ENTOMOLOGY

A new species of *Aprostocetus* (Hymenoptera: Eulophidae) gall inducer on *Astragalus alpinus* from Norway

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Abstract

A new species of *Aprostocetus* (Hymenoptera: Eulophidae), gall inducer on *Astragalus alpinus* (Fabaceae), is described from Norway. The eulophid causes an irregular round or spindle-shaped gall on stem, which shows a single internal cavity for the gregarious larvae. One generation is developed from spring to spring. The parasitoids *Macroneura vesicularis* (Retzius) and *Eurytoma* sp. emerged from the gall.

Introduction

Gall induction in the family Eulophidae (Hymenoptera: Chalcidoidea) has been summarized by Gates *et al*. (2020). Phytophagous species are listed in the subfamilies Opheliminae and Tetrastichinae (La Salle, 2005). Within the largest genus of Tetrastichinae, *Aprostocetus* Westwood, gall induction is rather rare, only five documented cases worldwide are documented (Gates *et al*., 2020).

In July 2019 the first author (A.F.) discovered some stem galls on *Astragalus alpinus* (Fabaceae) and supposed that the agent was probably a gall midge (Diptera: Cecidomyiidae) or perhaps an Agromyzidae. He dissected several galls, but no larvae of Diptera were found. In the single cavity of the gall several larvae of Hymenoptera were detected. From overwintering galls kept outdoor several Hymenoptera Chalcidoidea emerged, but the most abundant was an Eulophidae, tentatively identified as *Aprostocetus* sp. Several biological traits of the species prove that the species is a gall inducer. The species is here described as new and compared with the known species of *Aprostocetus* gall inducers on Fabaceae in Europe.

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Materials and Methods

The first stem galls on *Astragalus alpinus* were discovered in July 2019. Some were dissected and the larvae found inside were collected and preserved in alcohol 70%. In September 2019 other galls were collected and maintained in vials outdoor. From these galls specimens emerged in the spring 2020. They were all Hymenoptera Chalcidoidea. Specimens were collected and preserved in alcohol 70%. Some of the specimens were mounted on pin card and other mounted on slide. Adults and larvae to be slide mounted were placed in a 10% KOH solution and, after washing them in water, they were immersed in glacial acetic acid. Subsequently they were transferred in a solution of balsam phenol 50% and 100%. In the latter solution the specimens were dissect when needed. The dissected parts were mounted on slides using Canad balsam as permanent medium. The stay of mounting material in each of the mentioned solutions was longer for the adults in KOH solution (1-3 hours) and not more than a few minutes for the other steps.

The terms used in the description follow Graham (1987) for the adult and Viggiani (2015), for the larva.

Taxonomy

Aprostocetus (*Aprostocetus*) *astragali* Viggiani, sp.n.

DIAGNOSIS. Female (Figure 1A) of this species differs from the known European *Aprostocetus* gall inducers on Fabaceae and the

other described species of the genus by having the following combination of characters: antenna with 3-segmented funicle, F2 slightly longer than F1 and F3, each segment with 6-7 linear sensilla in two irregular rows, clava 3-segmented; mesosoma 0.5× as long as gaster, mid lobe of mesoscutum without a median line, with 5-6 adnotaular setae in one row on each side; propodeum with 2 setae on callus; fore wing 2.5× as long as wide, submarginal vein with 4-5 dorsal setae, marginal vein 3.8× as long as stigmal vein, subcubital line of setae reaching the distal base of speculum, discal frange with longest setae 1/5 of the stigmal vein; gaster narrow and acuminate, 5× as long as wide, with long and narrow postcercale, cerci with one longest seta, ovipositor robust, exserted about 0.15× of the gaster length. Body black with slight metallic reflections. Male with ventral plaque 1/3 as long as scape, funicle 4-segmented, with F1 shorter than F2, and clava 3-segmented with whorls of long setae on each segment; gaster oblong slightly longer than mesosoma, genitalia with three teeth on digitus.

