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Source: Florida Entomologist, 97(2) : 540-548

Published By: Florida Entomological Society

URL: <https://doi.org/10.1653/024.097.0227>

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## DESCRIPTION OF THE MATURE LARVA OF *SYNERGUS FILICORNIS* (HYMENOPTERA: CYNIPIDAE: SYNERGINI), WITH NOTES ON THE IMMATURE LARVAE

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Supplementary material for this article in Florida Entomologist 97(2) (June 2014) is online at <http://purl.fcla.edu/fcla/entomologist/browse>

### ABSTRACT

The final instar larva of *Synergus filicornis* (Cameron) (Hymenoptera: Cynipidae: Synergini) is described and illustrated. Morphological structures of a diagnostic value are discussed. The most remarkable character states shown by the mature larva of this species lie in the integument, which unlike the rest of described cynipoid mature larvae, presents tegumental differentiations. Additionally the antennal orbits, unlike those of other described *Synergini* mature larvae, did not present one seta on each antennal orbit. The immature larvae can be differentiated from the mature larva by the following characters: a) number and location of sensorial tegumental differentiations on the head, b) number of sensilla on the maxillary palpi, and c) morphology of the mandibles. The determination of the mandibles size, in this species, permits the estimation of the larval stages number.

Key Words: Cynipoidea, larval phase, morphology, phylogeny

### RESUMEN

Se describe la larva madura de *Synergus filicornis* (Cameron) (Hymenoptera: Cynipidae: Synergini). El carácter diagnóstico básico más relevante, mostrado por este último estado larvario, radica en que el tegumento, a diferencia del resto de larvas maduras descritas de Cynipoidea, presenta diferenciaciones. Adicionalmente, las órbitas antenales, a diferencia de otras larvas maduras descritas de Synergini, no presentan una seta en cada órbita antenal. Las larvas inmaduras se diferencian de las larvas maduras en los siguientes estados de carácter: a) número y situación de las diferenciaciones tegumentarias sensoriales de la cabeza, b) número de las sensilas de los palpos maxilares y c) morfología de las mandíbulas. El tamaño de las mandíbulas permite, en esta especie de inquilino, estimar el número de estados larvarios.

Palabras Clave: Cynipoidea, fase larvaria, morfología, filogenia

Gallwasps (Cynipidae) are divided into 2 main trophic groups: the gall inducers and the gall-associated inquilines, which together make up 8 tribes (Liljeblad et al. 2011; Pujade-Villar 2013). This classification reflects differences in the bi-

ology and host plant association in combination with some morphological features.

This study addresses the larval morphology of a species belonging to the tribe Synergini, *Synergus filicornis* (Cameron) (= *Synergus furnessana*,

Weld). The insects had been reared from galls of *Andricus quercuslaurinus* Melika & Pujade-Villar (Cynipidae: Cynipini) recently described from Mexico as a serious pest on *Quercus laurina* Humb. & Bonpl. (Fagales: Fagaceae) in the Hidalgo state (Melika et al. 2009; Pujade-Villar et al. 2012; Pujade-Villar 2013) and the presence of the inquiline, *S. filicornis*, increases the problem in the areas devastated by *A. quercuslaurinus* (Cebrian, unpublished data). *Synergus filicornis* was described from Guatemala by Cameron (1883); later Weld (1913) described a new species from Mexico (*S. furnessana*) and that later was synonymised to *S. filicornis* (Weld 1930). In Mexico this species develops in woody galls induce by *Callirhytis furnessae* 'sensu Weld' and *A. quercuslaurinus*.

Regarding the relevant biological features of *Synergus filicornis*, this species does not seem a lethal inquiline. However, this possibility cannot be completely ruled out, because although there is no evidence that the inquiline larva kills the inducer (*Andricus quercuslaurina*), it is possible that on laying their eggs- the first females of *S. filicornis*, which oviposit after the gall is induced, could kill the egg of the inducer. It is also possible that the first instar inquiline larva might emerge from the egg before the inducer and kill it. This situation cannot occur later, since *Andricus quercuslaurina* is never found at the ends of the galls. The tribe Synergini, includes *Synergus* Hartig genus with 109 known species worldwide (Penzés et al. 2012). Previous to this study descriptions of the pre-imaginal stages of only 3 *Synergus* species were carried out: the Nearctic *Synergus pacificus* McCracken and Egbert by Evans (1965), and the mature larvae of the Western Palaearctic *Synergus incrassatus* Hartig and *S. clandestinus* Eady by Nieves-Aldrey et al. (2005). The aims of this study are to describe the final instar larva of *Synergus filicornis*, to increase knowledge of this immature stage of inquilines, and to compare its morphology with that of other 3 known species.

Figs. 1, 3 and 5 are shown in color online in supplementary material in the Florida Entomologist 97(2) (June 2014) at <http://purl.fcla.edu/fcla/entomologist/browse>.

