis*** Meigen, 1822
– PRP: spiracular plate with three pairs of spiny projections in the margin 4

4. Head skeleton: one large and two small pairs of mouth-hooks ..... ***C. variabilis*** Panzer, 1789
– Head skeleton: three to four pairs of similar size mouth-hooks ..... ***C. illustrata*** Harris, 1780

5. PRP: lateral margins conspicuously raised (see Rotheray 1988: fig. 9) ..... ***C. grossa*** Fallén, 1817
– PRP: lateral margins not conspicuously raised 6

6. PRP: spiracular plate with indistinct and variable form of spiracular openings (see Rotheray 1988: fig. 6) ..... ***C. fraterna*** Meigen, 1830
– PRP: spiracular plate with three or more spiracular openings 7

7. PRP: spiracular plate with three spiracular openings 8
– PRP: spiracular plate with four or more spiracular openings 11

8. PRP: spiracular plate with curved serrated spiracular openings ..... ***C. impressa*** Loew, 1840
– PRP: spiracular plate with curved non-serrated spiracular openings 9

9. Head skeleton: with three pairs of mouth-hooks ..... ***C. pubera*** (Zetterstedt, 1838)
– Head skeleton: with four pairs of mouth-hooks 10

10. Head skeleton: small pair on the inner margin of the first large hook (not visible laterally) ..... ***C. antiqua*** Meigen, 1822
– Head skeleton: all pairs of mouth-hooks on the same margin ..... ***C. latifrons*** (Zetterstedt, 1843)

11. PRP: spiracular plate with curved serrated spiracular openings ..... ***C. bergenstammi*** Becker, 1894
– PRP: spiracular plate without serrated spiracular openings 12

12. Head skeleton: with only one pair of mouth-hooks ..... ***C. proxima*** (Zetterstedt, 1843)
– Head skeleton: with three or four pairs of mouth-hooks 13

13. Head skeleton: four pairs of mouth-hooks 14
– Head skeleton: three pairs of mouth-hooks 16

14. PRP: all surface wrinkled ..... ***C. lasiopa*** Kowarz, 1885
– PRP: surface with some smooth parts 15

15. PRP: wrinkled surface at the base, then smooth apically (see Brunel & Cadou 1990: fig. 2e) ..... ***C. vulpina*** Meigen, 1822
– PRP: smooth surface at the base, then wrinkled at the rest ..... ***C. vernalis*** (Fallén, 1817)

16. PRP dorsal view: tapering from the base to the tip ..... ***C. himantopa*** (Panzer, 1798)
– PRP dorsal view: rectangular form..... ***C. canicularis*** (Panzer, 1801)

Discussion

According to Ståhls *et al*. (2004), the sister genus of *Cheilosia* is *Rhingia* Scopoli, 1763. The immature stages of both genera can be differentiated by the trophic habits or developmental site. Immature stages of *Cheilosia* can be found in the cambium, leaf, stem, or roots, while larvae of *Rhingia* are associated with to fresh cow dung or moist accumulation of rotting foliage (Speight 2020; Zhao *et al*. 2024). This characteristic must be taken with caution, since it is known that *Rhingia rostrata* (Linnaeus, 1758) lays eggs on the underside of leaf overhanging the dung of large mammals, and then when the larva hatches, it drops down to the dung (Grunin 1939; Speight 2020). Regarding the morphology, *Rhingia campestris* Meigen, 1822, is the only species of the genus with an immature stage described. Based on this description, the immature stages of *Cheilosia* and *Rhingia* can be distinguished by the shape of the lappets and papillae which are cone-shaped in *Cheilosia* but stick-like in *Rhingia* (Rotheray 1993; Rotheray & Gilbert 1999).

Morphology of the *Cheilosia* immature stages

According to Rotheray & Gilbert (1999), the principal difference between all the *Cheilosia* feeding mode divisions is in the head skeleton, specifically in the number of pairs of mouth-hooks. *Cheilosia canicularis* and *C. vernalis* belong to the group that bores in roots and stems, with three and four pairs of mouth-hooks in the head skeleton respectively. In comparison, the leaf-mining group (e.g., *Cheilosia fasciata* Schiner & Egger, 1853) has six pairs, the cambium group (e.g., *Cheilosia morio* Zetterstedt, 1838) one large pair, the mycophagous group (e.g., *Cheilosia longula* Zetterstedt, 1838) with one small pair and the saprophagous group (e.g., *Cheilosia pagana* (Meigen, 1822)) with small inconspicuous mouth-hooks.

The head skeleton of *C. vernalis* and *C. canicularis* is typical for phytophages since it presents large heavily sclerotized mouth-hooks (similar to a fang), that are used to eat through live plant tissue (Rotheray 2019). In the aphidophagous head skeleton, the labrum and labium present a sharp end that is used to pierce the integument of prey (Hartley 1963), in the saprophagous there are pharyngeal ridges that concentrate the food (Dowding 1967) and in the mycophagous the mandible and mandibular lobes are slightly sclerotized, and the mandibular apodeme is not fused with the mandibular lobes (Rotheray & Gilbert 1999). Therefore, this 'fang' allows us to distinguish the head skeleton of these species from the other regimes.

