



Description of larval instars of *Dryinus tarraconensis* Marshall, 1868 and *Gonatopus baeticus* (Ceballos, 1927) (Hymenoptera: Chrysidoidea: Dryinidae), parasitoids of the genus *Dictyophara* Germar (Hemiptera: Auchenorrhyncha: Dictyopharidae)

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Abstract

The last immature stage and the mature larva of *Dryinus tarraconensis* Marshall, 1868 and *Gonatopus baeticus* (Ceballos, 1927) are described for the first time. Features of immature and mature larvae of both species are discussed in the context of their implications for characterizing the subfamilies Dryininae and Gonatopodinae.

Key words: Dryininae, Gonatopodinae, Fulgoromorpha, postembryonic development, morphology

Introduction

Dryinidae are distributed worldwide with approximately 1800 species belonging to 15 subfamilies (Olmi & Virla, 2014; Olmi *et al.*, 2014).

Within Dryinidae, Gonatopodinae (556 species) and Dryininae (342 species) after the Anteoninae (599 species) are the largest subfamilies (Olmi & Virla, 2014). Gonatopodinae include 11 genera, all extant (*Adryinus* Olmi, 1984, *Echthrodelphax* Perkins, 1903, *Epigonatopus* Perkins, 1905, *Esagonatopus* Olmi, 1984, *Eucamptonyx* Perkins, 1907, *Gonatopus* Ljungh, 1810, *Gynochelys* Brues, 1906, *Haplogonatopus* Perkins, 1905, *Neodryinus* Perkins, 1905, *Pareucamptonyx* Olmi, 1991, *Pentagonatopus* Olmi, 1984). Only two fossil species are known, tentatively attributed to *Gonatopus* and *Neodryinus*, respectively. *Gonatopus cretacicus* Olmi, Rasnitsyn & Guglielmino, 2010, was described from Siberia, Magadan Region, Obeschchayushchiy, in marl deposits from Upper Cretaceous (Cenomanian) (90–95 Ma) and *Neodryinus somniatus* Brues, 1933, was described from Baltic amber (40–45 Ma). Dryininae include at present five genera (*Thaumatomydryinus* Perkins, 1905, *Dryinus* Latreille, 1804, *Megadryinus* Richards, 1953, *Gonadryinus* Olmi, 1991 and *Pseudodryinus* Olmi, 1991) with fossil and extant species, and four fossil genera (*Palaeodryinus* Olmi & Bechly, 2001, *Harpactosphecion* Haupt, 1944, *Hybristodryinus* Engel, 2005 and *Cretodryinus* Ponomarenko, 1975). The fossil Dryininae are known from amber and the oldest ones, *Cretodryinus zherichini* Ponomarenko, 1975 and *Dryinus antiquus* (Ponomarenko, 1981), are attributed to the Cretaceous (Taimyr amber (Siberia): 78–115 Ma) (Olmi *et al.*, 2010).

Dryinidae are parasitoids and predators of Hemiptera Auchenorrhyncha (Guglielmino & Olmi, 1997, 2006, 2007; Guglielmino *et al.*, 2013). Their postembryonic development and biology are still poorly known, and the knowledge on the morphology of the immature and mature larvae is very scarce as well. In respect to the last topic, the major number of data regard three genera of Gonatopodinae: *Gonatopus*, *Haplogonatopus* and *Neodryinus* (for a review until 1998 see Guglielmino & Virla, 1998; further data are published in Guglielmino & Bückle, 2003; Guglielmino *et al.*, 2006; Mangione & Virla, 2004; Virla & Mangione, 2000). In Dryininae, the only biological data regard the genus *Dryinus*: *D. collaris* (Linnaeus, 1767) (Haupt 1932, as *D. (Lestodryinus) formicarius*

Latreille, 1805), *D. tarraconensis* Marshall, 1868 (Pillault 1951), *D. pyrrillae* (Kieffer, 1911) (Subba Rao 1957, as *Lestodryinus pyrrillae* Kieffer), *D. poecilopterae* (Richards, 1947) (Abril Ramírez 1992, as *Tridryinus poecilopterae* (Richards)), *D. stantoni* Ashmead, 1904 (Yang 1994, as *D. lycormae* Yang), and *D. latus* Olmi, 1984 (Yan *et al.* 2010). Information on larval morphology and anatomy is also very limited. The only, unfortunately very incomplete description of immature and mature larvae of Dryininae concerns *D. poecilopterae* (Abril Ramírez 1992, as *Tridryinus poecilopterae* (Richards)).

In this paper we present detailed descriptions of the external morphology of the immature and mature larvae of *Dryinus tarraconensis* Marshall, 1868, and *Gonatopus baeticus* (Ceballos, 1927) with the aim to provide larval characters with relevance in further studies on the relationships between different Dryinidae subfamilies and genera.

Material and methods

Dryinus tarraconensis and *Gonatopus baeticus* larvae were obtained from parasitized nymphs and adults of *Dictyophara pannonica* (Germar, 1830) collected in Hungary (Kiskunság National Park, Bugac puszta, in July 1998, Fig. 1) and *D. multireticulata* Mulsant & Rey, 1855, collected in Italy (Puglia: Taranto, Castellaneta, Bosco dei Terzi) in June 2011. *D. tarraconensis* was reared also from *D. europaea* (Linnaeus, 1767) in Italy (Lazio: Viterbo, Sutri, in July 2005 and Piemonte: Torino, Carignano, in September 2013). Description, drawings and measurements of the immature and mature larvae were made on specimens preserved in 70% ethanol.



FIGURE 1. Nymph of *Dictyophara pannonica* (Germar) parasitized by immature larva of Dryinidae.

For the examination of the body *in toto*, immature and mature larvae were put in glycerine and studied under a Zeiss SV11 stereomicroscope. Subsequently, the head region of some mature larvae was separated from the rest of the body, cleared for about 10 minutes in 10% KOH, and slide-mounted in Faure liquid for further study under a Leitz Laborlux 12 microscope and for drawing or photographing details.

For scanning electron microscopy (SEM), the immature and mature larvae were fixed for 2 h in 4% paraformaldehyde-5% glutaraldehyde, buffered with sodium cacodylate (0.1M and pH 7.3), rinsed in the same buffer overnight and, then, dehydrated in a graded ethanol series, critical point dried in CO₂ in a Polaron E 3000 Series II apparatus, sputter coated with gold/palladium in a Balzers SCD 030 Sputter-Coater, and investigated with a Cambridge Stereoscan 250 Mk 2.