## MATERIALS AND METHODS

Pre-imaginal stages of *Synergus filicornis* were obtained from the ends of the galls of *Andricus quercuslaurina*, collected from branches of *Quercus laurina* in Mexico (State of Hidalgo, Acaxochitlán township, La Victoria, [N 20° 10'16" and W 98° 11'25"], 2,120 m asl.). In this respect, it should be noted that the inquiline, *S. filicornis*, deposits its eggs on the developing gall of the host, *A. quercuslaurina*, and therefore the gall grows at the end points, at which new chambers form in which the inquiline develops alone. Only this kind of inquiline comes from this type of gall.

A total of 52 mature larvae and 67 immature larvae were cut out of galls on 25 Apr 2008 and preserved in 70% ethanol. Descriptions below, however, are based on a thorough study of several specimens only.

The length of the mandibles is defined as the maximum length measured from the head's articulation point to the tip of the basal tooth. For light microscopic preparation of larval stages, the methods described by Tormos et al. (2003, 2004, 2007, 2009a,b) were employed. Photos and measurements to the nearest 0.01 mm of different structures of the pre-imaginal stages were taken under a Leica MI65C microscope, equipped with a Leica EC3 camera using the Application Suite Version 3.6.0 (Imagine software Integrates, Leica Microsystems Imaging Solutions) (Instituto Valenciano de Investigaciones Agrarias, Valencia, Spain). The sketches were made with a drawing tube and use of the Adobe Illustrator CS5 application to create and manipulate the vector drawings. For scanning electron microscopy, the live samples were frozen in slush N<sub>2</sub> and attached to the specimen holder of a CT-1000C Cryo-transfer system (Oxford Instruments, Oxford, United Kingdom) interfaced with a JEOL JSM-5410 scanning electron microscope (SEM) (Universidad Politécnica de Valencia, Valencia, Spain). The samples were then transferred from cryostage to the microscope sample stage, where the condensed surface water was sublimed by controlled warming to -90° C. Then, the samples were transferred again to the cryostage and were sputter-coated with gold. Finally the samples were returned to the microscope sample stages for visualization at an accelerating voltage of 15 KeV. Images were also taken by using Oxford Instruments.

In the descriptions we essentially employed the terminology and organization used by Nieves-Aldrey et al. (2005) and Tormos et al. (2009a, b). In all cases the term "seta" is used in the meaning of setigerous sensilla. Data are presented as range and mean values  $\pm$  standard deviation (SD). In order to determine the possible underlying implicit grouping in the larval instars, with respect to mandible length, a cluster analysis was performed by using the hierarchical clustering method. The statistical analysis was performed using the SPSS (v15.0) package. Voucher specimens are deposited at the Fundación Entomológica "Torres-Sala" (Valencia, Spain).

## RESULTS

### Description of the Mature Larvae

General aspect (Fig. 1). Body (l = 1.65- 2.23 mm ( $\bar{x} \pm SD = 1.98 \pm 0.07$ ,  $n = 35$ ), maximum w = 0.5-1.4 mm ( $\bar{x} \pm SD = 0.95 \pm 0.17$ ,  $n = 29$ ) with a relatively large head, sub-cylindric, ventrally curved (Fig. 1a), slightly broader in the mid re-

