

Molecular identification of hymenopteran parasitoids and their endosymbionts from agromyzids — Supporting Information

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Coquilleau, Mallik B Malipatil and Peter M Ridland (2023)

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SEM and photomicrographs for *Neochrysocharis formosa* are given in Xu *et al.* (2022).

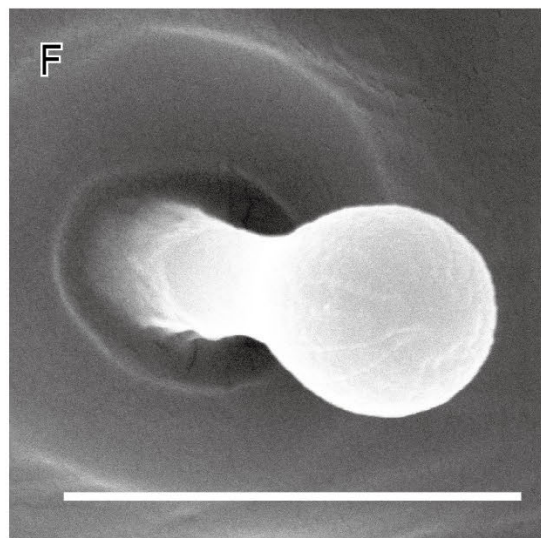
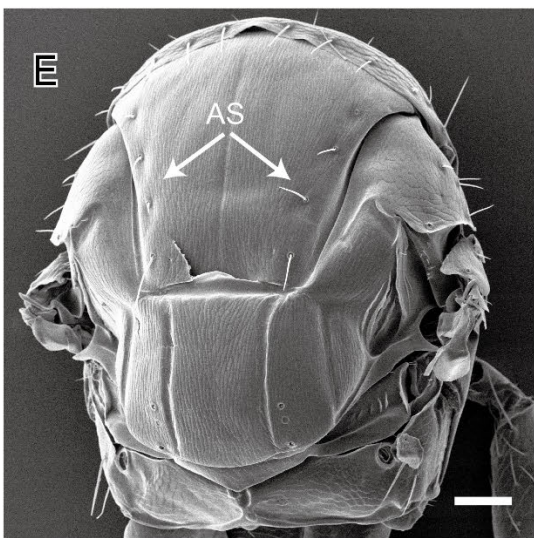
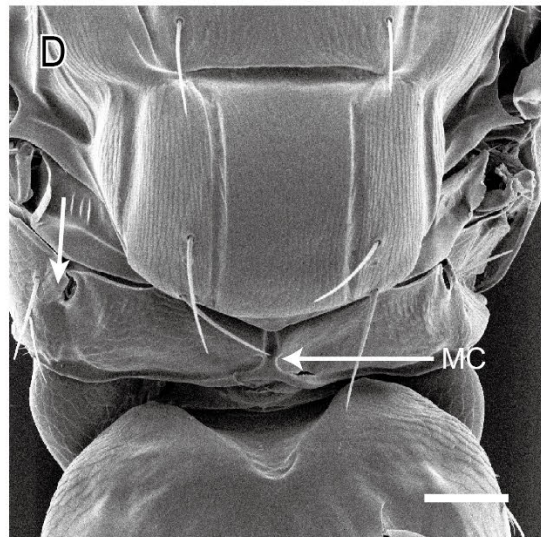
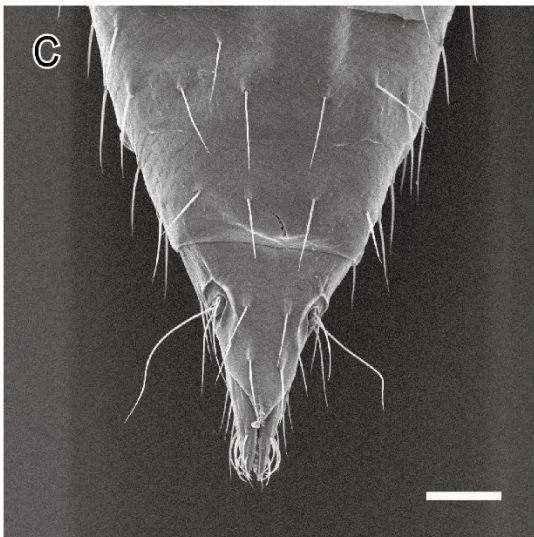
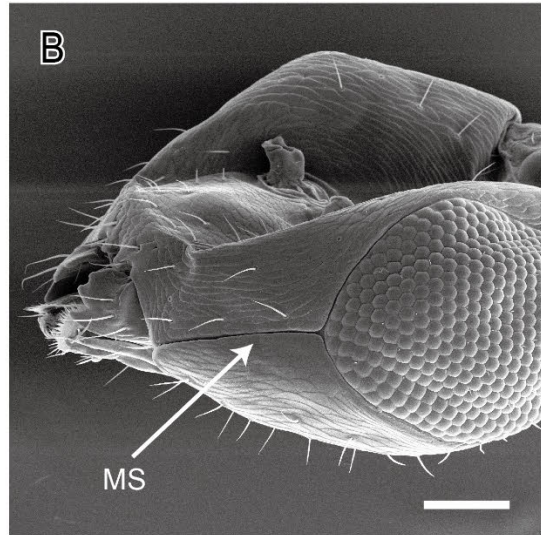
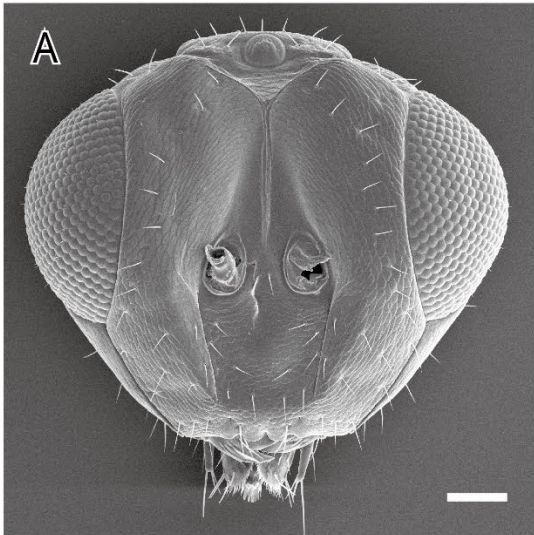
Terminology of characters for chalcidoids follows Gibson (1997), Gibson *et al.* (1998) and Hansson (2022); and for braconids follows van Achterberg (1993), Sharkey and Wharton (1997), Wharton (2006) and Karlsson and Ronquist (2012).

1. *Aprostocetus* sp. Westwood, 1833

(Hymenoptera: Eulophidae: Tetrastichinae)

Diagnostic characters (Bouček 1988; Reina & La Salle, 2006)

- i. Fronto-facial sutures near to anterior ocellus (Fig. S1-A)
- ii. Malar sulcus present and straight (Fig. S1-B)
- iii. Cercal setae unequal in length, with one being distinctly longer than the others and sinuate (Fig. S1-C)
- iv. Mesoscutellum with 2 pairs of setae and with 2 pairs of longitudinal lines (Fig. S1-D).
- v. Propodeum with median carina (MC) present with a raised lobe of the callus, which partially overhangs the outer rim of the spiracles (Fig. S1-D).
- vi. Mid-lobe of mesoscutum with median line and a single row of adnotaular setae just mesal to the notaulus. (Fig. S1-E).
- vii. Flagellum with 3 funiculars and 3 clavomeres (club) (Fig. S1-G); type 2 peg sensilla (Fig. S1-F).
- viii. Postmarginal vein (PMV) absent or less than 0.5 the length of stigmal vein (STV) (Fig. S1-H).
- ix. Three or more setae on submarginal vein (SMV) (Fig. S1-H).



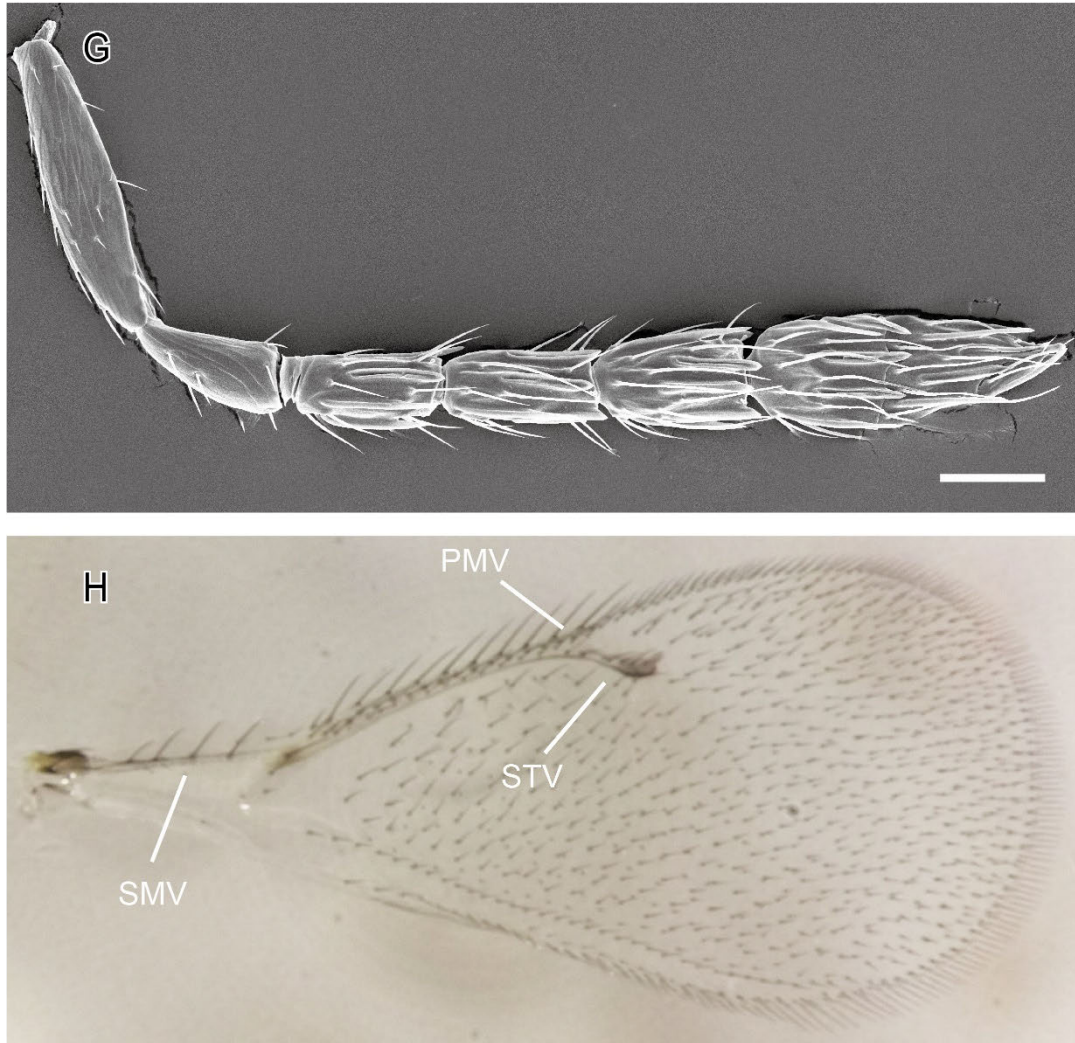


Figure S1. *Aprostocetus* sp. (A) ♀ head; (B) ♀ malar sulcus; (C) ♀ longest cercal setae; (D) ♀ scutellum; (E) ♀ mesoscutum; (F) ♀ peg sensilla on antenna; (G) ♀ antenna; (H) ♀ forewing. All scale bars are 50 µm except (F) is 4 µm. AS=adnotaular setae; MC=median carina; MS=malar sulcus; PMV=postmarginal vein; SMV=submarginal vein; STV=stigmatal vein.

2. *Asecodes* sp. Förster, 1856

(Hymenoptera: Eulophidae: Entedoninae: Entedonini)