The descriptions are based on two immature and five mature larvae of *Dryinus tarraconensis*, and three immature and six mature larvae of *Gonatopus baeticus*. The terminology follows Guglielmino & Virla (1998), Guglielmino & Bückle (2003, 2010, 2013) and Guglielmino *et al.* (2006).

Results

1. *Dryinus tarraconensis* Marshall, 1868 (Figs 2–5)

A. *Last immature larval instar* (Fig. 2). Maximal width: ca. 1.6 mm.

Brown in colour. Cephalic region with two sickle-shaped vesicles quite distant from each other (Fig. 2); posterior region of the body bent ventrally. Segmentation of the body indistinct, respiratory system peripneustic with nine pairs of lateral spiracles (one thoracic + eight abdominal). Ventral process absent.

Body partially covered by the discarded exuviae of the previous larval instars, immersed into the host's haemocoel with its anterior part, the posterior part protruding outside (Fig. 2). The position of the exuviae shows that for each moult the cuticle breaks along a preformed mediodorsal line.

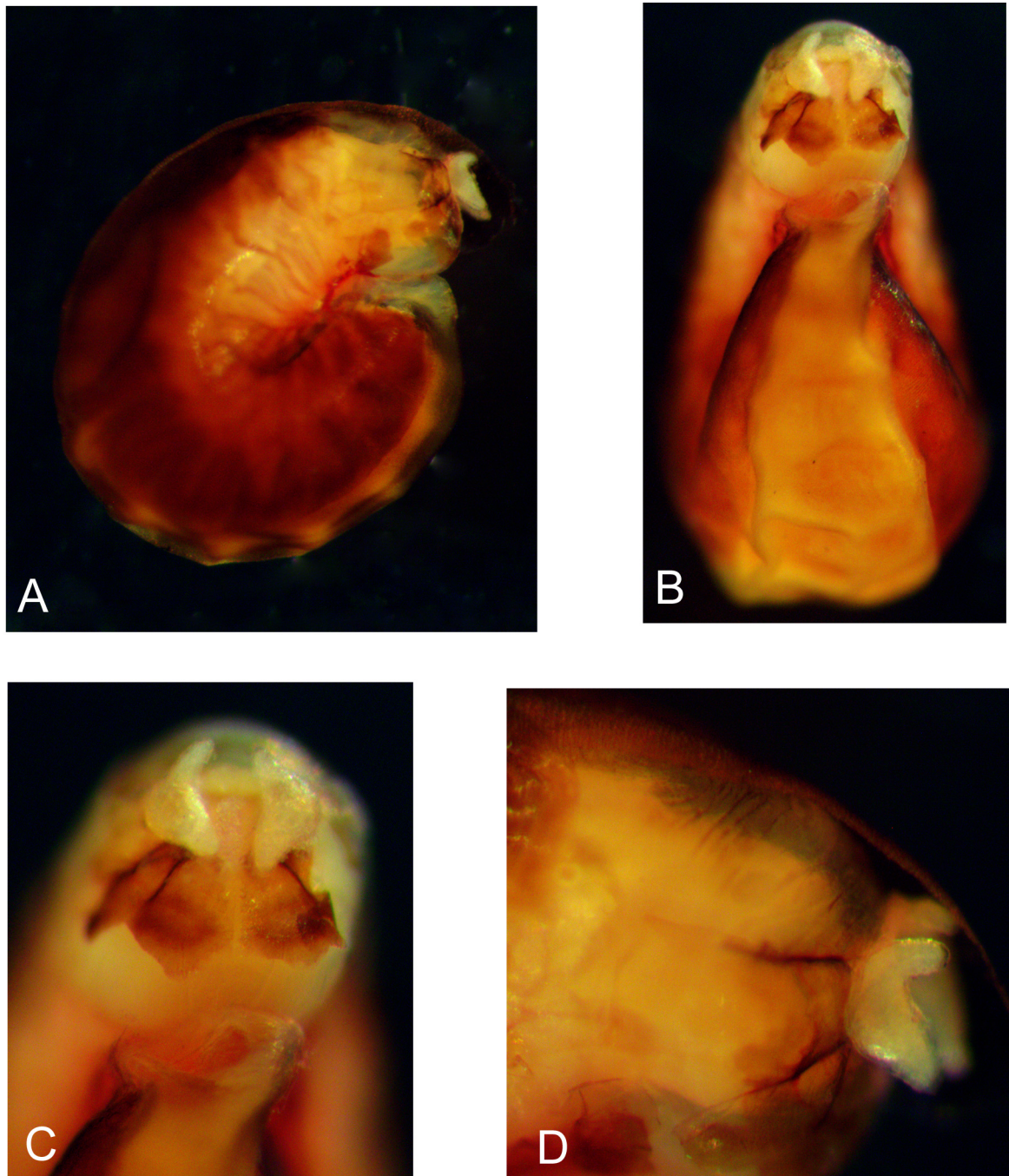


FIGURE 2. *Dryinus tarraconensis* Marshall, immature larva. A. Lateral view. B. Frontal view. C. Anterior region with cephalic vesicles, frontal view. D. Anterior region with cephalic vesicles, lateral view.

In the *Dictyophara* nymphs the dryinid larva protrudes between the host's metathorax and abdomen below the metathoracic wingpad (as in Fig. 1). In the rather rare case we found parasitized adults it was inserted in the abdomen and protected below the wings.