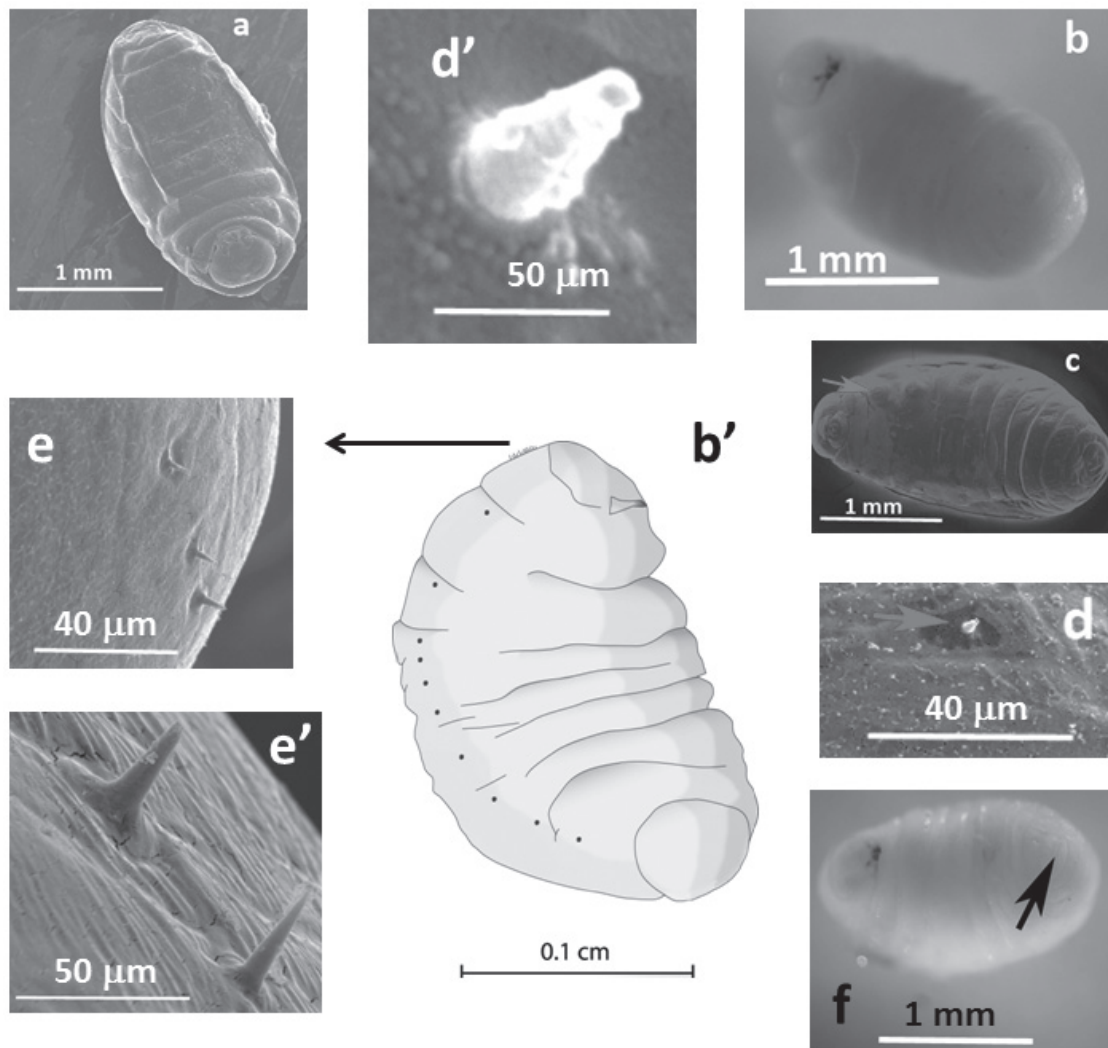


Fig. 1. *Synergus flicicornis*: mature larva: 1. General aspect showing the hymenopteriform morphology, with a detail of the ventrally curved body [1a (under SEM), the body is broadest around the middle and tapers gradually to the end], segmentation (1b') and colour (1b), pleural lobes [1c (under SEM), arrow], tegumental differentiations [1d (under SEM), 1d' (under SEM) (detail), 1e (under SEM) (on dorsum of prothorax), 1e' (under SEM) (on dorsum of prothorax, detail), and anal segment (1f, arrow)].

gion, with distinct segmentation, with the posterior segments relatively shorter than the anterior and the anal segment frequently coarcted. Color yellowish, to cream (Fig. 1b). Weakly sclerotized. Pleural lobes developed (Fig. 1c). Integument with tegumental differentiations (Figs. 1d,d',e,e'), some on dorsum of prothorax (Fig. 1e,e'). Anal segment (Fig. 1f) with a transverse slit.

Spiracles (Fig. 2) on mesothorax, metathorax (Fig. 2a) and on first 8 abdominal segments (Fig. 2b); atrium ( $l = 82 \mu\text{m}$ ,  $d$  maximum =  $37 \mu\text{m}$ ) simple, with peritreme (Fig. 2c) and asperities well differentiated; closing apparatus adjacent to atrium ( $l = 52 \mu\text{m}$ ;  $w = 22 \mu\text{m}$ ).

Cranium (Fig. 3) ( $w = 730 \mu\text{m}$ ,  $h$  (from apex of cranium to base of mandibles) =  $530 \mu\text{m}$ ) wider than high, narrower than the first thoracic segment (Fig. 1a), very weakly indented dorsally along its middle line (Fig. 3a), very weakly sclerotized except for apical half of mandibles (Fig. 3b). Parietal bands absent. Antennal orbits circular with 2 sensilla at the centre (Fig. 3c, e). It has one setae on each side lateral to antennal orbits (Fig. 3e, a), and one pair on the clypeus (Fig. 3c, e), 2 pairs on the labrum (Fig. 3c, e), and one on each gena (Fig. 3c, e). Epistoma complete. Labrum semicircular not covering the apices of the mandibles (Fig. 3a). Mandibles tridentate