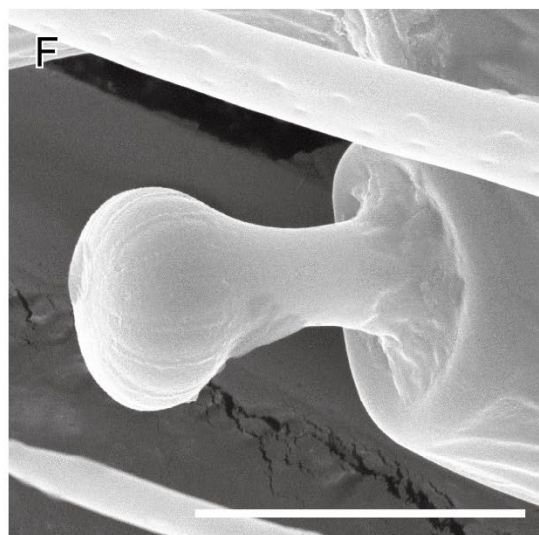
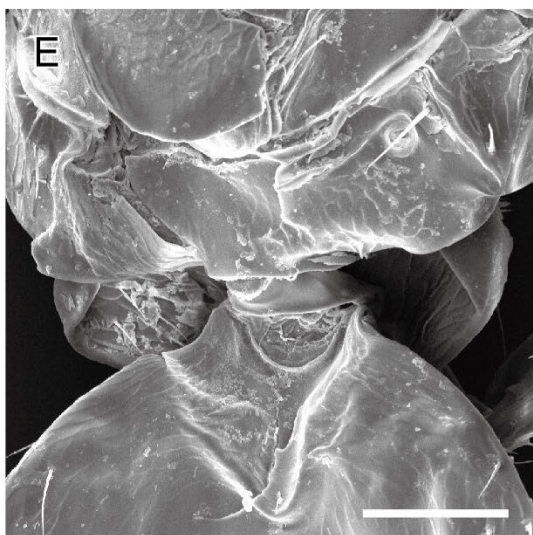
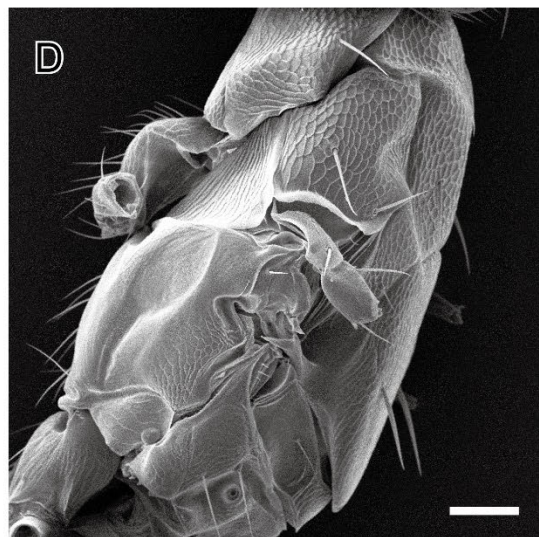
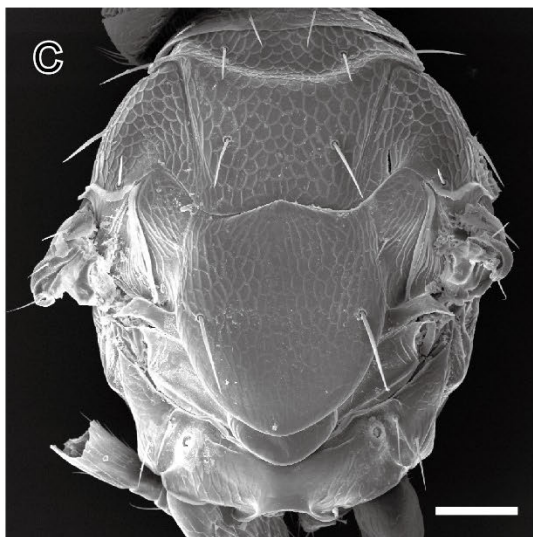
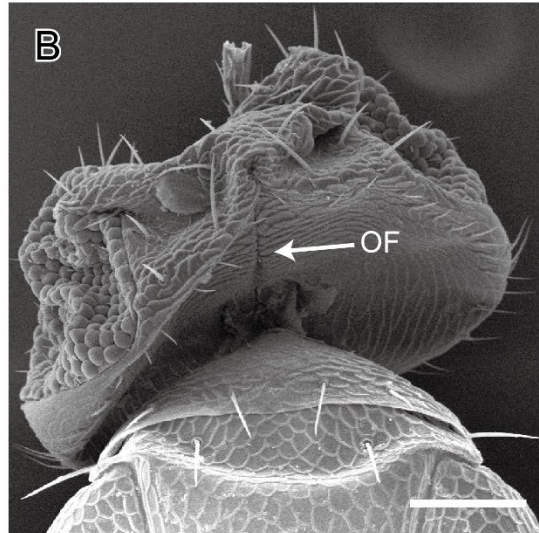
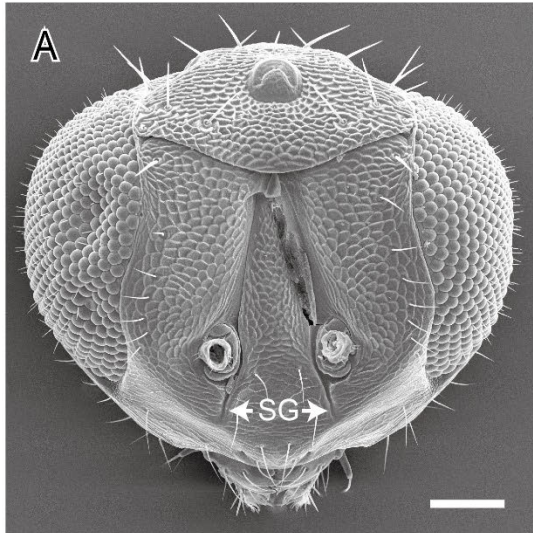
To date, all *Asecodes* spp. reared from agromyzids were originally considered to be in the genus *Teleopteris* (Hansson 1994). Bouček (1988) noted 3-4 species of *Teleopteris* in Australian collections, but *T. atripes* was the only one species described (Girault, 1915a; Dahms 1983). Subsequently, *Teleopteris* was synonymised

with *Asecodes* (Hansson 1996). Gumovsky (2001) then synonymised *Asecodes*, *Neochrysocharis*, *Hispinocharis* and *Mangocharis* with *Closterocerus*. However, molecular analysis led Burks *et al.* (2011) to remove *Neochrysocharis* and *Asecodes* from synonymy.

Belongs to Entedonini - two setae dorsally on SMV (Fig. S2-I), one pair of setae on mesoscutellum (Fig. S2-C).

Diagnostic characters (Bouček 1988; Hansson 1994, 1996; Fisher *et al.* 2005; Burks 2019)

- i. Subtorular grooves present (SG); fronto-facial suture distinctly separated from anterior ocellus and V or Y-shaped (Fig. S2-A).
- ii. Head with distinct occipital median furrow (OF) (Fig. S2-B).
- iii. Mesosoma slightly sculptured; pronotum very short and without transverse carina; notauli are incomplete; mesoscutum and scutellum without longitudinal grooves; propodeum smooth, shiny and without median carina and plicae. (Fig. S2-C; Fig. S2-D)
- iv. Petiole not distinct (Fig. S2-E).
- v. Flagellum with 2 funiculars and 3 clavomeres (Fig. S2-G, Fig. S2-H); ♂ flagellum with setae confined to a basal whorl on each segment (Fig. S2-H); (but Hansson (1994) noted that in some genera of Entedoninae, this character is present only in some species, so it is not a suitable character to define limits for a genus); type 2 peg sensilla on flagellum (Fig. S2-F).
- vi. Forewing with 2 indistinct stigmal hair lines (rows of setae radiating from STV; PMV shorter than STV (Fig. S2-I).



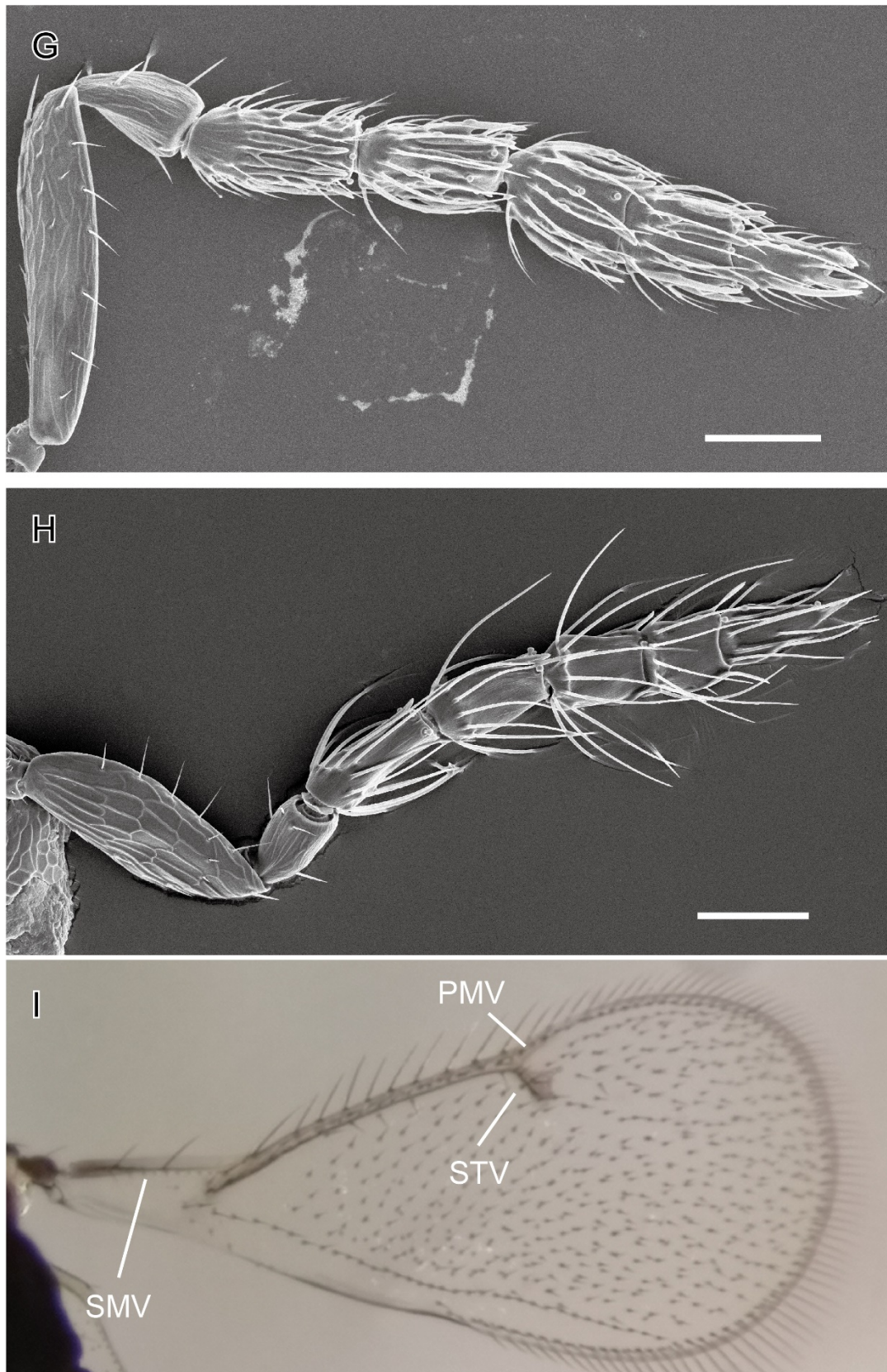


Figure S2. *Asecodes* sp. (A) ♀ head; (B) ♀ occipital grooves; (C) ♀ mesosoma, dorsal view; (D) ♀ mesosoma, lateral view; (E) ♂ petiole; (F) ♀ type 2 peg sensilla on

antenna; (G) ♀ antenna; (H) ♂ antenna; (I) ♂ forewing. All scale bars are 50 µm except (F) is 4 µm. OF=occipital median furrow; PMV=postmarginal vein; SG=subtorular grooves; SMV=submarginal vein; STV=stigmatal vein.

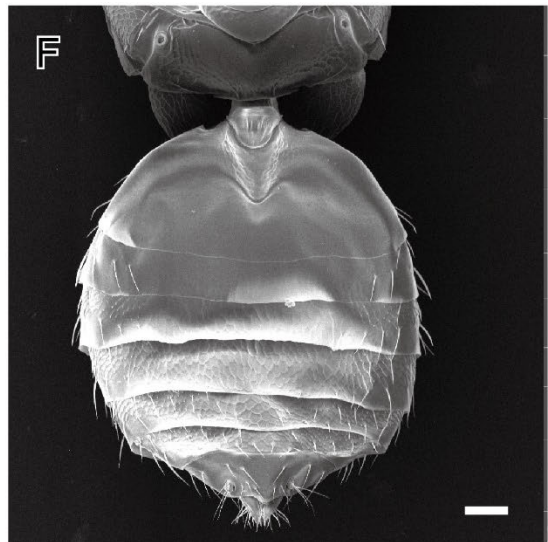
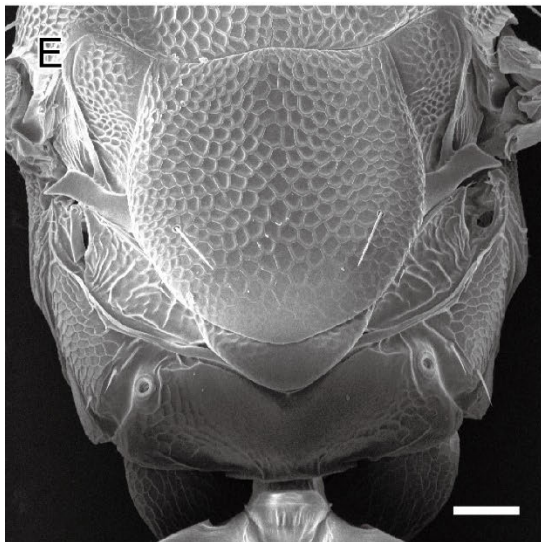
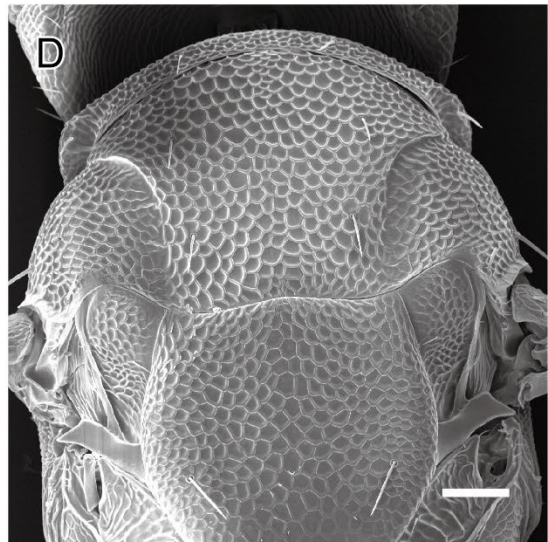
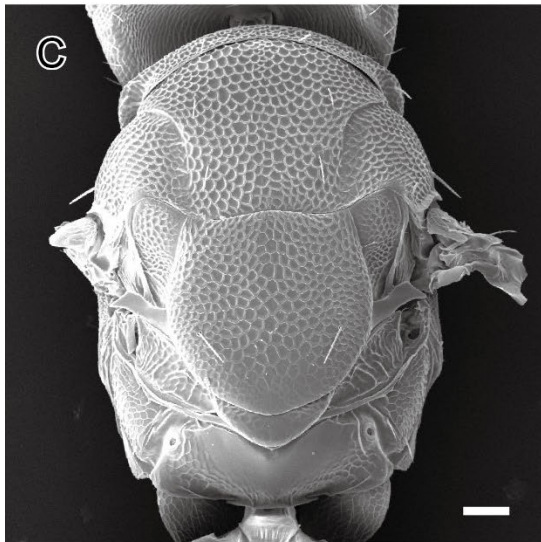
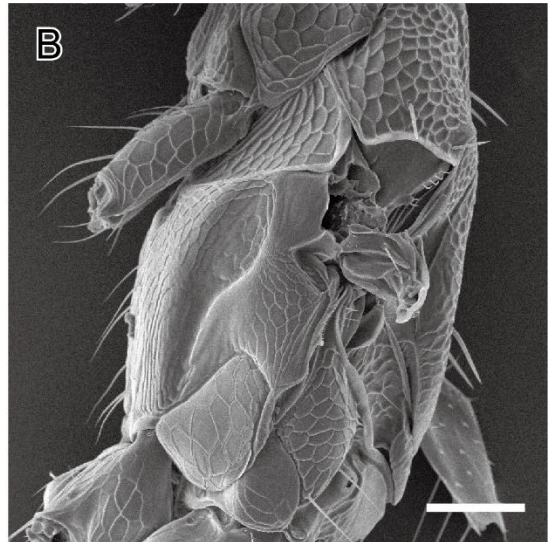
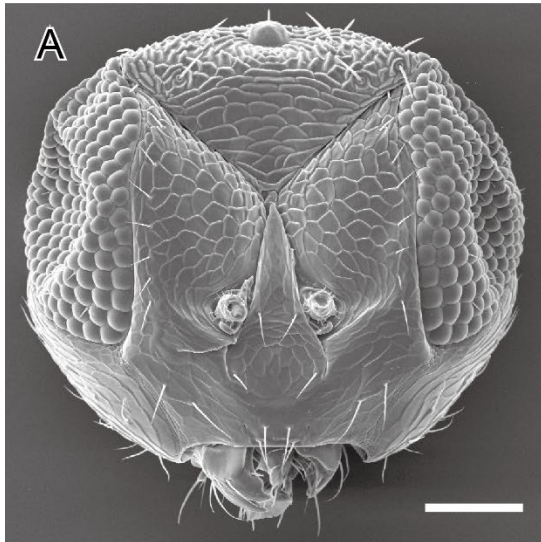
3. *Closterocerus mirabilis* Edwards & La Salle, 2004

(Hymenoptera: Eulophidae: Entedoninae: Entedonini)

Belongs to Entedonini - two setae dorsally on SMV (Fig. S2-I), one pair of setae on mesoscutellum (Fig. S2-C).