B. *Mature larva* (Figs 3–5). Length: ca. 4.6–6.5 mm; width of head 0.72–0.80 mm.

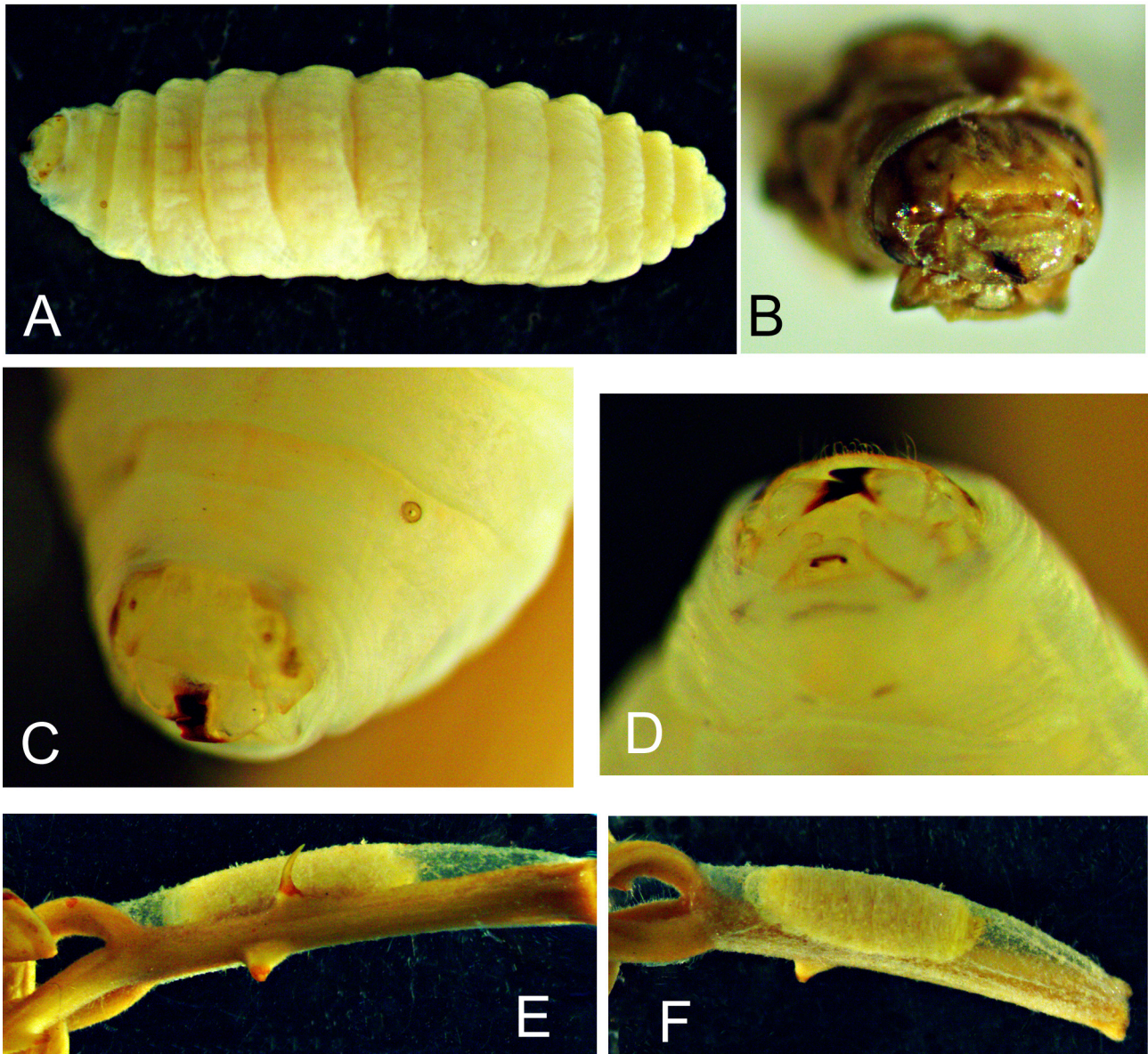


FIGURE 3. *Dryinus tarraconensis* Marshall. A–D. Mature larva. A. Dorsal view. B. Head, frontal view. C. Anterior region, dorsal view. D. Anterior region, ventral view. E, F. Cocoon on *Rubus*.

Larva apodous, white yellowish, subcylindrical (Fig. 3A). Head yellowish, well sclerotized, with barely visible medial ecdysial line posteriorly (Fig. 4A). Antennal orbit circular with two sensilla (Figs 4A, 5E). Head capsule with three pairs of long sensory bristles located in proximity of anterior margin and on each side one pair of sensory pits between antenna and anterior margin, a single small indistinct sensory pit more laterally and one pair of sensory pits in anterolateral position (Fig. 4A, arrow). Frontoclypeal suture distinct. Anterior margin of clypeus straight (Fig. 3B). Labrum large, wider than clypeus, anterior angles rounded, dorsal surface with two sensory pits and numerous long bristles irregularly distributed until the anterior margin and the distal region of epipharynx (Figs 3B, 4B). Epipharynx with basal large triangular lobe; lobe covered by scaly microsculpture as on surrounding posterior part of epipharynx, and with dorsad directed sagittal fold, visible ventrally as distinct groove; medioanterior region of epipharynx with pubescence of long robust bristles gradually decreasing in length basad

and laterad and within medioposterior part of pubescence with about four cone-shaped sensilla; posterior part of epipharynx covered by scaly microsculpture, scales of lobe anteriorly pectinate forming slightly curved transverse rows of spinules (Fig. 5A). Mandible strongly sclerotized, stout, securiform with broad base and apical portion divided in an internal simple and an external bidentate lobe (Figs 4C, 5B). Each mandible with one long sub-basal sensory bristle. Maxilla weakly sclerotized, with maxillary palp and five long sensory bristles (Figs 4D, 5D). Maxillary palp unsegmented, subcylindric, with distinct lateral sensory depression and with subapical sensory bristle (Figs 4D, 5C), apically with four sensilla of different size and structure: two uni-articulated, small and cone-shaped and two bi-articulated consisting of a long cylindrical basal and a small cone-shaped apical article. Labium sclerotized, distally with spinneret and subapically with labial palps (Figs 4D, 5F). Three sensory bristles in proximity of each palp, two in basal and one in lateral position. Labial palp (Figs 4D, 5F) unsegmented, with small circular sensory depression on midlength and with four distal sensilla structurally similar to those of maxillary palp. Spinneret with two sensory pits on each side (Figs 4D, 5G, arrows), apically opening by transverse slit.