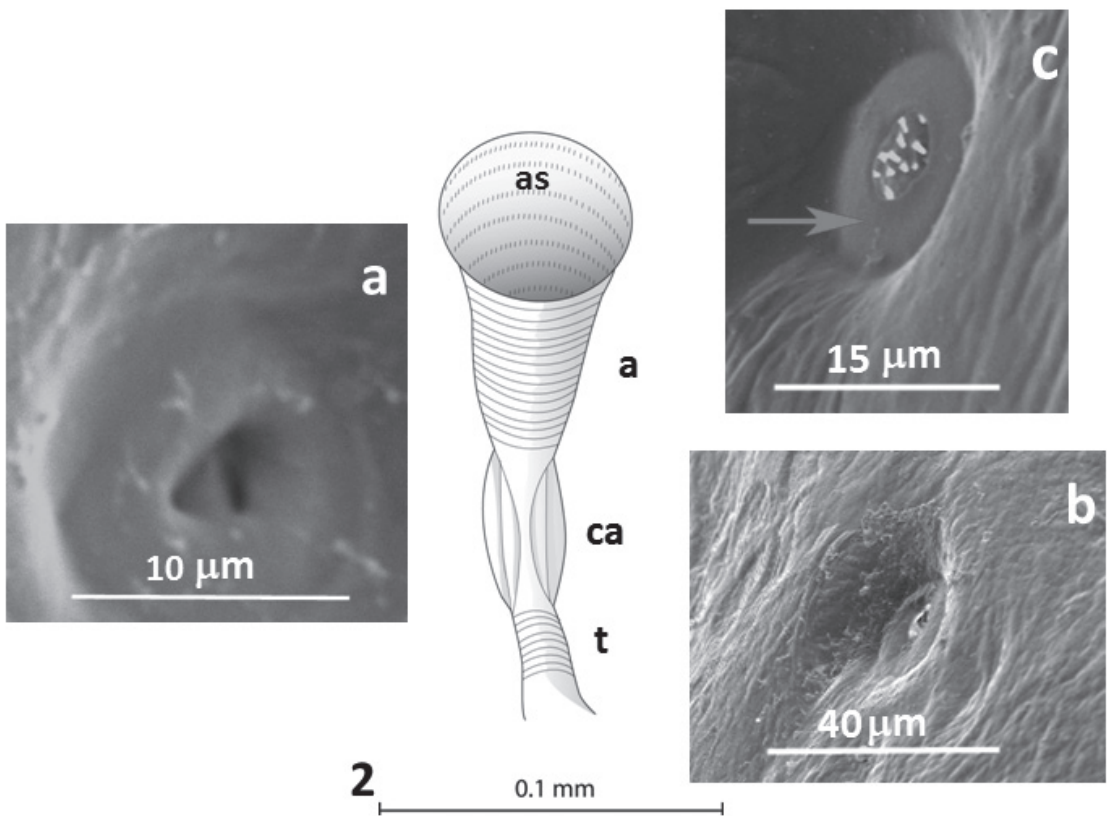


Fig. 2. *Synergus filicornis*: mature larva: Spiracle showing the atrium (a) with asperities (as), closing apparatus (ca), and beginning of the trachea (t), with detail of mesothoracic [2c (under SEM), peritreme –see arrow–], meta-thoracic (2a, under SEM) and abdominal [2b (under SEM), first segment] spiracles.

(Fig. 3b) [ $l = 120\text{--}190\ \mu\text{m}$  ( $\bar{x} \pm \text{SD} = 157.16 \pm 26.45$ ,  $n = 18$ )], with the second mandibular tooth broad and with sculpture on its base (Fig. 3c). Maxillae with 2 setae on external side (Fig. 3, 3c). Maxillary palpus slightly protruding with 3 sensilla at apex, one of them larger (Fig. 3, 3c). Labium with 4 setae (Fig. 3c), 2 behind palpi and 2 front palpi; labial palpus with 2 sensilla at apex (Fig. 3); salivary orifice small and rounded (Fig. 3c).

#### Description of the Immature Larvae

Numerous larvae were obtained with size [ $l = 1.25\text{--}2.18\ \text{mm}$  ( $\bar{x} \pm \text{SD} = 1.67 \pm 0.11$ ,  $n = 58$ ), maximum  $w = 0.46\text{--}1.25\ \text{mm}$  ( $\bar{x} \pm \text{SD} = 0.88 \pm 0.22$ ,  $n = 51$ )] and with a morphology similar to the mature larvae: general aspect fusiform (Fig. 4a); integument with tegumental differentiations (Fig. 4b); spiracles with peritreme, asperities differentiated and closing apparatus adjacent to atrium (Fig. 5c), and head wider than high (Fig. 5d), narrower than the first thoracic segment, very weakly indented dorsally along its middle line, very weakly sclerotized except for apical half of mandibles, with pa-

rietal bands absent, and antennal orbits circular with 2 sensilla at the centre, and labial palpi with 2 sensilla. Nevertheless, the immature larvae are distinguished from the mature larva in the following character states: a) number and situation of sensorial tegumental differentiations of the head, b) maxillary palpi with 2 sensilla, and c) mandibles with different morphology. In this respect, in the meconium 3 different mandibular models were identified (Fig. 8): a) one first model [ $l = 21\text{--}28\ \mu\text{m}$  ( $\bar{x} \pm \text{SD} = 25.52 \pm 2.20$ ,  $n = 22$ )] with only one tooth and slightly sclerotized, b) one second model [ $l = 33\text{--}41\ \mu\text{m}$  ( $\bar{x} \pm \text{SD} = 32.96 \pm 1.62$ ,  $n = 27$ )] with a well differentiated tooth and others lightly outlined and well sclerotized, and c) a third model [ $l = 61\text{--}66\ \mu\text{m}$  ( $\bar{x} \pm \text{SD} = 63.35 \pm 1.72$ ,  $n = 31$ )] with 3 well developed and well sclerotized teeth (Figures 4d y 5c' show this latter model in an immature larva). These 3 models could correspond with different larval instars. The cluster analysis (Fig. 6) carried out using the variable for mandible-length of the immature and mature larvae revealed a perfect delimitation of fourth groups, probably corresponding to larval instars present in the larval