Diagnostic characters (Edwards & La Salle, 2004)

- i. Frontal-facial suture V-shaped, connecting to scrobal grooves but not connected to each other medially; short subtorular grooves present (Fig. S3-A).
- ii. Mesosoma: Pronotum without a distinct transverse carina. The notauli on the mesoscutum are incomplete (Fig. S3-C, Fig. S3-D); pronotum, mesoscutum and mesoscutellum strongly and evenly reticulate dorsally and laterally, except posterior margin of mesoscutellum with transverse smooth band (Fig. S3-B, Fig. S3-C, Fig. S3-D, Fig. S3-E); mesoscutellum with a single pair of setae (Fig. S3-E).
- iii. Petiole not distinct (Fig. S3-F).
- iv. Antenna compressed, scape widest at apex; flagellum with 2 funicular segments (wider than long) and club 3-segmented. (Fig. S3-G).
- v. Forewing with a characteristic infusate pattern: a transverse stripe at the wing apex, a complete dark transverse stripe at level of stigmatal vein, a cloud beneath the marginal vein, and a stripe along the hind margin of the wing. Speculum is large. Cubital row of setae complete to basal vein. Single hairline from stigmatal vein. Radial cell bare. (Fig. S3-H).



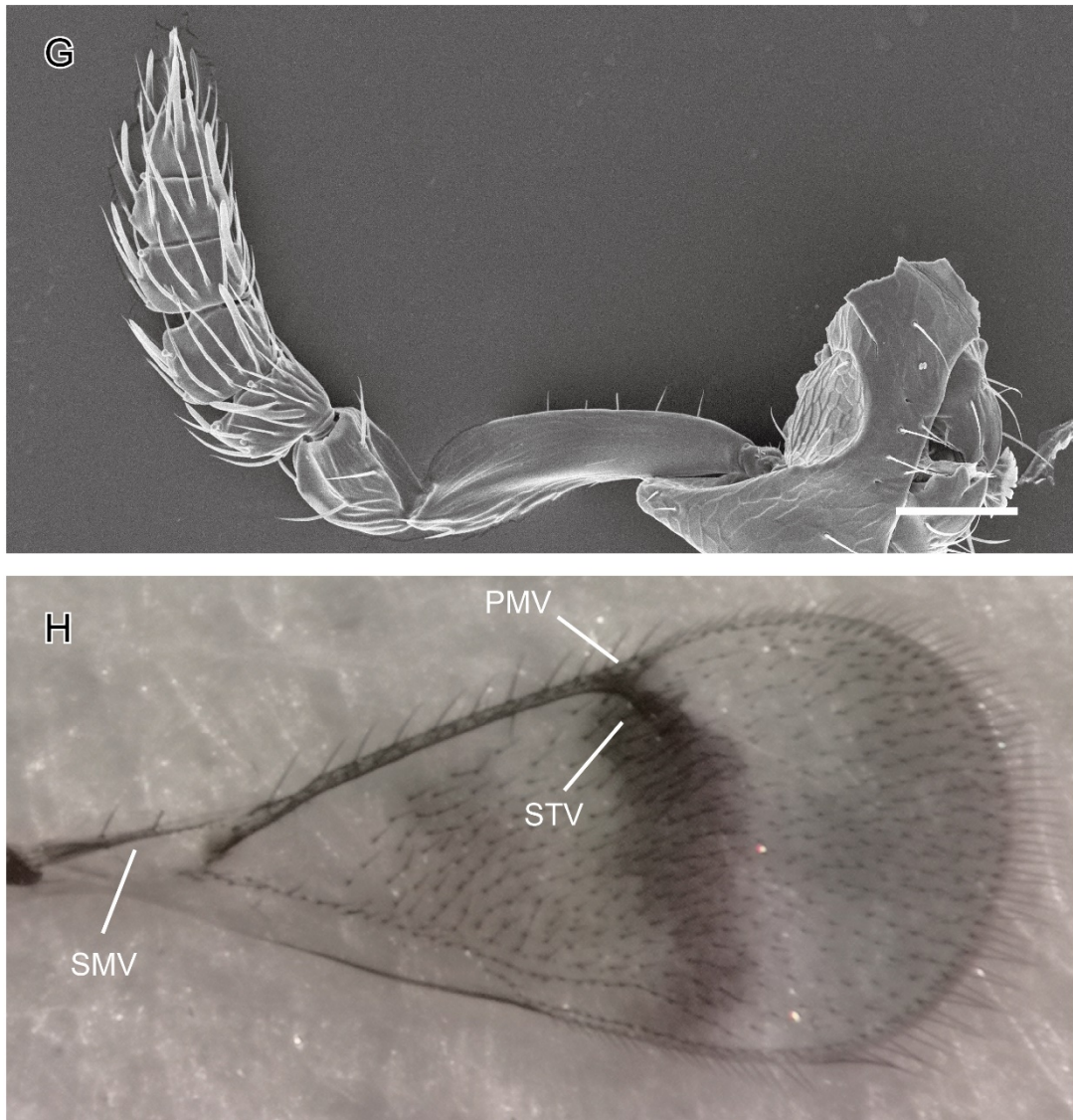


Figure S3. *Closterocerus mirabilis*. (A) ♀ head; (B) ♀ mesosoma, lateral view; (C) ♀ mesosoma, dorsal view.; (D) ♀ mesoscutum; (E) ♀ mesoscutellum and propodeum (F) ♀ metasoma, showing petiole. (G) ♀ antenna; (H) ♀ forewing; All scale bars are 50 µm. PMV=postmarginal vein; SMV=submarginal vein; STV=stigmatal vein.

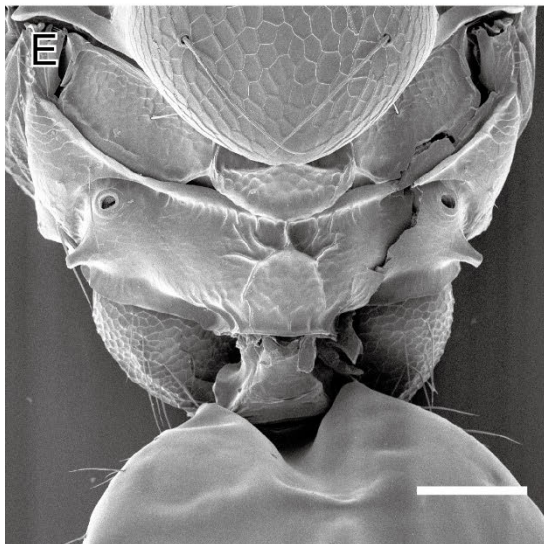
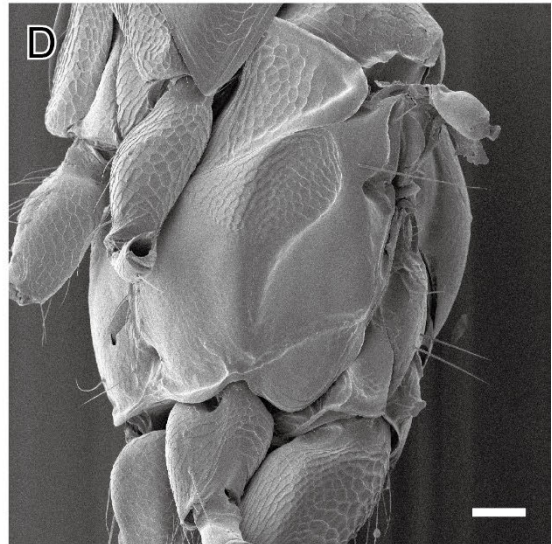
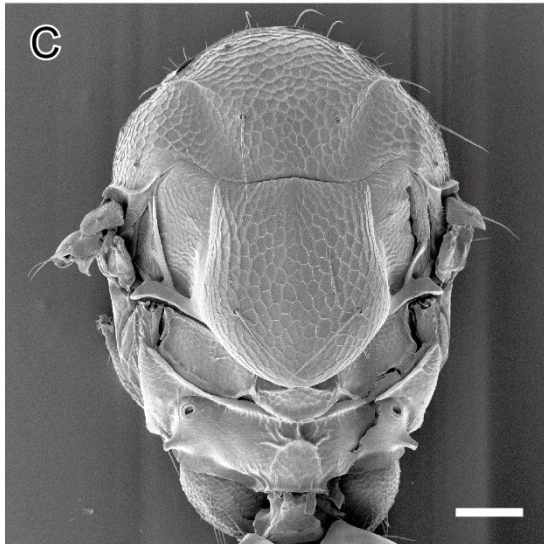
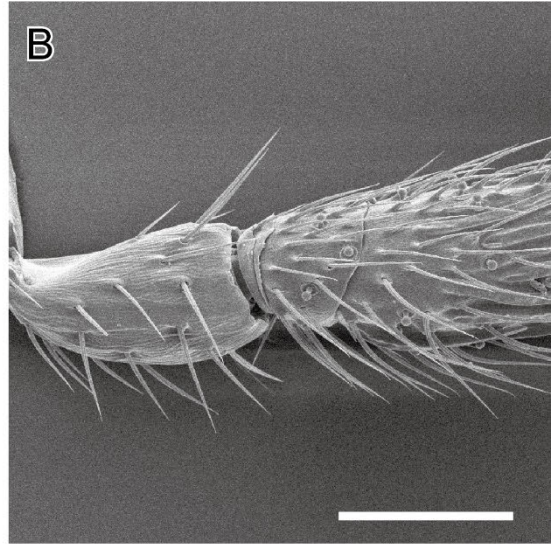
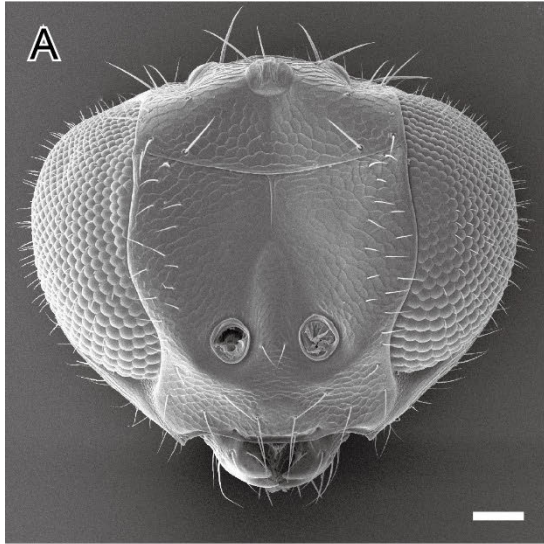
4. *Chrysocharis pubicornis* (Zetterstedt, 1838)

(Hymenoptera: Eulophidae: Entedoninae: Entedonini)

Belongs to Entedonini - one pair of setae on mesoscutellum (Fig. S4-C); two setae dorsally on SMV (Fig. S4-F),

Diagnostic characters (Hansson 1985; Ikeda 1996; Narendran et al. 2013)

- i. Head without a sulcus surrounding ocellar triangle; fronto-facial suture distinctly separated from anterior ocellus and usually V or Y-shaped (Fig. S4-A).
- ii. Type 1 peg sensilla on ♀ antennae. Third annellus enlarged (Fig. S4-B, Fig. S4-G)
- iii. Mesosoma slightly to strongly sculptured; pronotum with a low carina; notauli are incomplete; mesoscutum and mesoscutellum without longitudinal grooves; mesoscutellum with a single pair of setae (Fig. S4-C; Fig. S4-D).
- iv. Propodeum with raised structure on anterior part (Fig. S4-E); petiole shorter than propodeum, wider than long (Fig. S4-E).
- v. Relative measurements for length of marginal/ postmarginal/ stigmal veins (MV/PMV/STV): 7.1/2.1/1.0 (Hansson 1985) (Fig. S4-F).