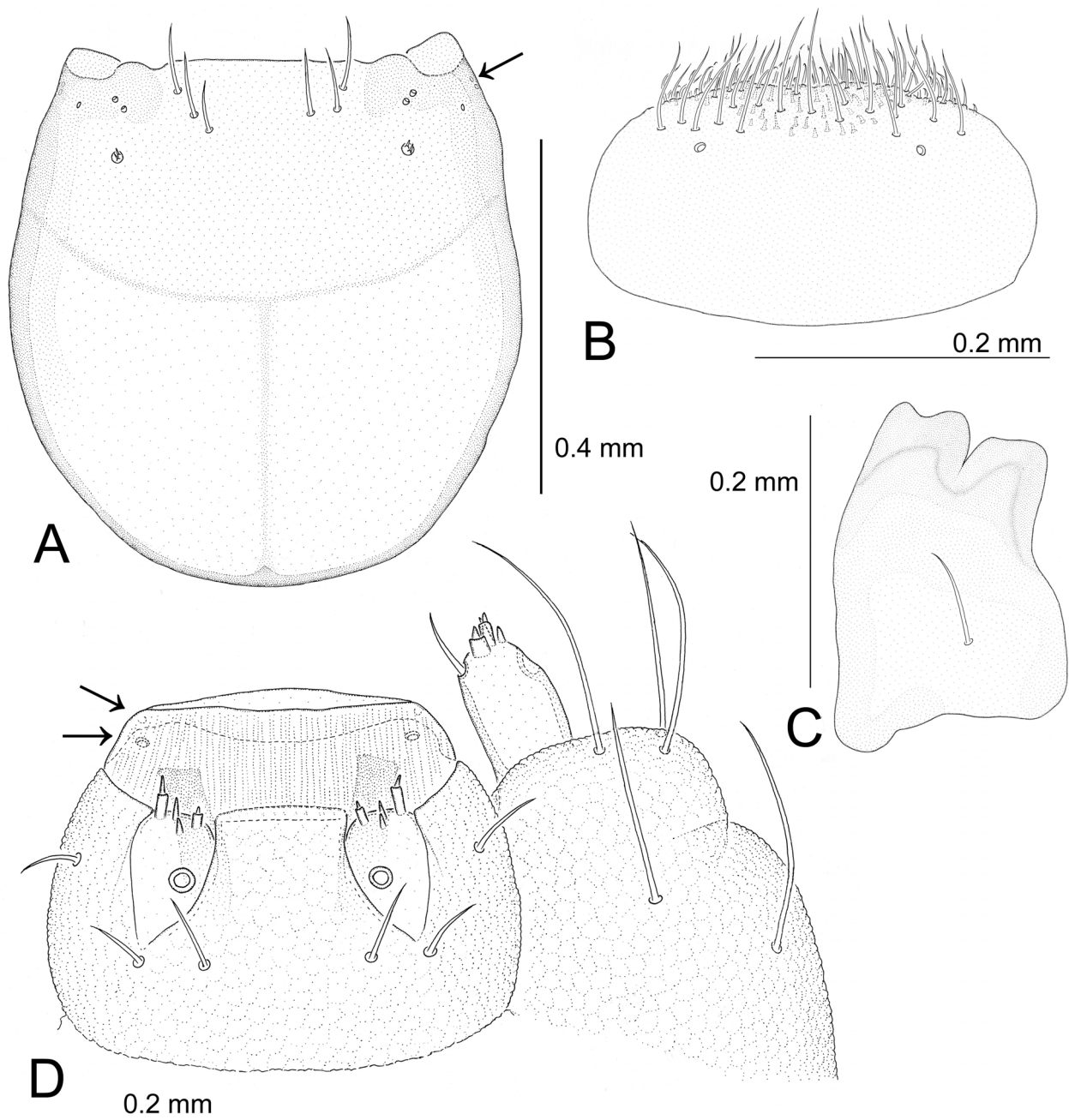


FIGURE 4. *Dryinus tarraconensis* Marshall, mature larva. A. Head capsule without clypeus, dorsal view. B. Labrum. C. Left mandible, dorsal view. D. Right maxilla and labium, ventral view.