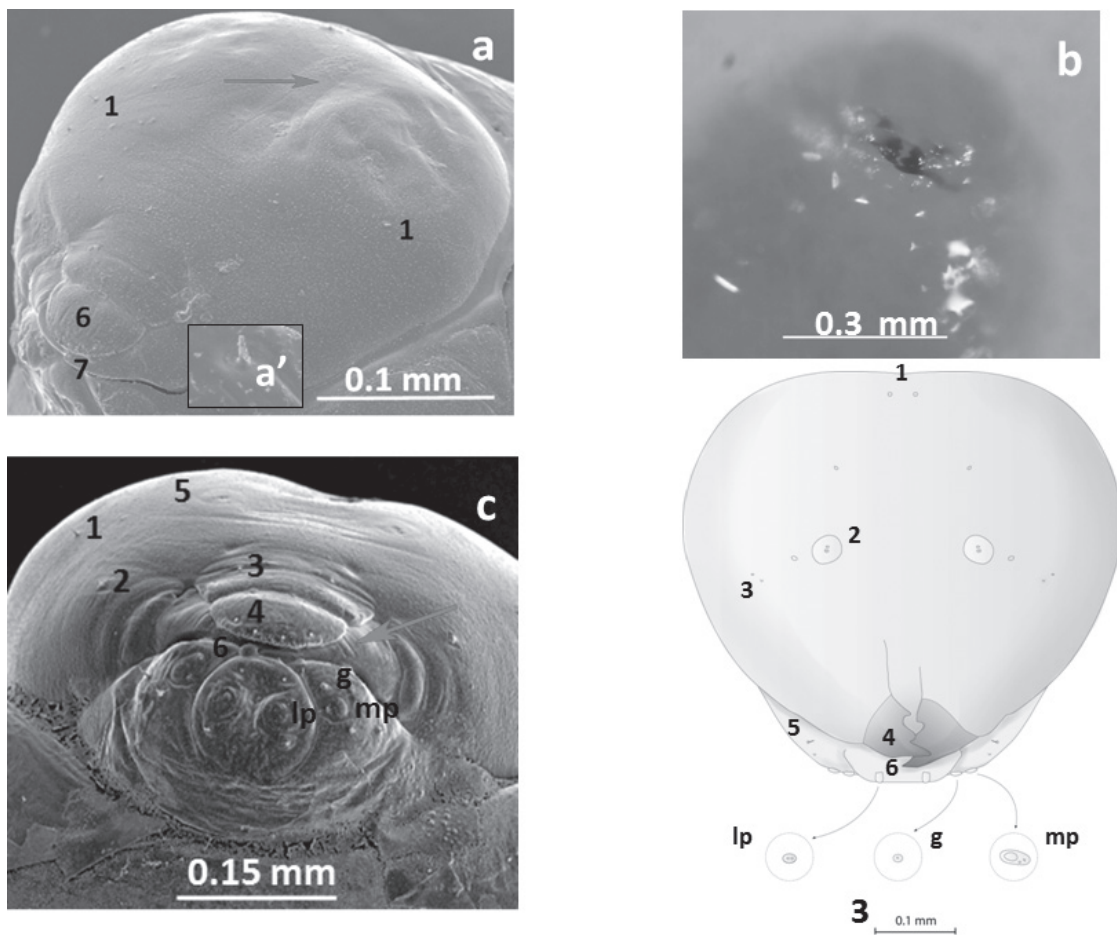


Fig. 3. *Synergus filicornis*: mature larva: Frontal view of the head with the mouthparts with detail of the vertex slightly incised [3 (1), 3a (under SEM), see arrow], antennal orbits [3 (2), 3c (under SEM): 5], sensorial structures: setae [3a, c (under SEM): 1, 3 (3), 3c (under SEM): 2,3,4; 3a' showed a detail of a seta of the labrum], labrum [3a (under SEM): 6], apex of the mandible [3 (4), 3a (under SEM): 7, 3b] and striation on its base [3a (under SEM), see arrow], maxillae [3 (5), 3c (under SEM) showed galea (g), maxillary palpi (mp), setae on external part (ssp) and apex of maxilla (6)], and labium [3 (6), 3c (under SEM) showed labial palpi; 3c show setae behind palpi (sbp)].

phase of *Synergus filicornis*. Therefore, the length and morphology of the mandibles of the larval stage allows the supposition of the existence in *S. filicornis* of at least 4 larval instars.