The morphology of the known immature stages of the root and stem-boring group is almost identical. The most useful morphological characteristics to separate species are the number of pairs of mouth-hooks of the head skeleton and the structure of the PRP. However, this must be taken with caution, since many species share the same number of pairs of mouth-hooks or the structure of the PRP is similar. For example, both *C. vernalis* and *C. vulpina* have four pairs of mouth-hooks, but they differ in the surface of the PRP. *Cheilosia canicularis* and *C. lasiopa* both have a PRP with wrinkled a surface, but they have different numbers of pairs of mouth-hooks. For this reason, these morphological characteristics must be taken as a whole and not individually.

The morphological variability of the puparium of *C. vernalis* (see Intraspecific variation within *Cheilosia vernalis* immature stages section) is in accordance with the work done on adults of *C. vernalis* by Ståhls *et al*. (2008). This is not the first time intraspecific variation of immature stages of syrphids has been observed in the pupal spiracles and the PRP (e.g., Orengo-Green *et al*. 2024). This variability makes it difficult to identify *C. vernalis* larva from other *Cheilosia* larva. On the other hand, the interspecific variability is low in terms of COI-5′ as well. The analysis of the barcodes revealed high similarities between *C. vernalis* and *C. reniformis* from a molecular point of view (Fig. 12). Likewise, Ståhls *et al*. (2008) reported no congruence between the observed morphological and molecular data. Similar studies

refer this high morphological variability to the Pleistocene glaciations and the subsequent speciation across Europe (Ståhls *et al*. 2008; Ballester-Torres *et al*. 2022). It might also be possible that larval feeding in these species is one of the triggers of the high variability of the corresponding adults. Larvae of *C. vernalis* can be found in different structures on a wide range of host plants (Stuke 2000; Speight 2020). This variability in larval feeding could be related to the morphological variability that the resulting adults exhibit (G. Ståhls pers. com.), as the same situation exactly occurs with species of the *Cheilosia longula* group, where larvae can be found in a wide variety of fungi species, and the adults also show high phenotypic diversity, particularly in *Cheilosia ruffipes* (Preyssler, 1793) (Clauβen & Ståhls 2007; Ballester-Torres *et al*. 2022). However, the variability found in this outlier specimen could simply be an isolated case of a possible malformation, since this larva was found on the same host plants as, and in a nearby location to, the rest of the specimens studied in this work. More pupae of *C. vernalis* are needed to discard this hypothesis definitively.

Integrative taxonomic studies should be the main tool to help to solve the taxonomic problem of the *C. vernalis* group. Studies including wing geometric morphometrics are proven to be very helpful to solve taxonomic difficulties in other hoverfly genera as *Merodon* Meigen, 1803 (Ačanski *et al*. 2023), as the results were concordant with the corresponding molecular studies. Other characters have also been used for geometric morphometrics, such as the surstylus shape of the male genitalia and the larval PRP (Ačanski *et al*. 2016; Radenković *et al*. 2018; Aracil *et al*. 2022), but they only work well when large numbers of larvae (PRP shape) or male adults (genitalia) from all species of the group are available: in contrast, wing geometric morphometrics only requires adults, regardless of sex.

Cheilosia canicularis* vs *Cheilosia himantopa

In Rotheray (1990), a description of the L3 of *C. canicularis* collected in former Yugoslavia in September, 1987 was made from reared adults in laboratory conditions. However, according to Stuke & Clauβen (2000), the larva described by Rotheray (1990) was not that of *C. canicularis*, but that of *Cheilosia himantopa* (Panzer, 1798). This assumption of Stuke & Clauβen (2000) was only based on the month when the described larva was collected, since *C. himantopa* larvae are more common in September than those of *C. canicularis*. Additionally, Stuke (2000) made an illustration of the PRP of *C. himantopa* (as *C. canicularis*) but did not make a description of the larva/puparium. However, in our work, we demonstrate that this supposition was an error since we reared larva of *C. canicularis* to make a correct identification and we found that the description of Rotheray (1990) matches our specimens. In addition, the drawing of the PRP of Rotheray (1990) and Stuke (2000) (see Stuke 2000: fig. 10, *C. canicularis* as *C. himantopa*) perfectly matches the PRP of our specimen. Martin Speight provided a puparium and an adult of *C. himantopa* to compare with our puparium of *C. canicularis*, and the conclusion was that we had the correct identification. Some works have already demonstrated that *C. canicularis* and *C. himantopa* are different species, using molecular markers (Milankov *et al*. 2005) or wing geometric morphometrics (Ludoški *et al*. 2008). The present paper represents further evidence that both taxa are actually distinct, even though they have a similar larval and adult biology and phenology (Stuke & Clauβen 2000). In view of the aforementioned error of Stuke we also remark how complicated it is to identify larvae and how important knowledge of the biology of the immature stages of syrphids is.

In this work, we present the first taxonomic key to the immature stages of root and stem-boring *Cheilosia*, and clarify the identity of the immature stages of *C. canicularis*/*C. himantopa* totally or partly described in literature. Additionally, the description of the immature stages of *C. vernalis* could contribute to a solution for the taxonomic problems in this and allied species of the *melanura* group, i.e., similarity between *C. vernalis*/*C. reniformis* and morphological variability of *C. vernalis*. Further efforts are needed since there are still many immature stages of *Cheilosia* yet to be described, and with each description made, our understanding of the morphology and biology of this genus increases.

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Supplementary file

Supp. file 1. List of barcode sequences. https://doi.org/10.5852/ejt.2025.1004.2979.13417