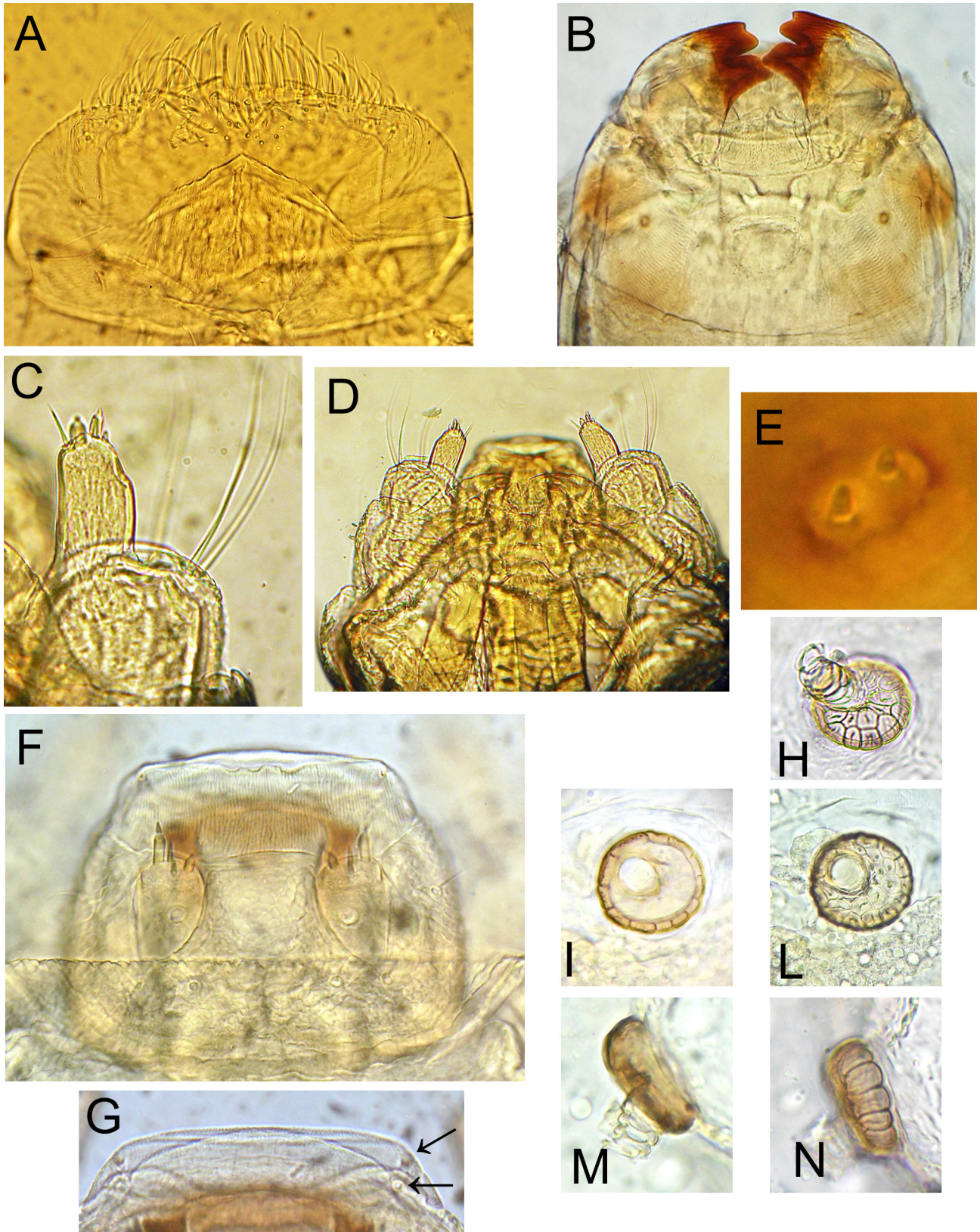


FIGURE 5. *Dryinus tarraconensis* Marshall, mature larva. A. Epipharynx. B. Mandibles, dorsal view. C. Right maxillary palp, dorsal view. D. Maxillae and labium, dorsal view. E. Antenna. F. Labium, ventral view. G. Apex of spinneret. H. Thoracic spiracle, from inside. I. Exterior margin of thoracic spiracle, from outside. L. Atrium of thoracic spiracle, from outside. M. Thoracic spiracle and part of trachea, lateral view. N. Exterior margin of thoracic spiracle, lateral view.

Thorax and abdomen with indistinct segmentation (Fig. 3A). Numerous and very small sensory bristles (ca. 10–15 μm) arranged in transverse rows around each thoracic and abdominal segment. Tracheal system with one pair of large thoracic spiracles (pro-mesothoracic) (Figs 3C, 5H–N) and eight pairs of smaller abdominal ones located on the first eight abdominal segments. Atrium of thoracic spiracles formed by about 20 cells (Figs 5H, 5L), with exterior margin consisting of about 12 cells (Fig. 5I, 5N). All spiracles simple, without bristles, hairs or other particular protection structures.

2. *Gonatopus baeticus* (Ceballos, 1927)

(Figs 6–9)

C. Last immature larval instar (Figs 6, 7A). Maximal width: ca. 1.5 mm.

The external morphology of the immature larvae of *Gonatopus baeticus* is similar to other Gonatopodinae larvae, in particular to those without a ventral tubercle (e.g. *G. lunatus* Klug, 1810: Guglielmino & Virla, 1998; *G. chilensis* (Olmi, 1991): Virla & Mangione, 2000; *G. bonaerensis* Virla, 1997: Mangione & Virla, 2004). Cephalic vesicles kidney-shaped, well developed, touching each other at their base (Fig. 6).

Position of the insertion of the larva in the host apparently as in *Dryinus tarraconensis*.

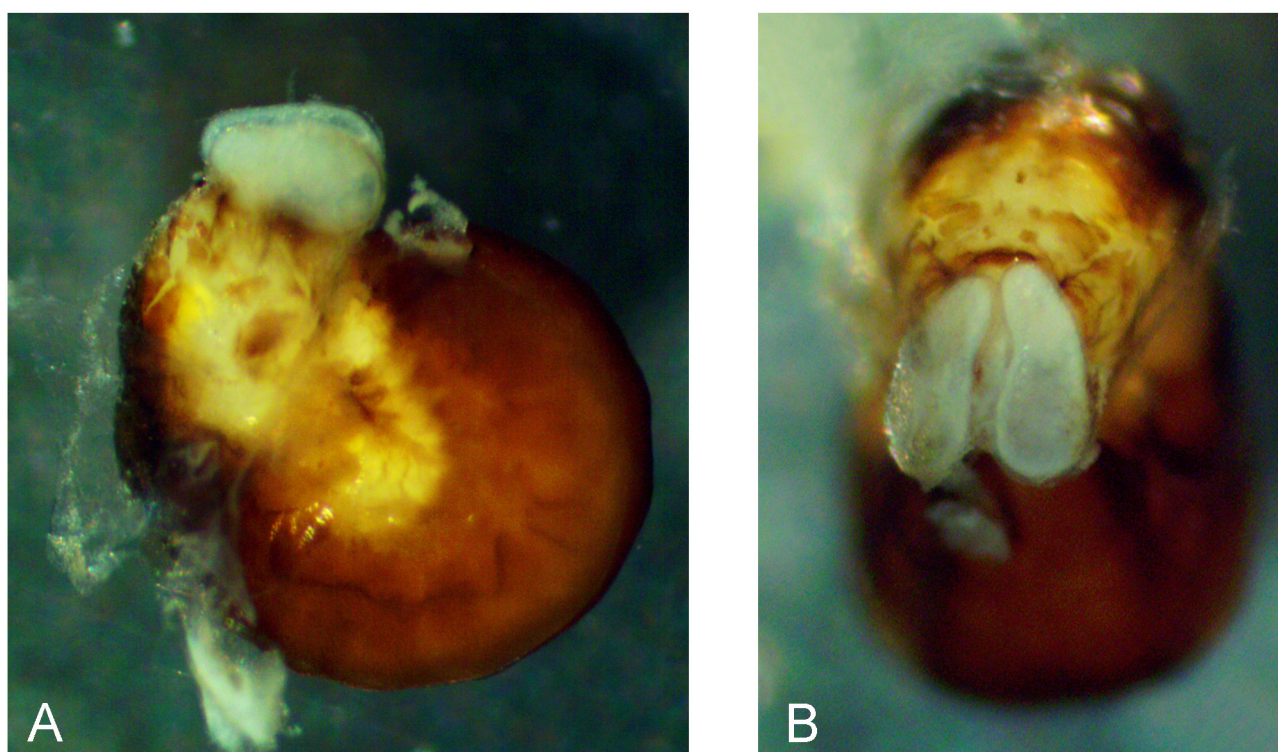


FIGURE 6. *Gonatopus baeticus* (Ceballos), immature larva. A. Lateral view. B. Anterior region with cephalic vesicles, frontal view.