#### DISCUSSION

The mature larva of *Synergus filicornis* is similar in general appearance to other Cynipoidea (Evans 1987; Nieves-Aldrey et al. 2005). The general aspect is hymenopteriform, with 13 segments and yellowish color. Nevertheless, the integument, unlike the rest of described cynipoid mature larvae, presents tegumental differentiations. It shares a number of character states with the mature larvae of the inquiline cynipids

(tribe Synergini) (Nieves-Aldrey et al. 2005; see Table 1), among which we highlight the following: 1) body generally ventrally curved, fusiform, 2) head quite large relative to the body, 3) vertex generally incised, 4) antennal area small and inconspicuous, 5) maxillae triangular, 6) sensorial tegumental differentiations on: a) each side lateral to the antennal area (one), b) clypeus (1 pair), b) labrum (2 pairs), c) each gena (one), d) each maxilla (2) and e) each side of the labium (2); 7) maxillary and labial palpi large, conspicuous, and more or less protruding, 8) mandible tridentate with a broad second tooth, and with striations on the base. Additionally, in the *Synergus* species the labrum does not cover at all the apices of the mandibles. Nevertheless, unlike the rest of mature larvae of Synergini described, *Synergus filicornis*

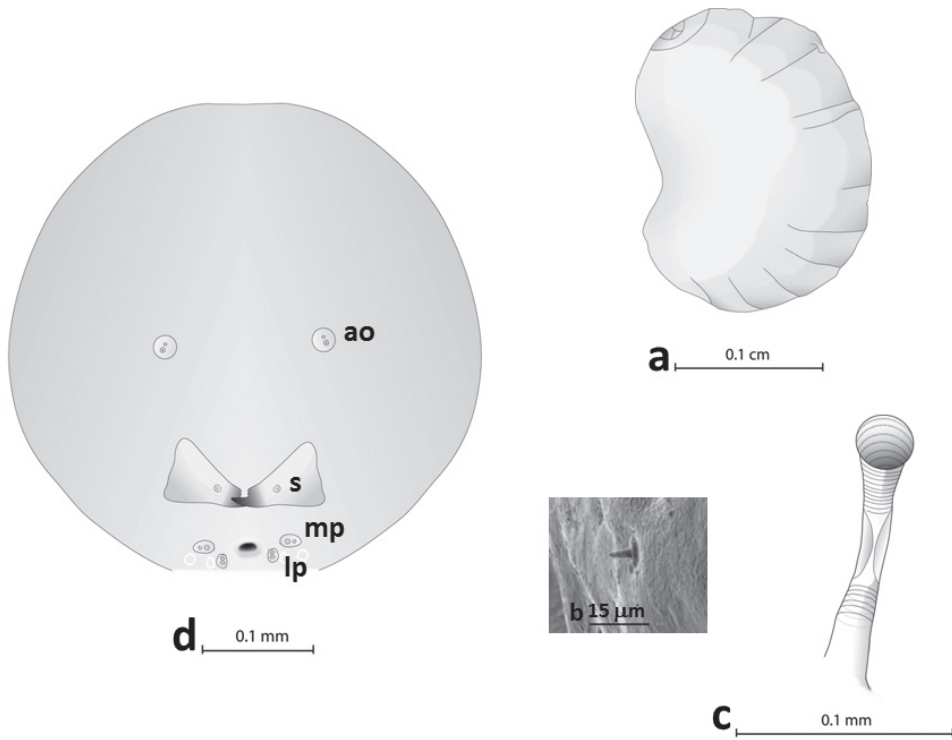


Fig. 4. *Synergus filicornis*: immature larva: General aspect (a), tegumental differentiation (b, under SEM), spiracle (c), and head (d) [antennal orbits (ao), maxillary (mp) and labial palpi (lp), sensillum (s)].