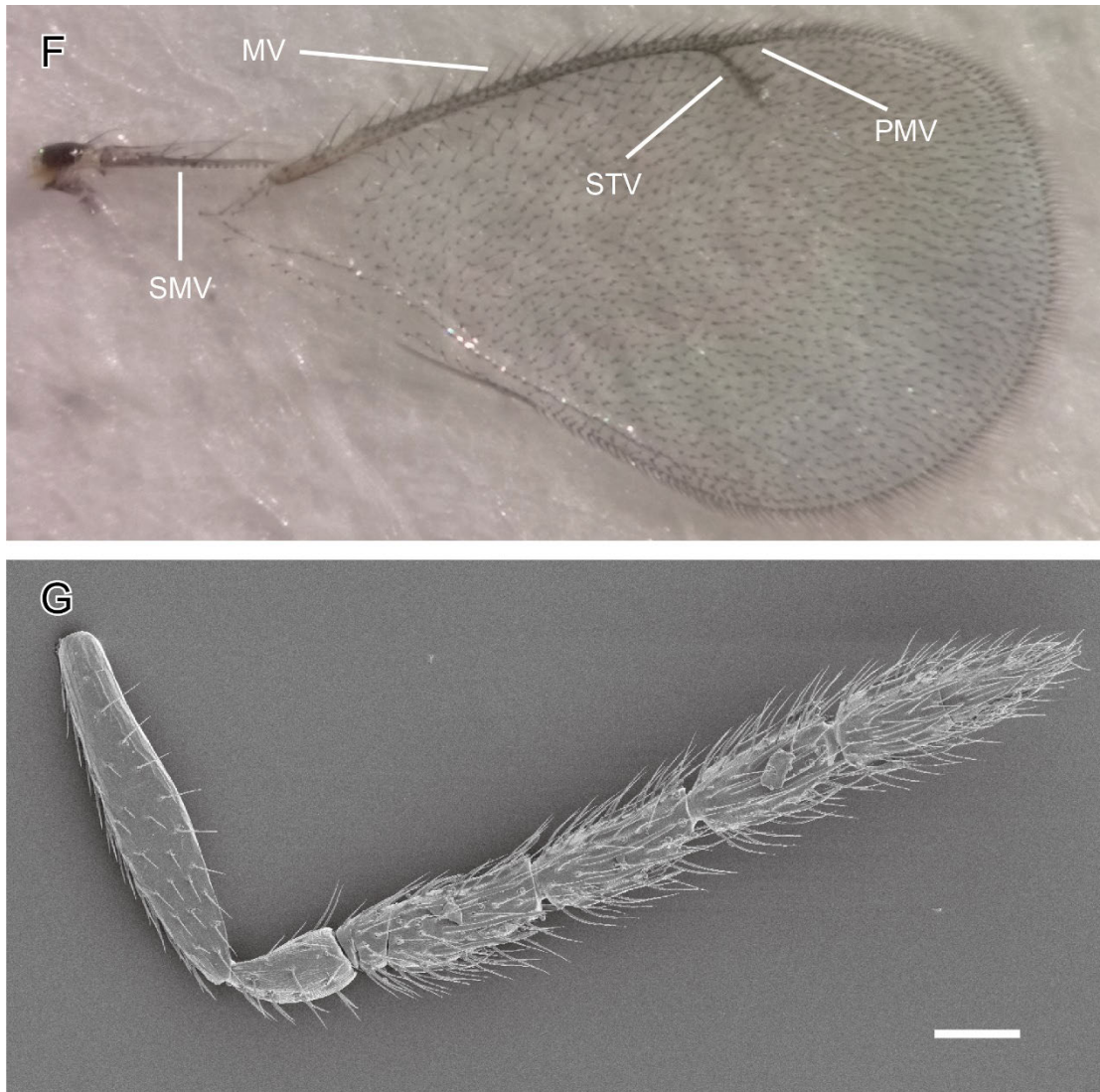


Figure S4. *Chrysocharis pubicornis*. (A) ♀ head; (B) ♀ peg sensillae on antenna; (C) ♀; mesosoma, dorsal view; (D) ♀ mesosoma, lateral view; (E) ♀ propodeum; (F) ♀ forewing; (G) ♀ antenna. All scale bars are 50 µm. MV=marginal vein; PMV=postmarginal vein; SMV=submarginal vein; STV=stigmatal vein.

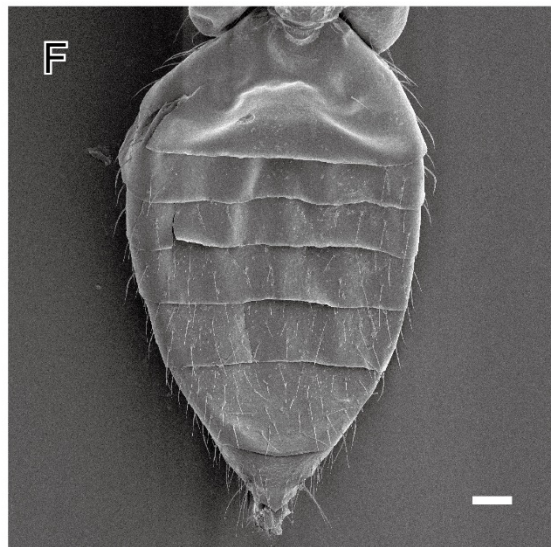
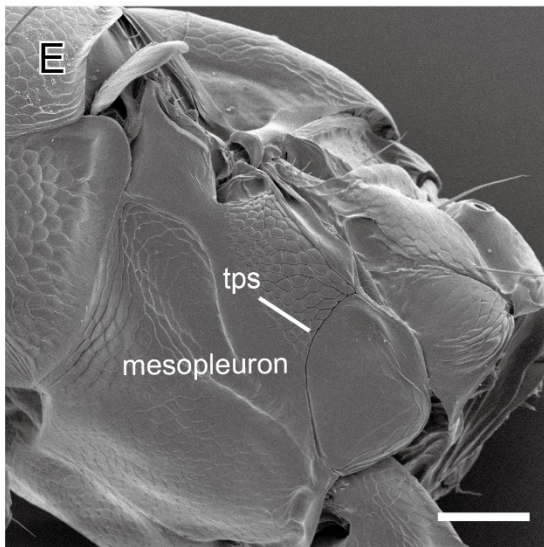
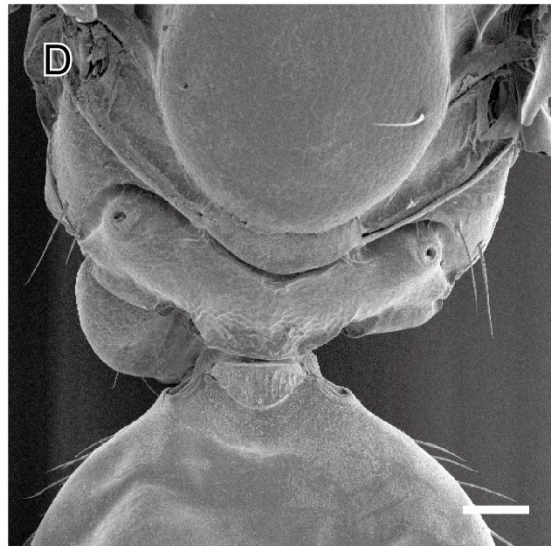
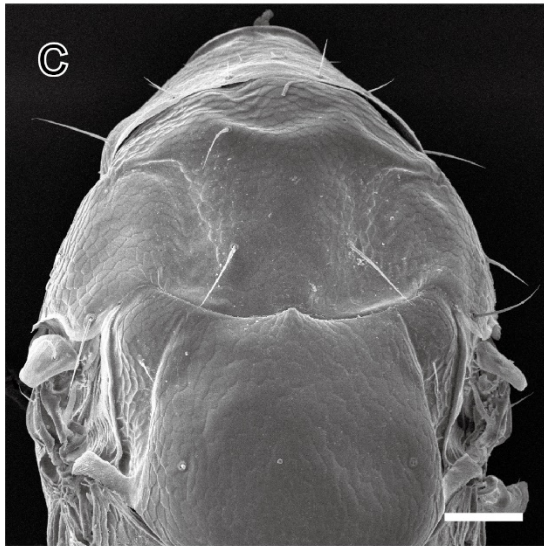
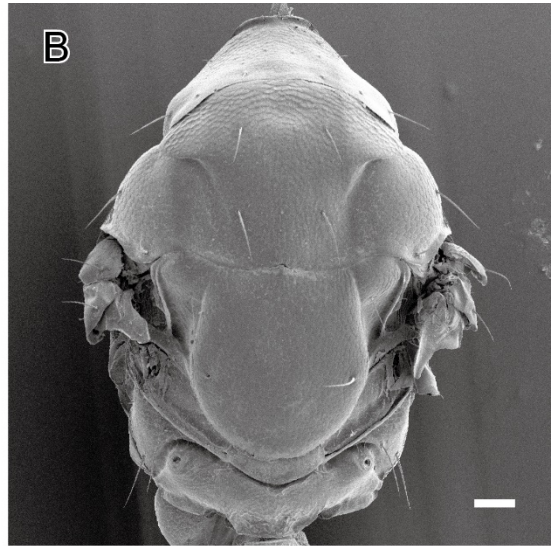
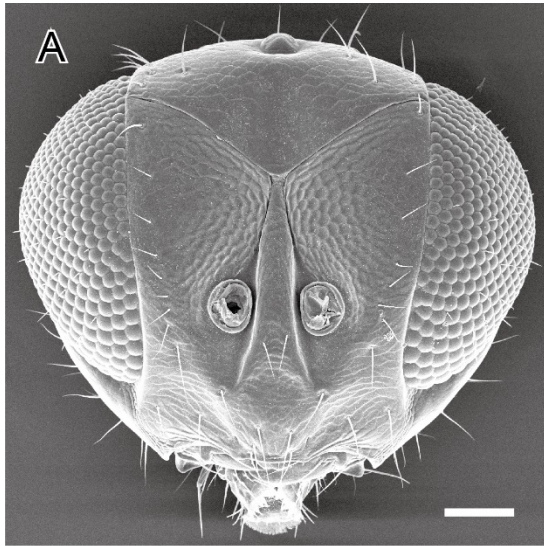
5. *Neochrysocharis okazakii* Kamijo, 1978

(Hymenoptera: Eulophidae: Entedoninae: Entedonini)

Belongs to Entedonini - two setae dorsally on SMV (Fig. S5-I), one pair of setae on mesoscutellum (Fig. S5-B, S5-C)

Diagnostic characters (Kamijo 1978; Hansson 1990; Fisher & La Salle 2005; Fisher *et al.* 2005)

- i. Head with fronto-facial suture distinctly separated from anterior ocellus and V or Y-shaped (Fig. S5-A).
- ii. Pronotum without transverse carina (Fig. S5-B); mesoscutum and scutellum without longitudinal grooves and notauli are incomplete (Fig. S5-D); transepimeral sulcus (tps) curved postero-dorsally (Fig. S5-E).
- iii. propodeum smooth, shiny and without median carina and plica (Fig. S5-F); callus with 2 setae; petiole not distinct (Fig. S5-F); mesosoma and gaster relatively smooth and shiny, without distinct reticulate sculpture. (Fig. S5-D, S5-H).
- iv. Flagellum with 2 funiculars and 3 fused clavomeres (Fig. S5-G, S5-H).
- v. PMV present and shorter than STV. No infumation associated with stigmal vein. Forewing about 2.0 times longer than wide, using the HW/LW definition given by Hansson (1990) (LW=length of forewing, measured from base of marginal vein to the place on the wing farthest away from base of marginal vein; HW=height of forewing, represented by the line right-angular to the line representing LW) (Fig. S5-I).