D. Mature larva (Figs 7B–E, 8, 9). Length: ca. 6 mm; width of head: 0.75 mm.

Larva apodous, white yellowish, subcylindrical. Head brown-yellowish, well sclerotized, without parietal bands and without distinct medial ecdysial line posteriorly. Antennal orbit circular, slightly convex, with two sensilla (Figs 7B, 8E). Chaetotaxy of head capsule characterized on each side by four or five sensory bristles situated between antennae, in proximity of posterior margin of clypeus and base of mandible (Fig. 7B). Head capsule (Figs 7B, 8A) with medially one pair of sensory pits, on each side one pair of sensory pits between antenna and anterior margin and one pair of sensory pits in anterolateral position (Fig. 7B, arrow). Frontoclypeal suture indistinct. Anterior margin of clypeus straight. Labrum large, anterior angles rounded, dorsal surface with a basal row of six-seven sensory pits and numerous long sensory bristles irregularly distributed until the anterior margin (Figs 7C, 8B). Epipharynx apparently without basal lobe, anterior area with numerous lying thin hairlike setae and

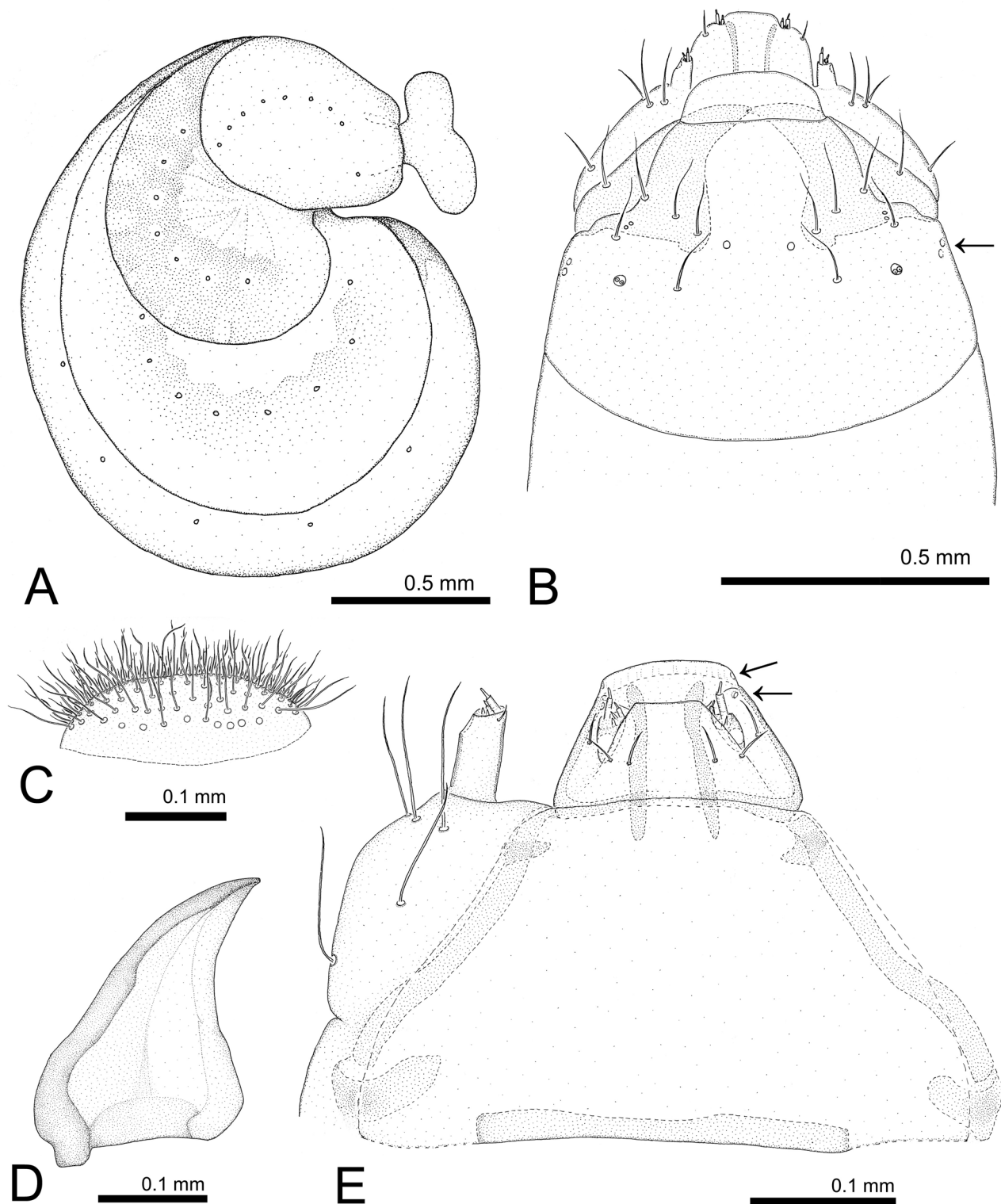


FIGURE 7. *Gonatopus baeticus* (Ceballos). A. Immature larva, lateral view. Mature larva: B. Head, dorsal view. C. Labrum, dorsal view. D. Right mandible, ventral view. E. Right maxilla and labium, ventral view.

with four sensilla proximally of this area; posterior area not pubescent, but covered by scaly microsculpture, scales anteriorly distinctly pectinate forming slightly curved transverse rows of spinules (Fig. 8C). Mandible strongly sclerotized, triangular, with broad base and apical portion pointed and curved in medial direction. Each mandible with long sub-basal sensory bristle (Figs 7D, 9B). Maxilla weakly sclerotized, with maxillary palp and five long sensory bristles (Fig. 7E). Maxillary palp (Figs 7E, 8F, 9A, 9C) unsegmented, subcylindric, with large lateral

sensory depression (Fig. 9D) and with subapical sensory bristle, apically with delimited area including four sensilla of different size and structure: two uni-articulated, small and cone-shaped and two bi-articulated consisting of a long cylindrical basal and a small cone-shaped apical article. Labium sclerotized, distally with spinneret and subapically with labial palps (Figs 7E, 8D, 9A). Three sensory bristles in semicircle in proximity of base of each palp. Labial palp (Figs 7E, 8D, 9E) unsegmented, with large proximal sensory depression (Fig. 9F) and with four distal sensilla structurally similar to those of maxillary palp. Spinneret with two sensory pits on each side (Figs 7E, 8D, arrows), apically opening by transverse slit.