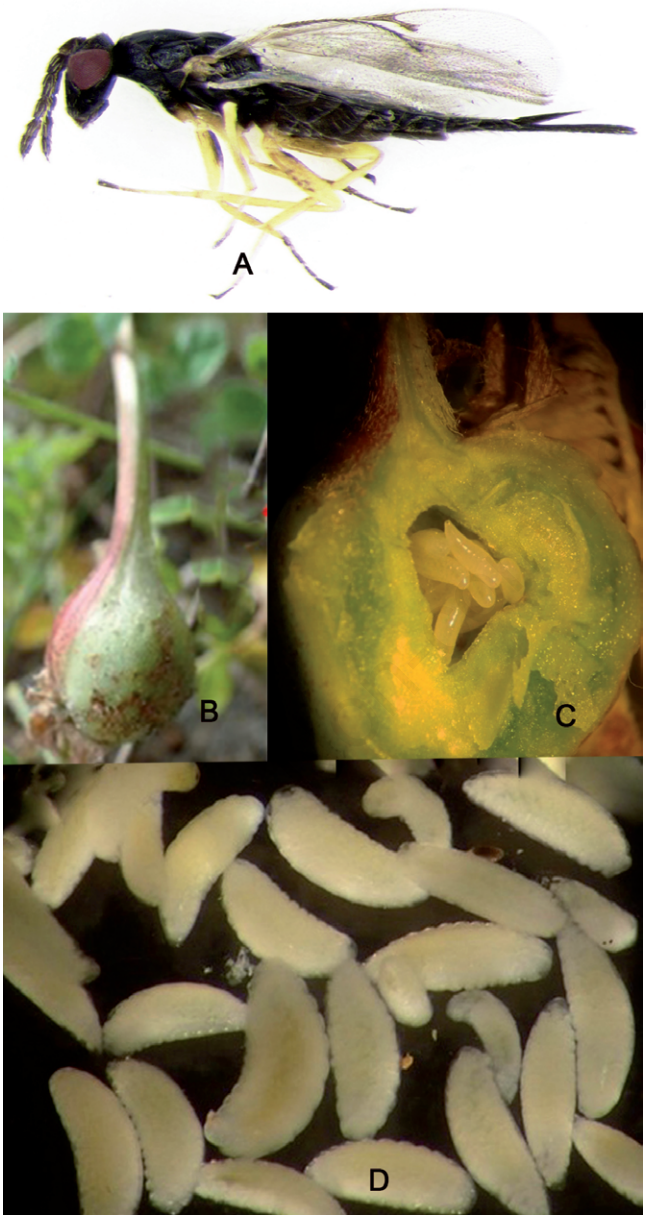

Figure 1. A) *Aprostocetus astragali* sp. n. female; B) stem gall on *Astragalus alpinus*; C) dissected gall; D) larvae of *Aprostocetus astragali*.

DESCRIPTION. Female. Body black with slight metallic reflections; eyes red; antennae black; tegulae yellow; dorsellum concolor with body or yellowish; fore wings hyaline with light brown veins; legs yellow, with fore legs having coxae basally black, femora in the basal half with brown, tibiae with the internal margin brown; tarsi brown; middle legs with coxae black, femora with basal half brown, distal 1-2 tarsomeres brown; hind legs as the middle legs, but tibiae without brown. Length: 1.8-2.4 mm (average: 2.22; SD: ±0.187; n: 10).

Head slightly wider than thorax (13:12), about 2× as wide as long; width slightly broader than height; POL 1.3× as OOL; malar space 0.7× height of eye (distance between dorsal and ventral margin; eye length slightly longer than malar space (7:6); mouth opening) as wide as malar space; malar sulcus straight without a fovea. Margin of clypeus in the middle bilobed (Figure 2A). Mandible (Figure 2B) with 2 teeth and a truncation. Maxillary palp (Figure 2C) unisegmented, 4-5× as long as wide. Antenna (Figure 2D) with