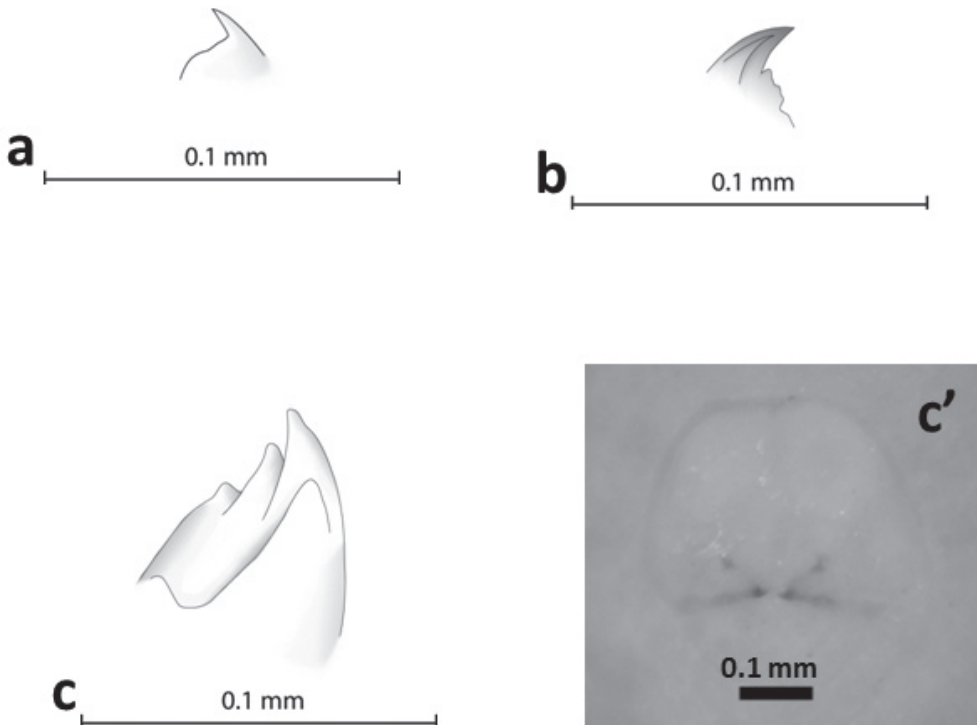


Fig. 5. *Synergus filicornis*: Mandibles with different morphology present in the meconium (a-c). Head of immature larva with detail of mandibles (c').

TABLE 1. MORPHOLOGICAL CHARACTERS OF SYNERGINI LARVAE WITH PHYLOGENETIC VALUE (NIEVES-ALDREY ET AL., 2005). THE CHARACTER STATES PRESENTED BY SYNERGINI *GUS FILICORNIS* ARE INCLUDED<sup>1</sup>.

Species	Characters																														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
<i>P. brandtii</i>	0	0	0	0	0	1	1	1	1	0	0	1	1	1	1	0	0	1	0	0	1	0	0	1	1	0	0	1	1	0	1
<i>S. politus</i>	1	0	0	1	0	0	1	1	0	0	1	0	1	0	1	1	0	0	1	0	0	0	0	1	1	0	0	1	1	0	1
<i>S. claudestinus</i>	0	0	0	0	0	1	1	1	1	0	0	1	0	1	1	1	0	0	0	0	0	0	1	0	1	0	0	0	0	1	1
<i>S. incrassatus</i>	0	0	0	0	0	1	1	1	1	0	0	1	0	1	1	1	0	0	0	1	0	0	1	0	0	1	0	0	0	1	1
<i>S. filicornis</i>	0	0	0	0	0	1	1	0	1	0	0	1	0	1	1	1	0	0	0	0	0	0	0	0	1	0	0	0	0	1	1

<sup>1</sup>**Larval body:** 1. Gradually tapering towards the posterior end (0) (Fig. 1a,b, b', c)/abruptly tapering towards posterior end (1). 2. With a fusiform appearance (0) (Fig. 1a, b', c)/with other appearances. 3. Fourth body segment (ventral view) is: not narrower than the 5th segment (0) (Fig. 1a)/clearly narrower than the 5th segment. 4. Head width (ventral view): clearly more than half the width of the first body segment (0) (Fig. 1a, b)/less than half the width of the first body segment (1). 5. Anterior and posterior margins of ventral part of first body segment: are more or less parallel, side forming an even edge posterior to the head or converge slightly to a thinner ventral area (0) (Fig. 1a)/are almost touching at the ventralmost point.

**Head region:** 6. Vertex: rounded (0)/incised medially (1) [Fig. 3 (1), a, cl]. 7. Antennal area: large and conspicuous/small and inconspicuous (1) [Fig. 3 (2), c (5)]. 8. Antennal seta: absent/present (1). 9. Lateral seta of antennal area: absent/present (1) [Fig. 3a (1), c (1)]. 10. Parietal bands: absent (0)/present. 11. Transversal crest on upper frons: absent (0)/present.

**Cephalic sclerites:** 12. Clypeal seta: present (0) [Fig. 3c (3)]/absent. 13. Lateral margins of labrum: parallel or subparallel/converging towards the basal end (1) [Fig. 3a (6)]. 14. Apical margin of labrum: straight (0) [Fig. 3a (6)]/other morphology (1)/15. Ventrolateral seta of labrum: absent/present (1) [Fig. 3c (4)]. 16. Medioapical seta of labrum: absent/present (1) [Fig. 3c (4)]. 17. Mandible: more or less covered by labrum (0)/exposed (1) (Fig. 3b). 18.

**Mouthparts:** apex of maxilla: pointed (0) [Fig. 3c (6)]/rounded. 19. Apical half of maxilla: not marked, evenly sloping inwards (0) (Fig. 3c)/markedly bulging outwards before curving inwards (1). 20. Maxillary palpi: conspicuous (0) [Fig. 3 (mp), c (mp)]/absent or inconspicuous. 21. Area surrounding salivary opening: smooth (0) (Fig. 3c)/with tuberculate sculpture (1). 22. Labial sclerite: well defined (0) [Fig. 3 (6), cl]/not marked (0). 23. Labial palpi: clearly present (0) [Fig. 3 (6), c]/absent or inconspicuous (1). 24. Left and right mandibles: more or less symmetrical (0) (Fig. 3b)/strongly asymmetrical (1). 25. Structure of mandibular incisor: sharp, thin and slender/strong and blunt (1) (Fig. 3b). 26. Number of teeth on left mandible: 3 or more (0) (Fig. 3b)/less than three. 27. Structure of apical tooth: straight, even inner margin (0) (Fig. 3b)/serrated inner margin. 28. Second tooth of right mandible: reaching at least to the middle of the apical tooth (0) (Fig. 3b)/clearly shorter than half the length of the apical tooth (1). 29. Shape of apex of second tooth of the right mandible/rounded (0) (Fig. 3b)/other morphology (1). 30. Deep and wide gap separating the second and the third tooth: absent (0)/present (1) (Fig. 3b). 31. Surface of mandible: more or less smooth/sculptured at the base of the teeth with strong longitudinal and/or transversal striations (1) (Fig. 3c, see arrow).