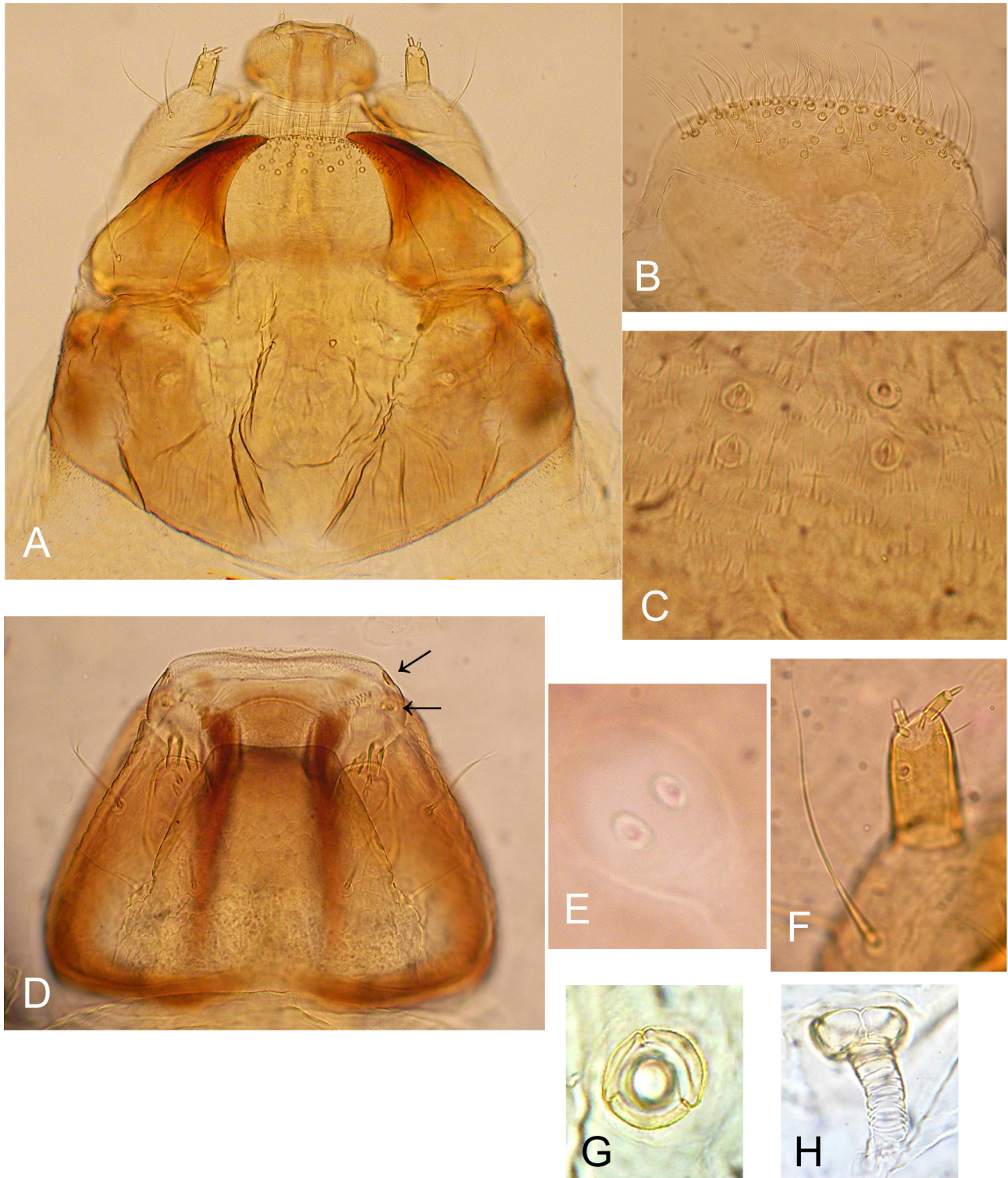


FIGURE 8. *Gonatopus baeticus* (Ceballos), mature larva. A. Head, dorsal view. B. Labrum. C. Sensilla of epipharynx. D. Labium, ventral view. E. Antenna. F. Right maxillary palp, ventral view. G. Thoracic spiracle, from outside. H. Thoracic spiracle, lateral view.