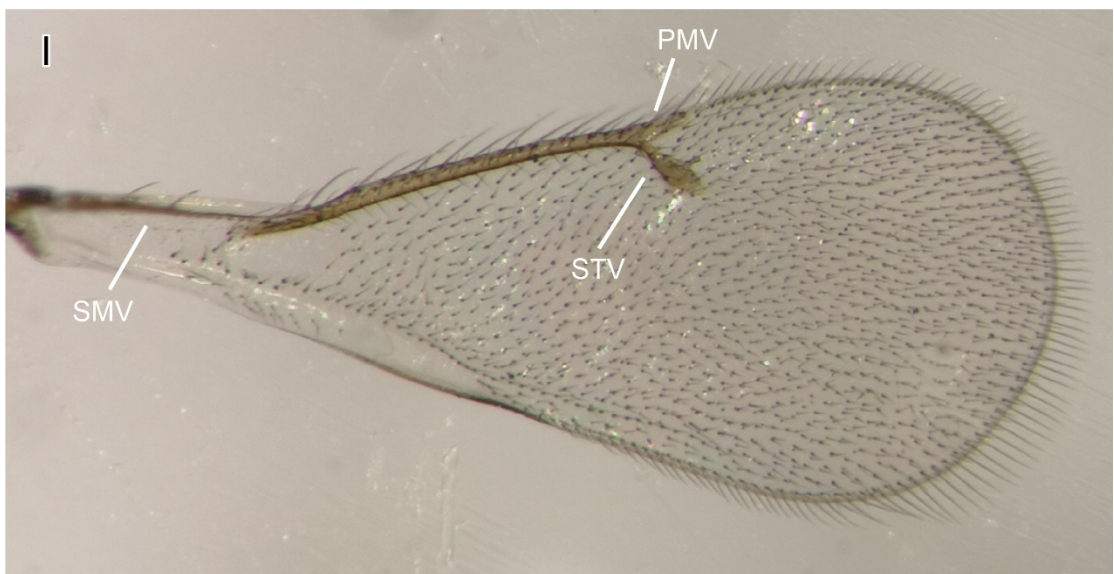
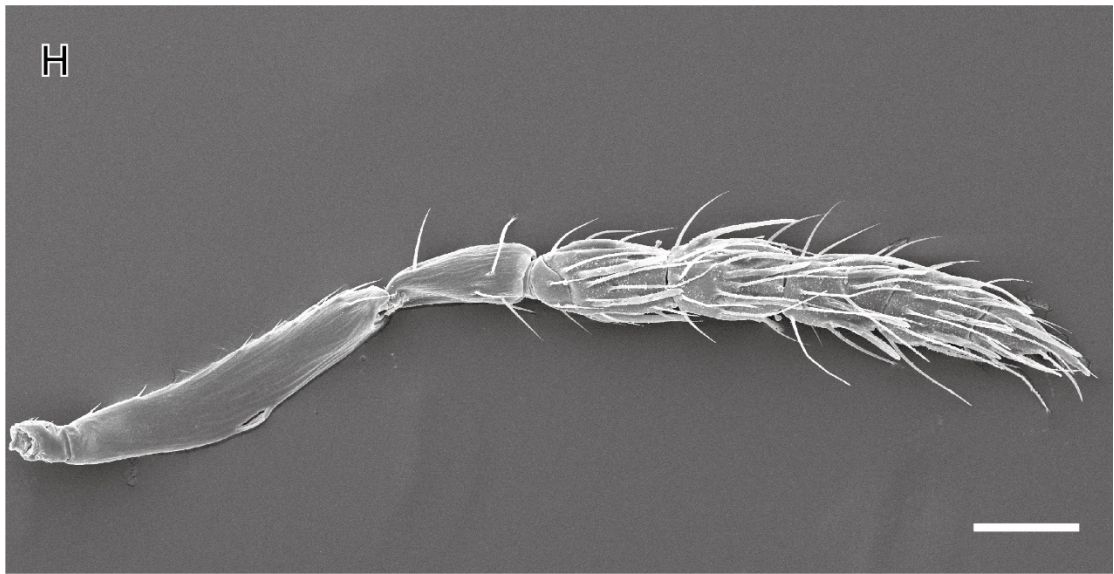
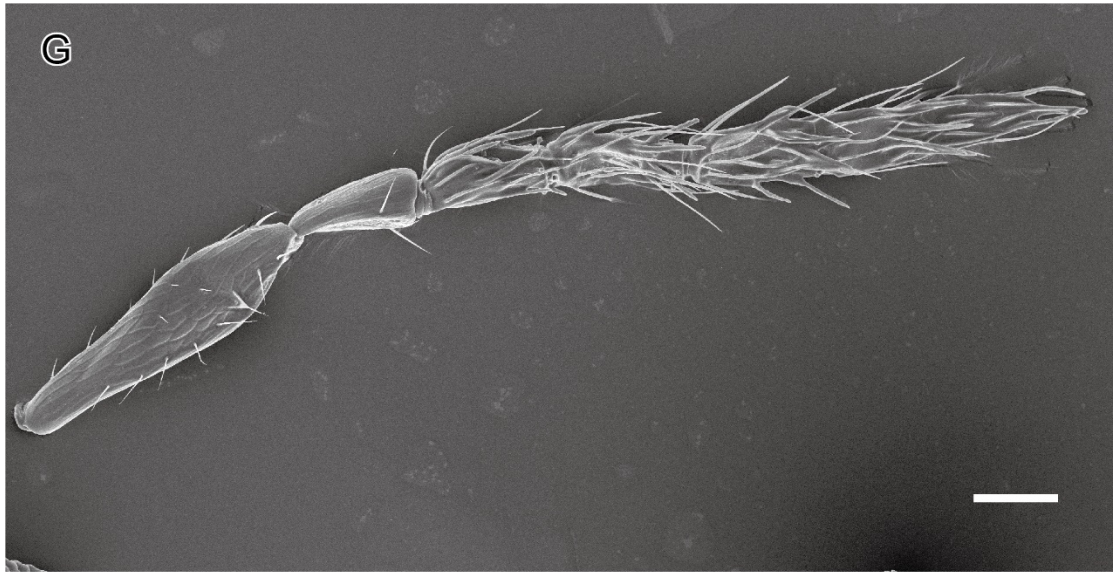


Figure S5. *Neochrysocharis okazakii*. (A) ♀ head; (B) ♀ mesosoma, dorsal view; (C) ♂ mesoscutum; (D) scutellum and propodeum; (E) ♀ mesosoma, lateral view; (F) ♀ gaster. (G) ♂ antenna; (H) ♀ antenna; (I) ♂ forewing. All scale bars are 50 µm. SMV=submarginal vein; PMV=postmarginal vein; STV=stigmatal vein.

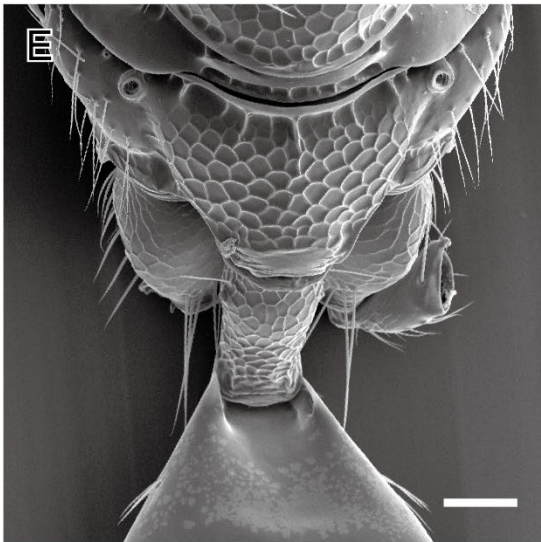
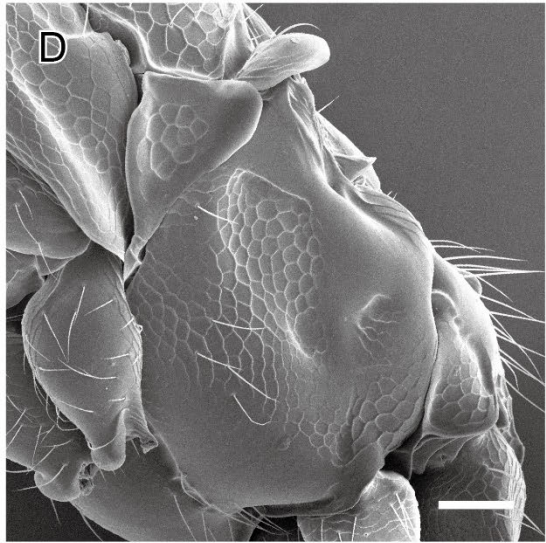
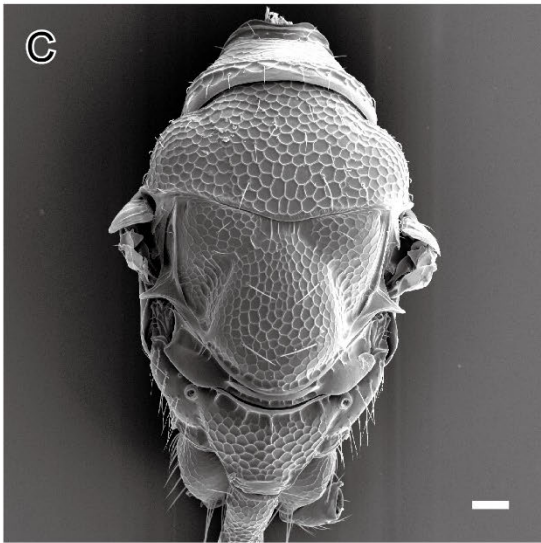
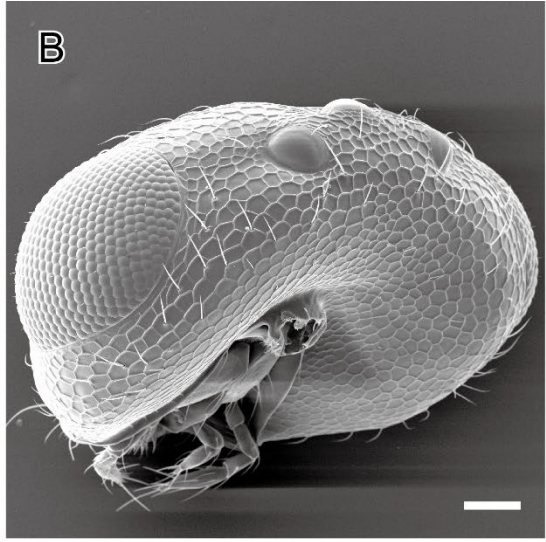
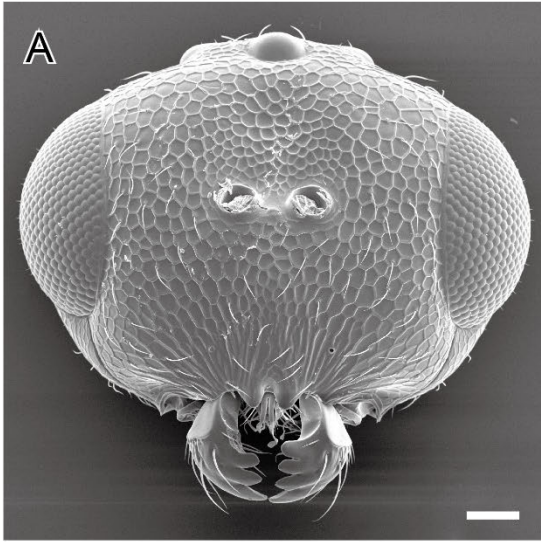
6. *Trigonogastrella parasitica* Girault, 1915

(Hymenoptera: Pteromalidae: Pteromalinae)

Diagnostic characters (Girault 1915b; Bouček 1988; Fisher *et al.* 2005)

- i. Forewing with PMV and STV well-developed; PMV elongate, as long as the MV; the STV distinctly shorter than both MV and PMV, PMV twice as long as STV (Fig. S6-G).
- ii. Flagellum with 6 funiculars, 3 clavomeres; two annelli (Fig. S6-H).
- iii. Mandibles four-dentate (Fig. S6-A).
- iv. No post-occipital carina (Fig. S6-B).
- v. Mesosoma and propodeum punctate; propodeum with a complete median carina and straight spiracular sulci (Fig. S6-C; Fig. S6-D).
- vi. Petiole present and distinct (Fig. S6-E).
- vii. Second gastral tergite shorter than first tergite; first gastral tergite not broadly concave (Fig. S6-I).
- viii. Scape of ♂ is entirely light brown, with the flagellum dark brown (Fig. 6-F). In contrast, the only the basal part of the scape of ♀ is light brown. The clava of the ♀ is more distinct than the clava of the ♂ (Fisher *et al.* 2005).

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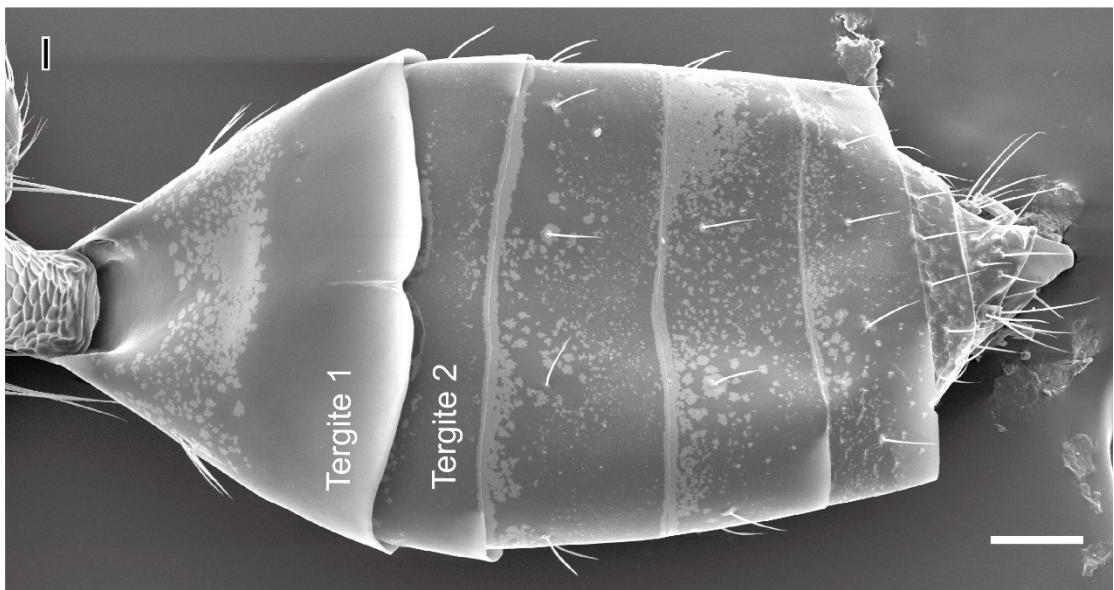
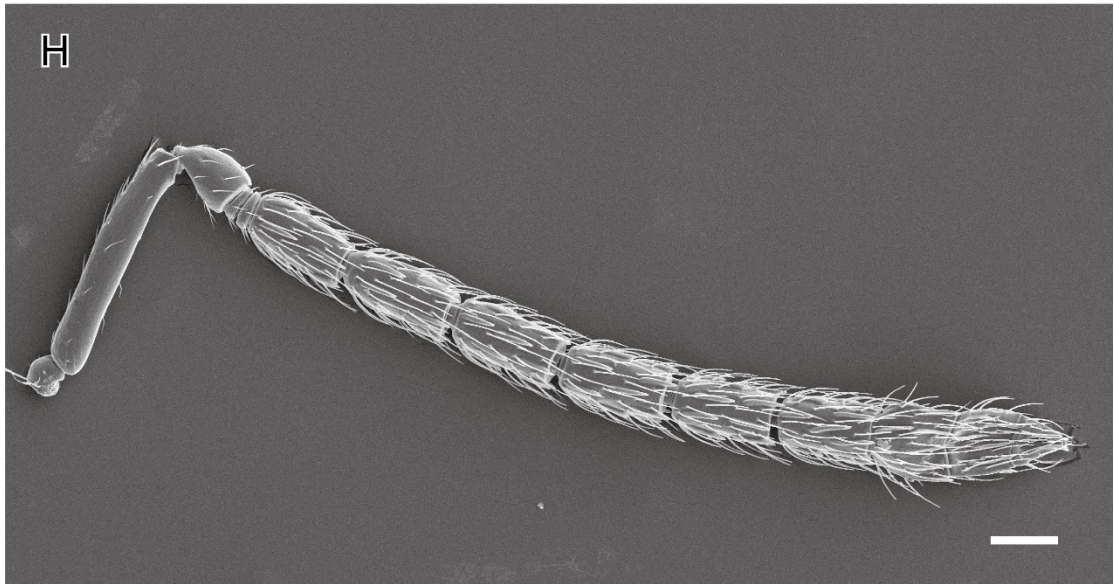
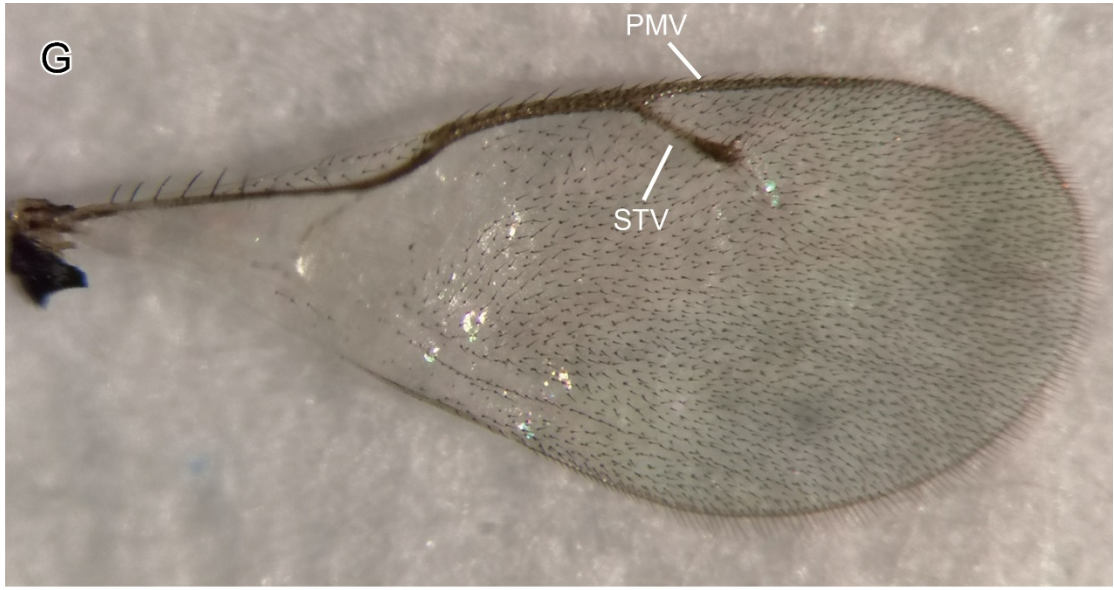