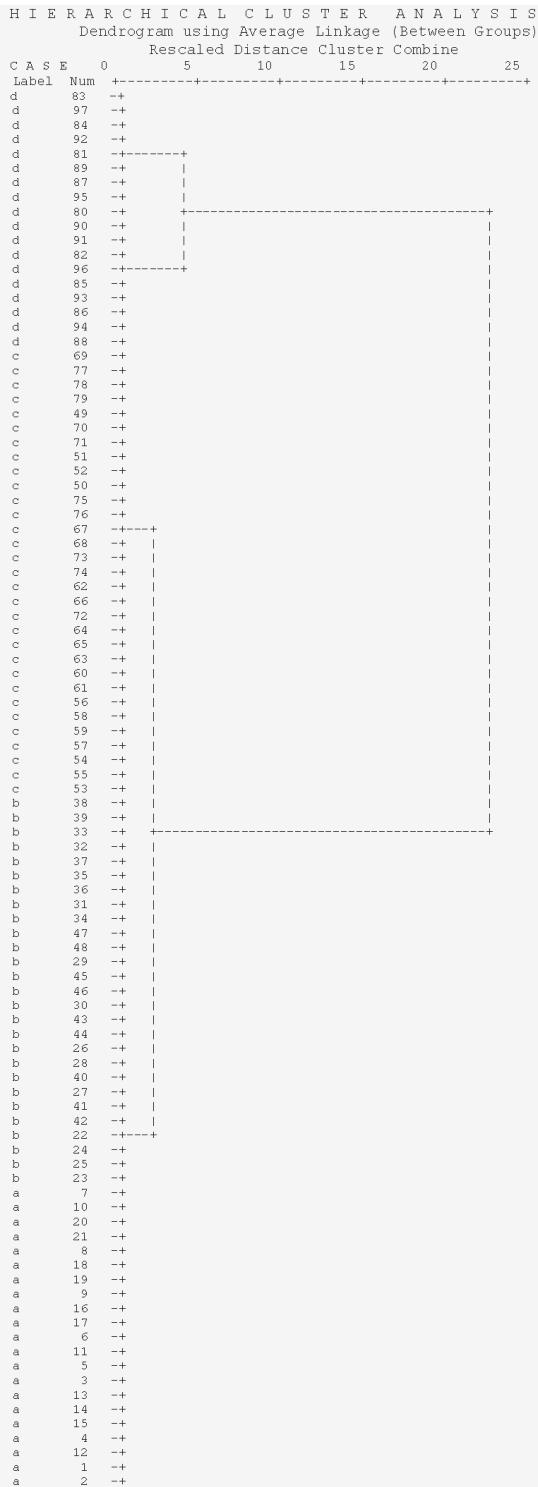


Fig. 6. *Synergus filicornis*: Dendrogram from the cluster analysis using the mandible length as clustering variable (a, b, c, d correspond to the probable larval instars).

does not present 1 seta on each antennal area. The lack of detail in the description of *S. pacificus* McCracken and Egbert (Evans 1965) does not allow for a in depth comparative analysis, and thus we can only state that this species shares with *Synergus filicornis* all character states mentioned in its description.

*Synergus filicornis* presents the 2 autapomorphies that allow characterizing the mature larvae of the tribe Synergini (Nieves-Aldrey et al. 2005): a) second mandibular tooth broader and blunter than incisor, and b) presence of the sculpture on the base of the mandible.

The immature larvae are similar to those already described for *Synergus pacificus* McCracken and Egbert (Evans 1965). In *Synergus filicornis*, different mandibles lengths corresponds to different larval instars, which confirms that so far, as happens in parasitoids (Onagbola & Fadamiro 2008; Tormos et al. 2009a, b) this variable can also be used to corroborate the number of states of the larval phase, in certain inquilines. Unlike of other species of Cynipidae (Viggiani & Nugnes 2010), in the different larval instars of this species were not observed mandibular asymmetry nor variation in number and position of the spiracles.

With this description the authors concur with the statement by Nieves-Aldrey et al. (2005): "Undoubtedly, further study of immature stages of Cynipoidea will be rewarding from the perspectives of both phylogeny and larval identification. In particular, there is an urgent need to study a better sample of the larval phase of the insect parasitic cynipoids. Even for the phytophagous gall-inducers and inquilines, larvae of many important taxa remain to be described in detail".

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