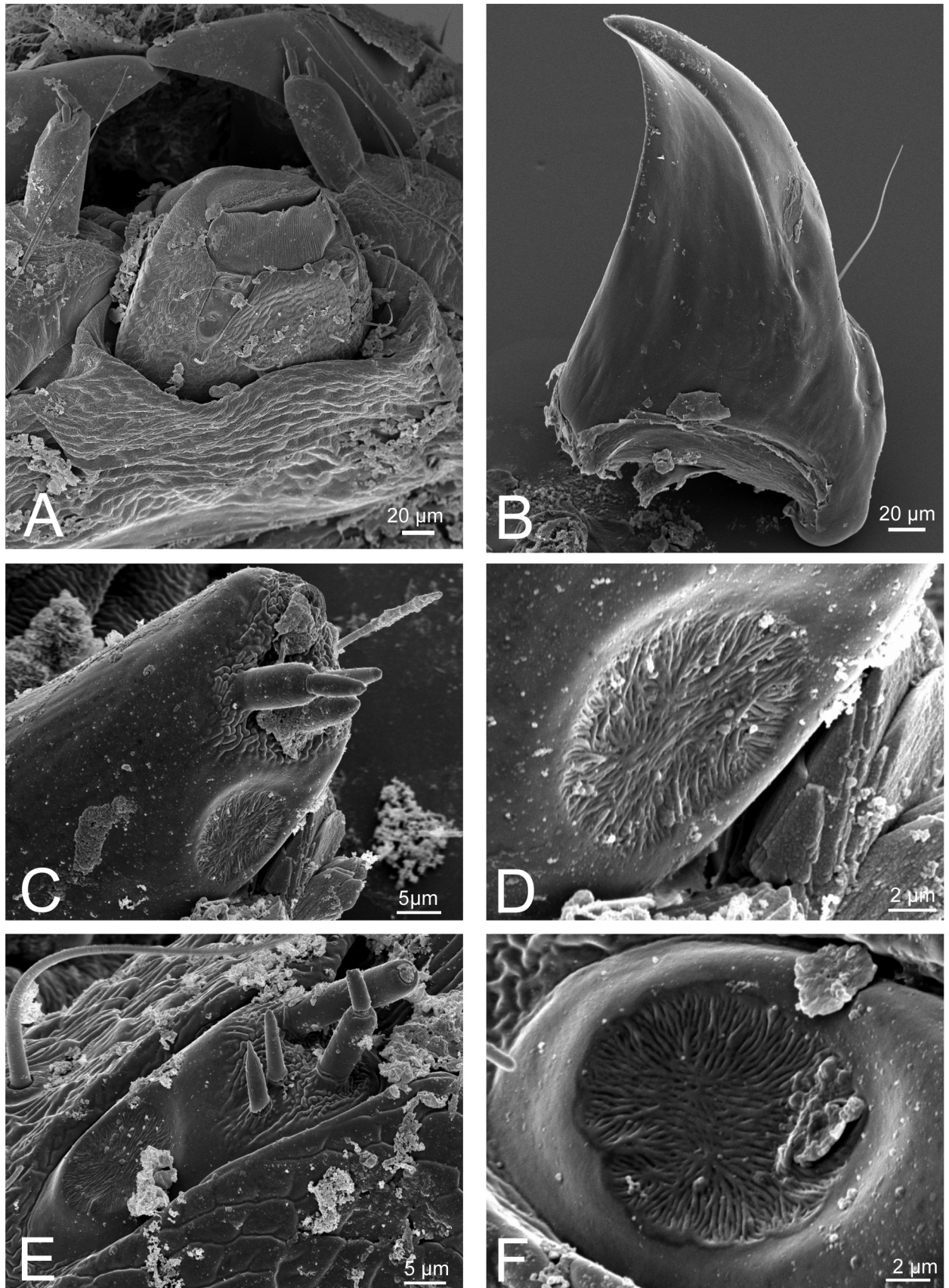


FIGURE 9. *Gonatopus baeticus* (Ceballos), mature larva. A. Maxillae and labium, ventral view. B. Left mandible, ventral view. C. Right maxillary palp, dorsolateral view. D. Detail of Figure C: Large lateral sensory depression. E. Right labial palp, medioventral view. F. Large lateral sensory depression of right labial palp.

Thorax and abdomen with indistinct segmentation. Thorax with small sensory bristles in proximity of the posterior margin of the head capsule and a few longer ones (ca. 40–50 µm) on the rest of the thoracic segments. Abdomen with numerous small sensory bristles (ca. 10–15 µm) arranged in transverse rows around each segment. Tracheal system with one pair of large thoracic spiracles (pro-mesothoracic) (Figs 8G, 8H) and eight pairs of smaller abdominal ones located on the first eight abdominal segments. Atrium of thoracic spiracles formed only by few (3–4) cells, all of them reaching exterior margin (Figs 8G, 8H). All spiracles simple, without bristles, hairs or other particular protection structures.

Discussion

Comparing the immature larva of *Dryinus tarraconensis* with that of *D. poecilopterae* described by Abril Ramírez (1992), as *Tridryinus poecilopterae*, we may observe that in both larvae the cephalic vesicles have a sickle-shape (“media luna”-shape according to Abril Ramírez). It is unclear if the peculiar shape of the cephalic vesicles in the immature larva is exclusive to the genus *Dryinus* or if this character is present also in other Dryininae genera as data about the morphology of the immature and mature larvae of other Dryininae are missing. The main differences between the immature larvae of the two *Dryinus* species consist in the size of the cephalic vesicles in proportion to the cephalic region (very large in *D. poecilopterae*, see Figs 3–5 in Abril Ramírez, 1992, smaller in *D. tarraconensis*, see Fig. 2) and in the presence/absence of the ventral process (present in *D. poecilopterae*, see Figs 3–5 in Abril Ramírez, 1992, absent in *D. tarraconensis*, see Fig. 2A).

Unfortunately, Abril Ramírez’s description of the mature larva of *D. poecilopterae* is rather poor and lacks many details, in particular of maxillae and labium. The mature larvae of *D. tarraconensis* and *D. poecilopterae* seem to share their head chaetotaxy, with only three pairs of sensory bristles situated in proximity of the posterior margin of clypeus, and the securiform bidentate mandibles (Figs 4A, 4C; see also Fig. 6 in Abril Ramírez). We observed this type of mandible also in *D. orophilus* (Benoit), *D. collaris* (L.), *D. corsicus* Marshall and *D. zambeziacus* Olmi. Very probably it is a peculiar character of the genus *Dryinus*, and perhaps of other Dryininae genera too.

The few data about the external morphology of Gonatopodinae and Dryininae larvae, do not allow an exhaustive comparison between the larvae belonging to these two subfamilies. The only significant difference between the immature larvae of *G. baeticus* and *D. tarraconensis* consists in the shape of the cephalic vesicles (kidney-shaped, well developed and basally touching each other in *G. baeticus*, Fig. 6; sickle-shaped, quite distant from each other in *D. tarraconensis*, Fig. 2). The mature larvae differ in the shape of the mandibles (triangular, with broad base and apical portion pointed in *G. baeticus*, Figs 7D, 9B; securiform with broad base and apical portion divided in an internal simple and an external bidentate lobe in *D. tarraconensis*, Figs 4C, 5B) and in the size of the proximal sensory depression of the labial palp (large in *G. baeticus*, Figs 9A, 9E, 9F; smaller in *D. tarraconensis*, Figs 4D, 5F). In addition, it would be interesting to check if the different structure of the thoracic spiracles in *D. tarraconensis* and *G. baeticus* may be a significant character to distinguish the two subfamilies. Other characters, for example the chaetotaxy of head and body, and the structure of the epipharynx, vary at least within Gonatopodinae and are probably not adequate for defining the Dryinidae subfamilies.

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