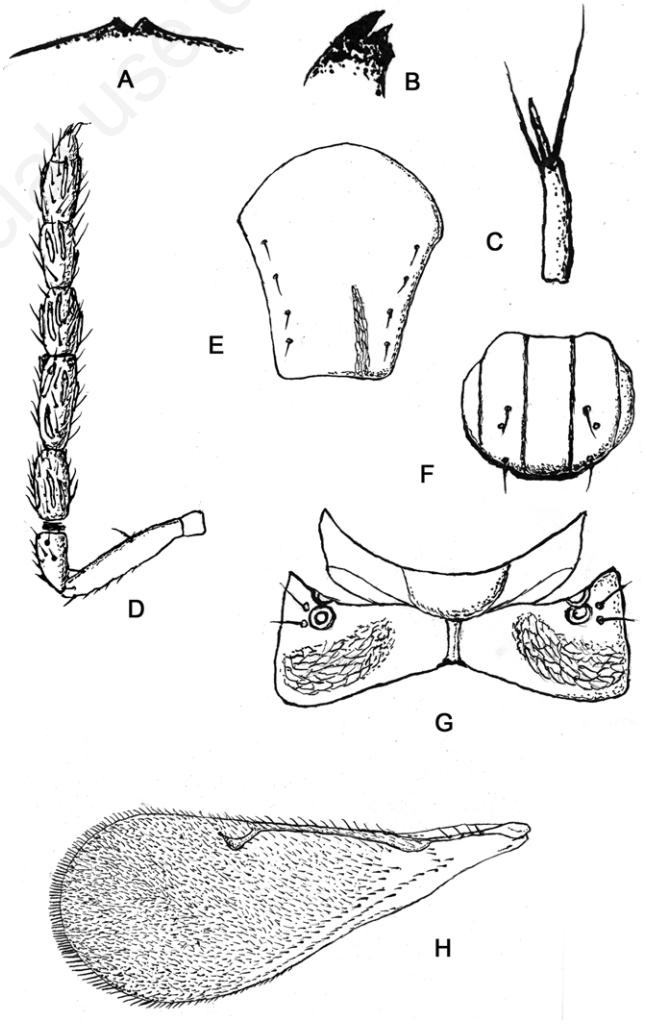

Figure 2. A) *Aprostocetus astragali* sp. n., female, margin of clypeus; B) mandible; C) maxillary palp; D) antenna; E) mid lobe of mesoscutum; F) scutellum; G) metanotum and propodeum; H) fore wing.

pedicel plus flagellum 1.3-1.6× the width of mesoscutum, scape slight shorter than eye height (7:8), about as long as eye length, just reaching vertex or slightly below, 4× as long as wide, with a row of short setae on dorsal margin and 2 setae ventrally; pedicel 0.5× as long as scape, 0.8× as long as F1; 2-3 discoidal anelli, very short; funicle 3-segmented, each segment about 2× as long as wide, F2 slightly longer than F1 and F3, each segment with 6-8 linear sensilla in two irregular rows and short setae; clava 3-segmented, 0.6× as long as funicle, C1 as long as C2, sligthly shorter than F3 (15: 17) with linear sensilla and setae as on funicle segments, C3 small, 0.4× as long as C2, with terminal spine, 1/3 as long as the corresponding segment with a short apical seta. Mesosoma 3× as long as head, 0.5×as long as gaster, moderately arched; pronotum short, campaniform; mid lobe of mesoscutum (Figure 2E) about as long as wide, without a median line and showing a superficial reticulation with engraved, elongate areoles, about 2× as long as wide, with 4-6 adnotaular setae in one row on each side; scutellum (Figure 2F) shorter than mid lobe of mesoscutum (7:9) with 2+2 setae, submedian lines not or only slightly nearer to sublateral lines than to each other; dorsellum short, about 2× as wide as long; propodeum (Figure 2G) in the middle as long as dorsellum, deeply emarginated, median carena slightly raised, spiracle of moderate size, almost touching metanotum, callus with 2 setae. Fore wing (Figure 2H) 2.5× as long as wide, costal cell narrow, with a row of 3-4 ventral setae at distal end, submarginal vein 0.6× as long as stigmal vein, with 4-5 dorsal setae, marginal vein moderately enlarged at base, 3.8× as long as stigmal vein, with short front setae, premarginal vein shorter than stigmal vein (3:5), discal ciliation rather dense, speculum extending at the base of the marginal vein, postmarginal vein very short, not longer than 1/5 of the stigmal vein, subcubital line of setae reaching the distal base of speculum, fringe very short, longest setae 1/5 of the stigmal vein. Hind wing distally pointed, with disc rather densely ciliated, from the base of the marginal vein, longest setae of the fringe 1/3 of the discal width. Legs normal, with basitarsus slightly shorter than the following segment (fore, 2:3; middle and hind, 3:4), middle spur about as long as corresponding basitarsomere.

Gaster (Figure 3A) narrow and acuminate, 5× as long as wide, 1.4-1.6 as long as head plus mesosoma, with a long and narrow postcercale, 0.34-0.46× the pre-cerci gaster length, 5× as long as inter-cerci distance, cerci with one longest seta, straight or slight kinked, 2× of the next longest; petiole very short, transverse; tergites with a reticulate sculpture, more engraved laterally and with rather short setae; hypopygium (Figure 3B) with tip placed at 0.27 lenght of gaster, bilobed and with 2 pairs of setae in the middle of distal margin; ovipositor robust, inserted at base of gaster and slightly exerted, about 0.15× of the gaster length; postcercale 1.6× exerted part of ovipositor sheaths; postcercale+ ovipositor sheaths as long as hind tibia.

Male. Colour and main features as female, but antenna (Figure 3C) with scape 3×as long as wide, ventral plaque 1/3 as long as scape, placed mostly below the middle on the ventral margin, with row of short setae on dorsal margin and ventrally with a subapical seta and 2 setae near the base of the ventral plaque; funicle 4-segmented, F1 shorter than pedicel (9: 12), F2 twice as long as F1, 2.2× as long as wide, F3 and F4 same size of F2; C1 shorter than F4 (13:18), C2 slightly longer than C1 (15:13), as long as F4, C2 as long as F1, C3 shorter than C1 (9:13), apical spine straight, short, 1/4 than C3, apical seta about as long as apical spine; whorls of long setae on funicle and club segments, except C3, with setae about twice as long as F2, and 2-3 linear sensilla on each segment.