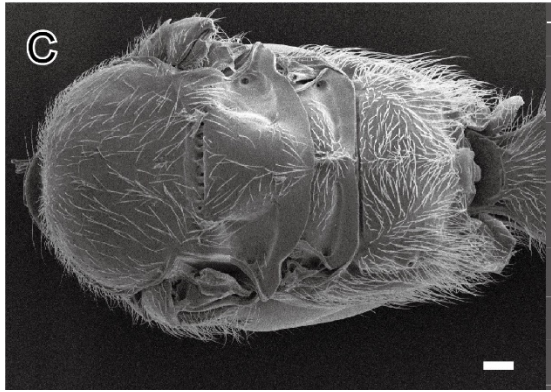
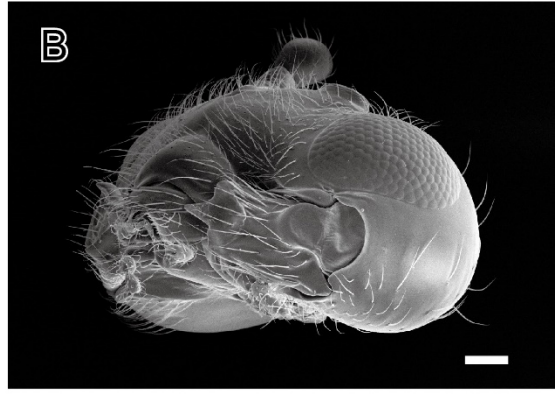
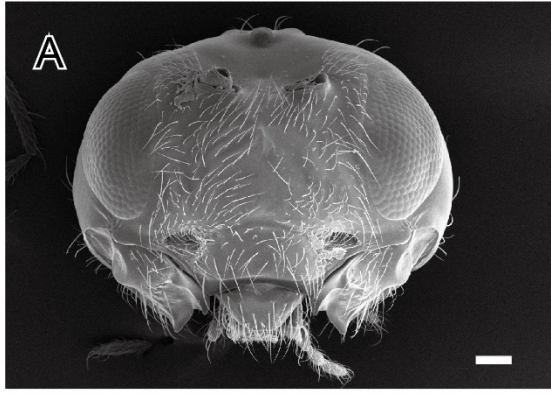
Figure S6. *Trigonogastrella parasitica*. (A) ♂ head; (B) ♂ occiput; (C) ♂ mesosoma, dorsal view; (D) ♂ mesosoma, lateral view; (E) ♂ petiole; (F) ♂ scape; (G) ♀ forewing; (H) ♂ antenna; (I) ♂ gaster. All scale bars are 50 µm. PMV=postmarginal vein; STV=stigmatal vein.

7. *Dacnusa areolaris* (Nees ab Esenbeck, 1812)

(Hymenoptera: Braconidae: Alysiinae)

Diagnostic characters (Griffiths 1966; Wharton & Austin 1991; Fisher *et al.* 2005; Berry 2007)

1. Mandibles exodont - pointing outwards and not coming close to meeting medially (Fig.S7-A).
2. Mandible with 3 teeth; tooth 1 and 3 rounded, tooth 2 long and acutely pointed (Fig.S7-B).
3. Mesonotum, metapleuron, and petiole densely setose (Fig. S7-C; S7-D)
4. Forewing: stigma long and narrow, at least 10 times longer than wide; vein *r* arising in basal 1/5 of stigma (Fig. S7-E).
5. Hindwing: 1st sub-basal cell slightly more than 1/2 the length of the basal cell (Fig. S7-F).



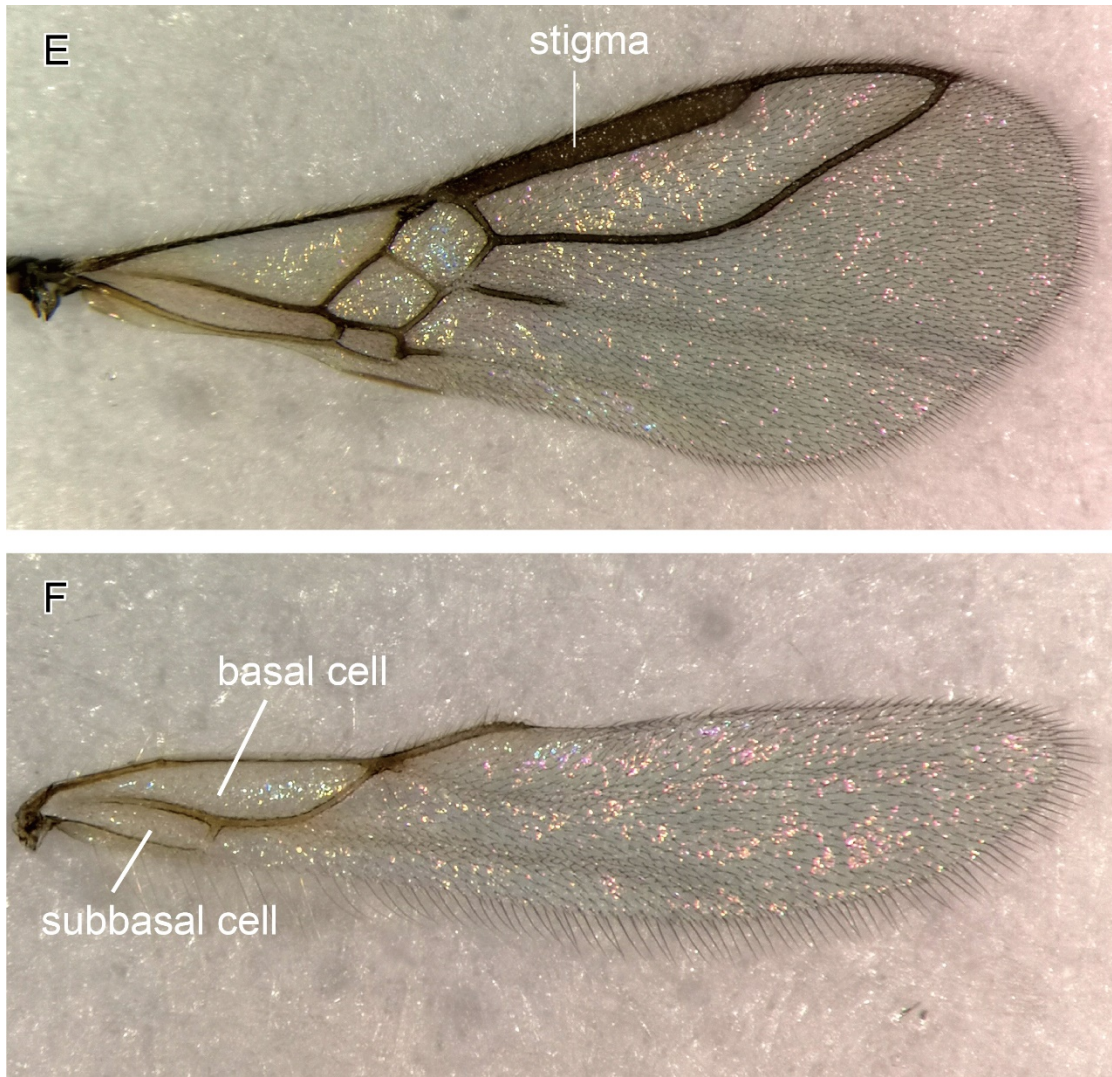


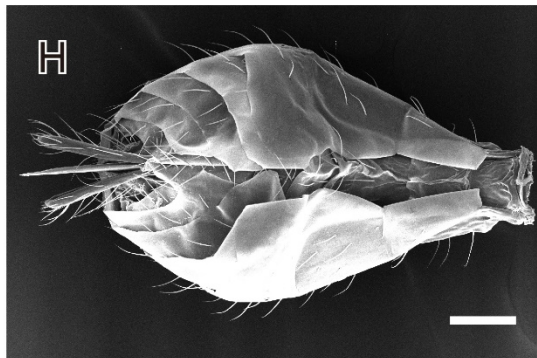
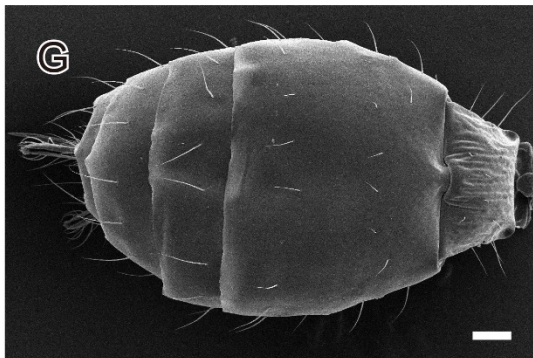
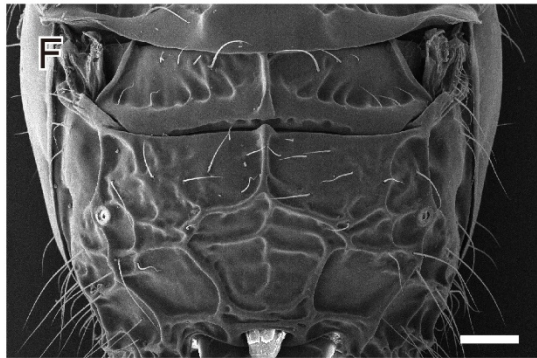
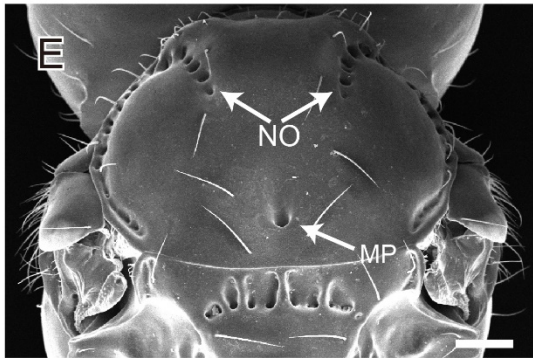
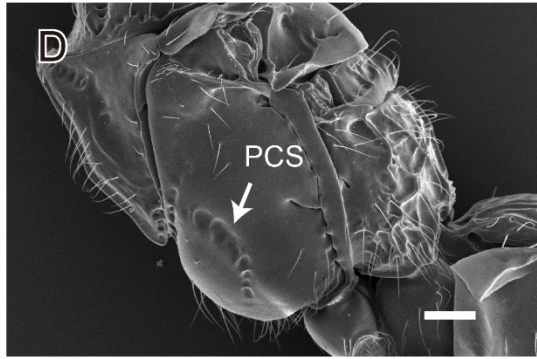
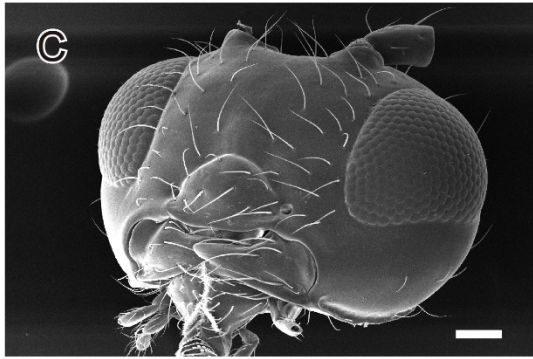
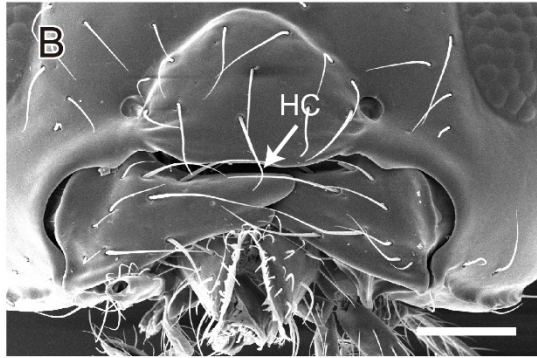
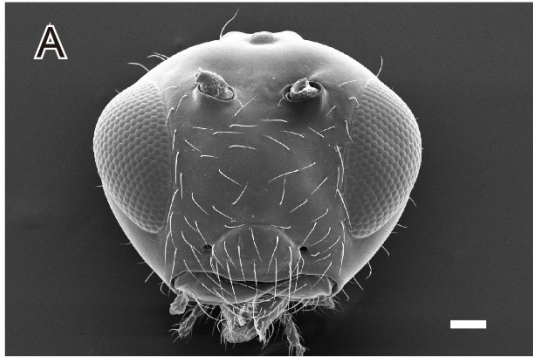
Figure S7. *Dacnusa areolaris*. (A) ♂ head; (B) ♂ mandible; (C) ♂ mesonotum; (D) ♂ lateral view of body showing metapleural setae; (E) ♂ forewing; (F) ♂ hindwing. All scale bars are 50 μm .

8. *Opius cinerariae* Fischer 1963

(Hymenoptera: Braconidae: Opiinae)

Diagnostic characters (Belokobylskij *et al.* 2004)

- i. Gap between clypeus and mandible absent or narrow (Fig. S8-A, Fig. S8-B) when mandibles are closed.
- ii. The malar suture is absent (Fig. S8-C).
- iii. Precoxal sulcus (PCS) deep and distinctly sculptured (Fig. S8-D).
- iv. Mesonotum with distinct median pit (MP) and the notauli (NO) are sculptured over the anterior half of the mesoscutal disc (Fig. S8-E).
- v. Propodeum rugose (Fig. S8-F).
- vi. First metasomal tergite densely striate; second metasomal tergite very finely coriaceous; remaining tergites smooth (Fig. S8-G, Fig. S8-H, Fig. S8-I, Fig. S8-J)
- vii. The radial cell of forewing is shortened (Fig. S8-K)
- viii. Nervellus vein (cu-a) and submedial (sub-basal) cell of hind wing absent (Fig. S8-L).



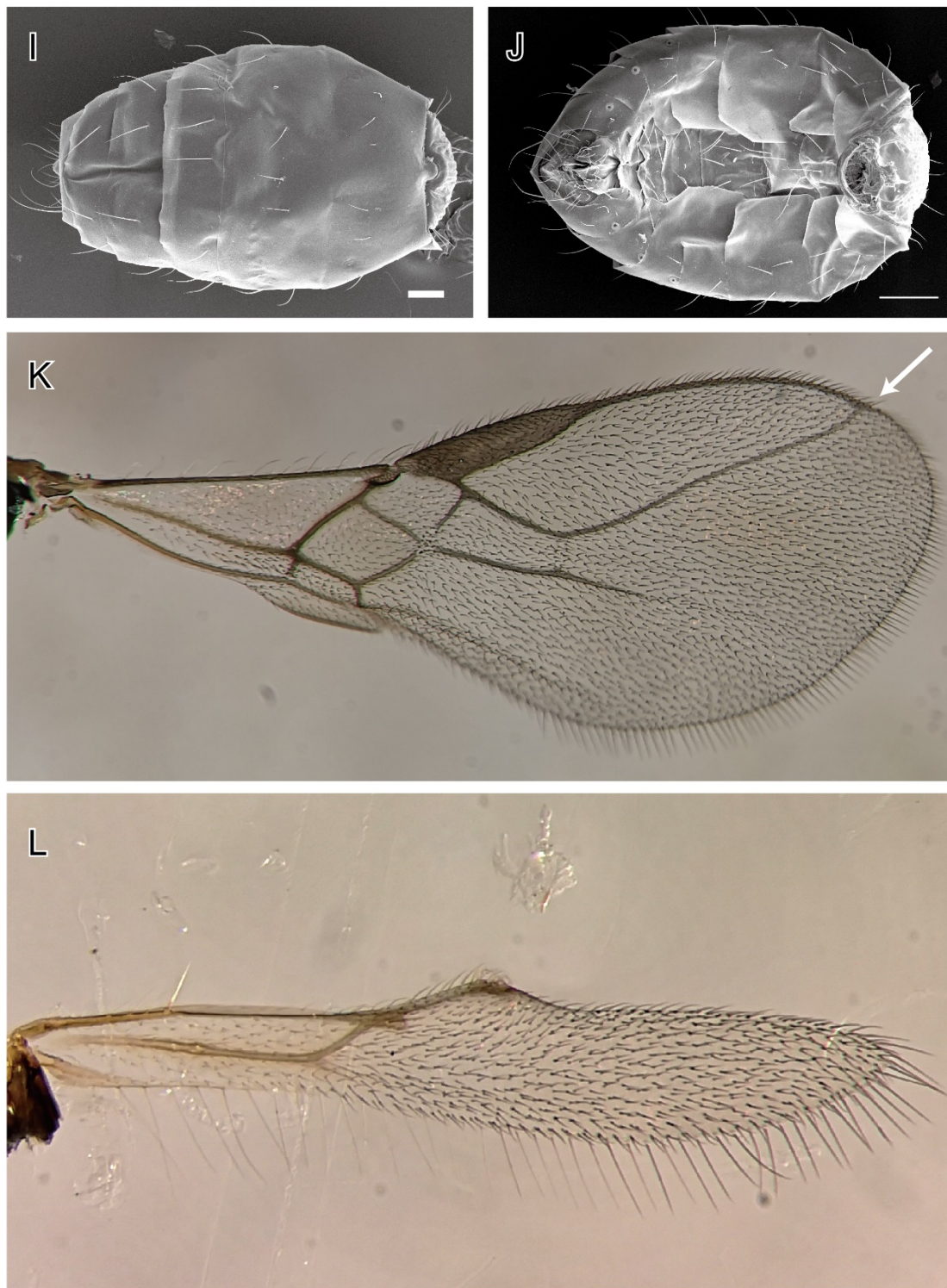


Figure S8. *Opius cinerariae*. (A) ♀ head, frontal view; (B) ♀ clypeus and mandibles showing hypoclypeal cavity; (C) ♀ head, lateral view (no malar sulcus); (D) ♂ mesopleuron; (E) ♂ mesonotum showing median pit and notauli; (F) ♂ rugose propodeum; (G) ♀ metasoma (dorsal); (H) ♀ metasoma (ventral); (I) ♂ metasoma

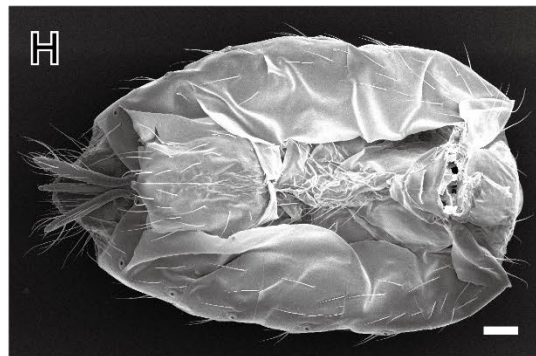
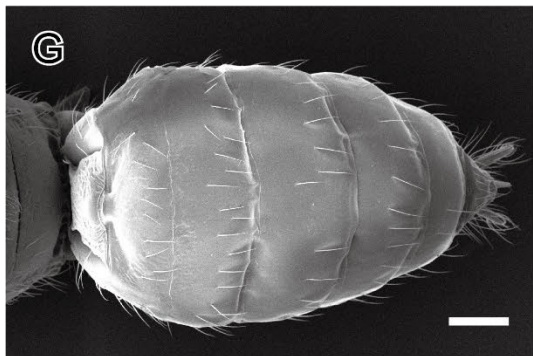
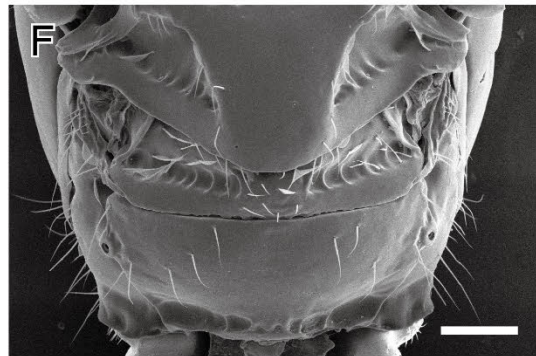
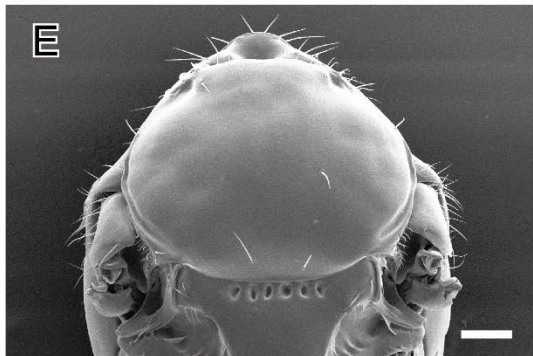
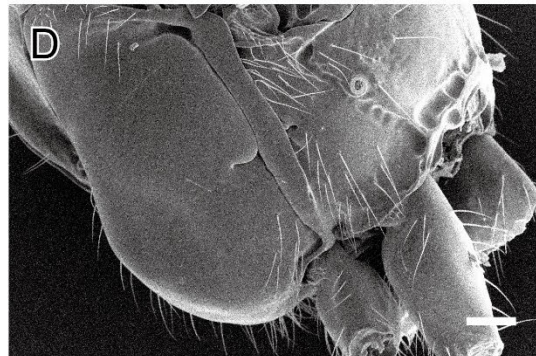
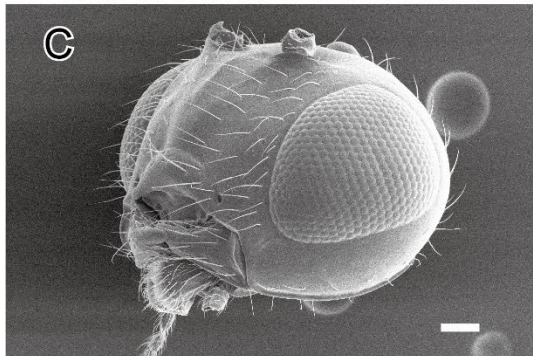
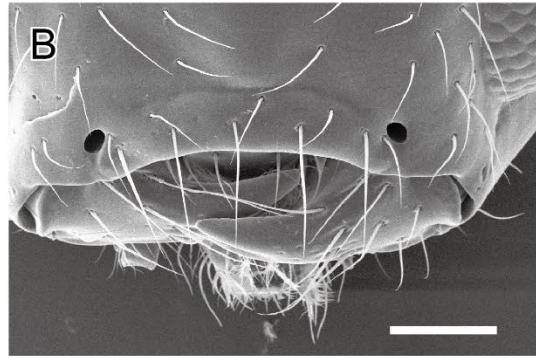
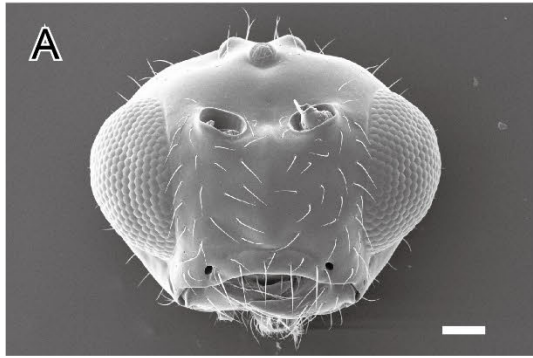
(dorsal); (J) ♂ metasoma (ventral); (K) ♀ forewing; (L) ♂ hindwing. All scale bars are 50 µm. HC =hypostomal cavity; NO =notaulus; MP =median pit on mesonotum; PCS=precoxal sulcus.

9. *Opius* sp. 1

(Hymenoptera: Braconidae: Opiinae)

Opius sp.1 is identical to *Opius* morphospecies 1 (Coquilleau 2020). It is very close to *Opius atricornis* Fischer (Belokobylskij *et al.* 2004).

- i. Clypeus narrow and labrum broadly exposed below clypeus (Fig. S9-A, Fig. S9-B) when mandibles are closed.
- ii. The malar suture is absent (Fig. S9-C).
- iii. Precoxal sulcus indistinct (Fig. S9-D).
- iv. Mesonotum without distinct median pit and the notauli are not sculptured over the anterior half of the mesoscutal disc (Fig. S9-E).
- v. Propodeum unsculptured and not rugose (Fig. S9-F).
- vi. First metasomal tergite is not densely striate; second and third metasomal tergites are smooth and polished (Fig. S9-G, Fig. S9-H, Fig. S9-I, Fig. S9-J)
- vi. The radial cell of the forewing is not shortened cf. *O. cinerariae*. The 3M vein is tubular and extends nearly to the margin of the wing; the 2CUb vein is visible for half of the distance to the margin of the wing (Fig. S9-K).
- vii. Nervellus vein (cu-a) and submedial (sub-basal) cell of hind wing absent (Fig. S9-L).
- viii. Body and head burnt orange, majority of the mesoscutum shiny black, with some variation to a lighter brown colour. Antennae brown, proximal half of the scape burnt orange. Coxae and legs, first and second metasomal tergites are pale orange, yellowish. Remaining tergites are brown. (Fig. S9-M)



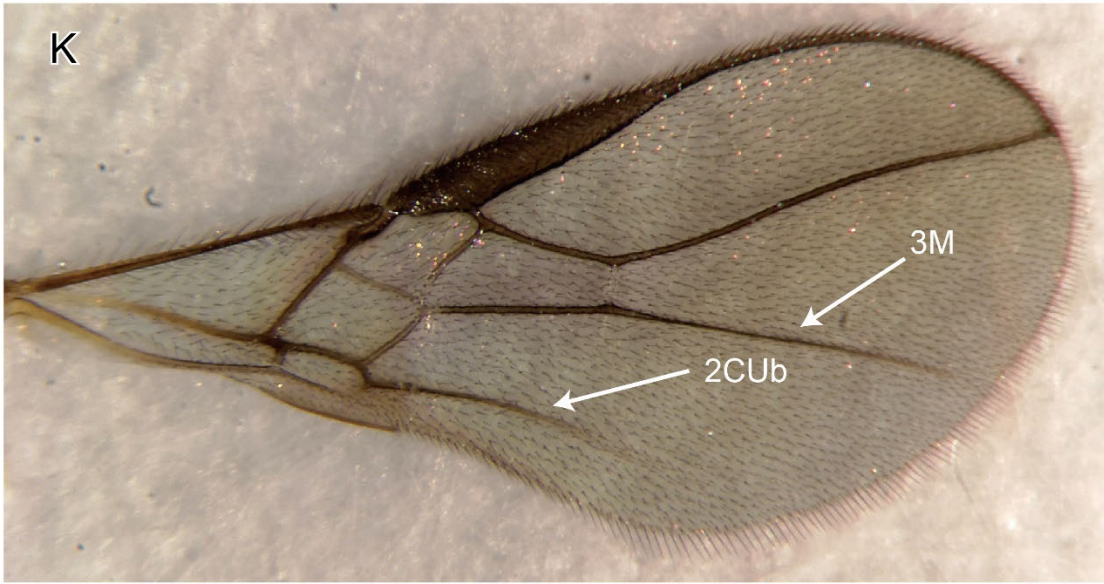
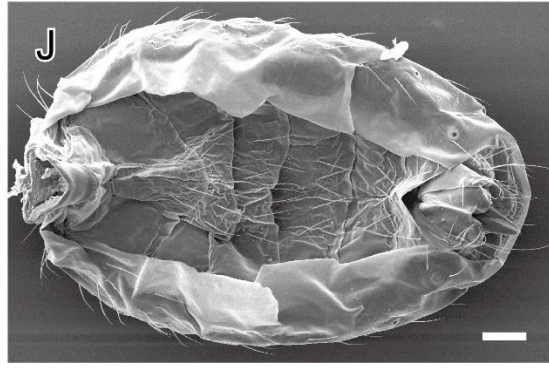
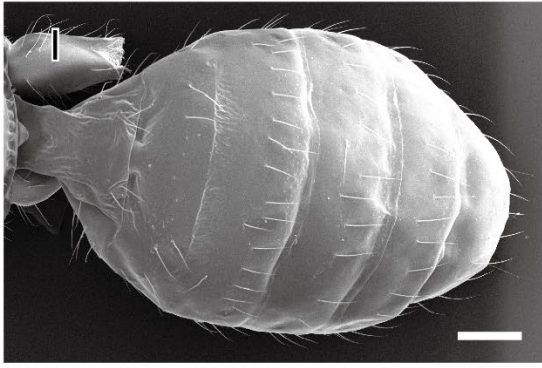




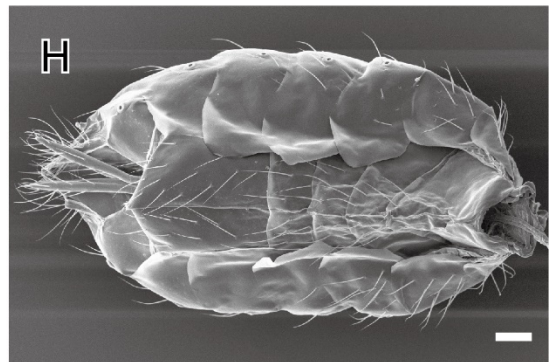
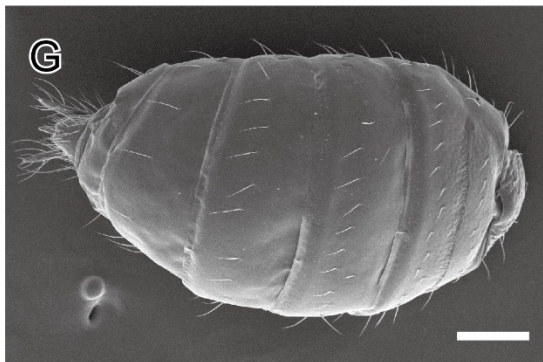
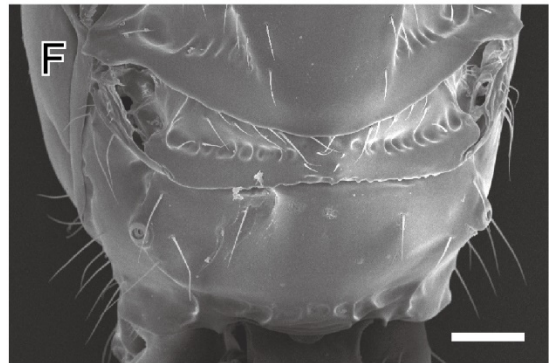
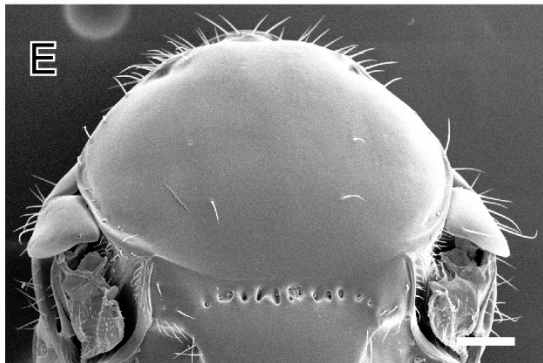
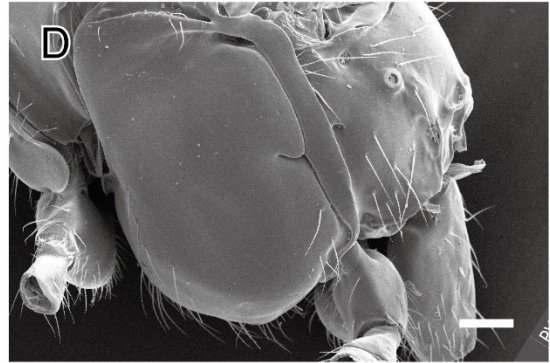
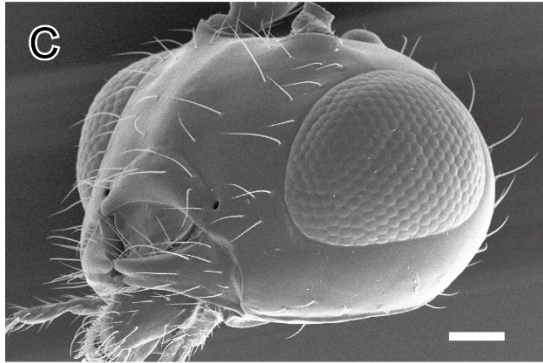
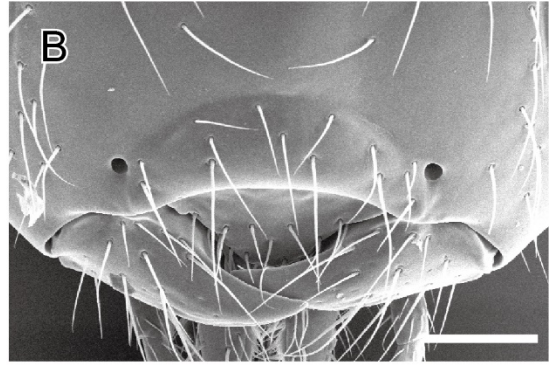
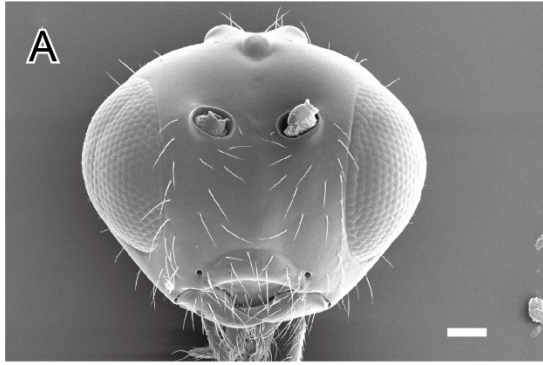
Figure S9. *Opius* sp. 1. (A) ♀ head, frontal view; (B) ♀ clypeus narrow and crescent shaped, mandibles, hypoclypeal cavity; (C) ♀ head, lateral view; (D) ♀ mesopleuron; (E) ♀ mesonotum; (F) ♀ propodeum unsculptured; (G) ♀ metasoma (dorsal); (H) ♀ metasoma (ventral); (I) ♂ metasoma (dorsal); (J) ♂ metasoma (ventral); (K) forewing; (L) hindwing; (M) ♂ habitus. All scale bars are 50 µm.

10. *Opius* sp. 2

(Hymenoptera: Braconidae: Opiinae)

Opius sp. 2 is identical to *Opius* morphospecies 2 (Coquilleau 2020). It differs from *Opius* sp. 1 primarily by colour and the transparency of some veins in the forewing.

- i. Clypeus narrow and labrum broadly exposed below clypeus (Fig. S10-A, Fig. S10-B) when mandibles are closed.
- ii. The malar suture is absent (Fig. S10-C).
- iii. Precoxal sulcus indistinct (Fig. S10-D).
- iv. Mesonotum without distinct median pit and the notauli are not sculptured over the anterior half of the mesoscutal disc (Fig. S10-E).
- v. Propodeum unsculptured and not rugose (Fig. S10-F).
- vi. second and third metasomal tergites are smooth and polished (Fig. S10-G, Fig. S10-H, Fig S10-I, Fig. S10-J).
- vi. The radial cell of forewing is not shortened cf. *O. cinerariae*. The 3M vein is nebulous and foreshortened; the 2CUb vein is nebulous (Fig. S10-K).
- vii. Nervellus vein (cu-a) and submedial (sub-basal) cell of hind wing absent (Fig. S10-L).
- viii. Body shiny black. Antennae and scape brown. Coxae and legs yellowish. First mesosomal tergite with light brown tinge, remaining tergites brown nearly black (Fig. S10-M)



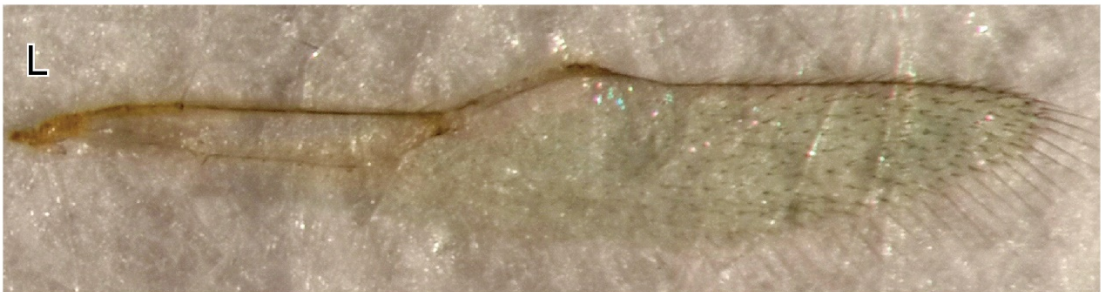
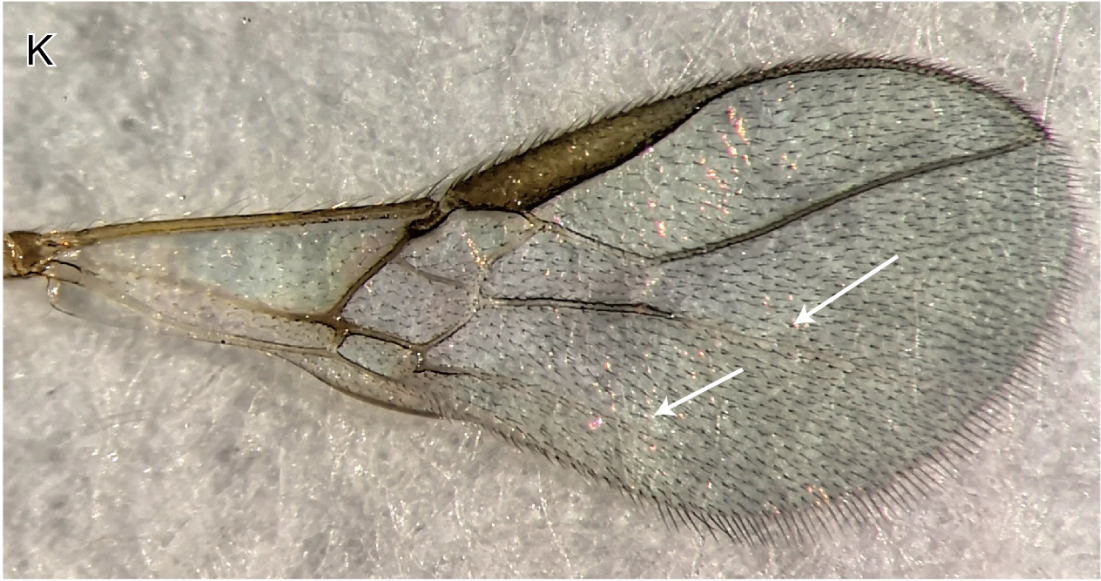
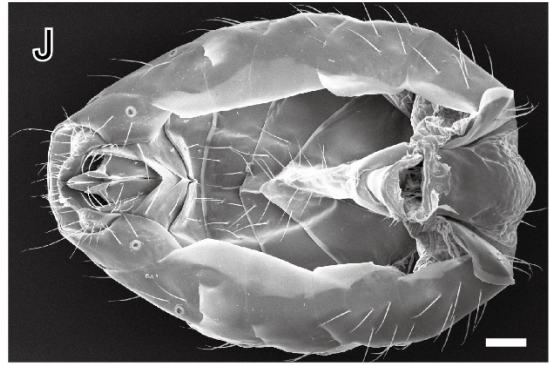
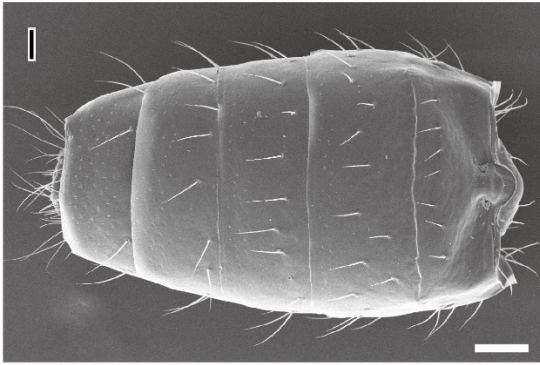




Figure S10. *Opius* sp. 2. (A) ♀ head, frontal view; (B) ♀ clypeus narrow and crescent shaped, mandibles, hypoclypeal cavity; (C) ♂ head, lateral view (no malar sulcus); (D) ♂ mesopleuron; (E) ♂ mesonotum (no medial pit); (F) ♂ propodeum; (G) ♀ metasoma (dorsal); (H) ♀ metasoma (ventral); (I) ♂ metasoma (dorsal); (J) ♂ metasoma (ventral); (K) ♀ forewing; (L) ♀ hindwing; (M) ♂ habitus. All scale bars are 50 µm.

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