Gaster oblong, slightly longer than mesosoma; genitalia (Figure 4A), 4× as long as wide, digitus (Figure 4B) with three teeth.

Type material. Holotype (on slide) ♀, Norway, Lom, NW of Årsjø, 61.8455°N, 8.5774°E, laboratory emergence, iv. 2020, ex galls on *Astragalus alpinus* collected in ix.2019, A. Fjellberg leg. Paratypes. 6 ♀ (3 on slide and 3 on pin card), 1 ♂ (on slide), same data as the holotype.

Additional material. 3 larvae on slide.

Holotype and paratypes will be preserved in the entomological collection of the Dipartimento di Agraria, Università degli Studi di Napoli " Federico II", located in Portici (NA).

Distribution. Europe, Norway. Apart from the type locality, similar stem galls on *Astragalus alpinus*. were also observed by the first author along roadsides in Vågå, about 30 km east of Lom (61.86855°N, 9.07213°E). These localities are situated in the central part of South Norway having a continental climate with low precipitation and cold winters. The type locality is situated 365m asl., with annual mean temperature 2.6°C and annual mean precipitation 321 mm. The host plant typically grows as prostrate cushions on very dry ground in scree slopes, river banks, roadsides and dry meadows. The type locality is originally a fluvial deposit of sand and gravel along the Otta River, now a ruderate area with disturbed ground and patchy vegetation .

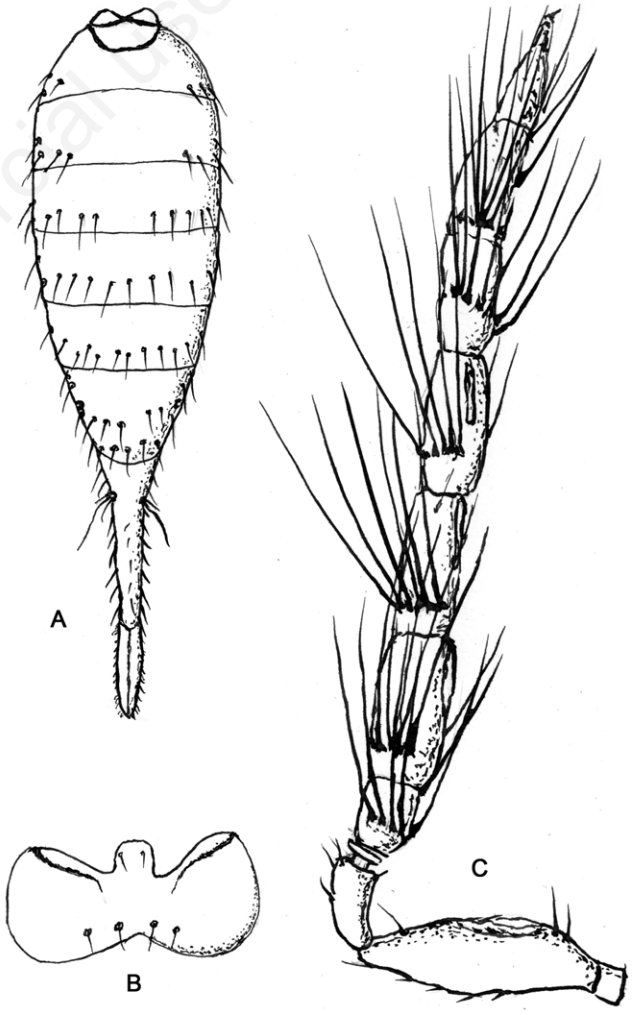

Figure 3. A) *Aprostocetus astragali* sp. n., gaster of the female; B) hypopygium of the female; C) antenna of the male.

Etymology. The species epithet is derived from the host name.

Remarks. The new species, according to the key in Graham (1987), is identified as *Aprostocetus* (subgenus *Aprostocetus*). Among the 9 species-groups recognized by the same author, *A. astragali* shares several characters with the species included in the *caudatus*-group, mostly parasitoids of Diptera Cecidomyiidae. The new species runs near *A. anodaphus*, *A. cultratus*, *A. salictorum* and *A.verutus* in having the female with rather long antennae and acuminate gaster, and male with sensorial plaque on the scape placed mostly below the middle of the ventral margin, funicle with F1 shorter than F2, all funicular segments with a whorl of long setae. However, in female of *A. anodaphus* the postcercale + ovipositor sheaths are 0.45-0.75× as long as hind tibia (in *A. astragali* postcercale + ovipositor sheaths as long as hind tibia); in *A. cultratus* the hypopygium reaches 0.60-0.65 the length of gaster, postcercale no longer than 2× as long as wide (in *A. astragali*, tip of hypopygium at 0.27 length of gaster, postcercale 5× as long as wide; in *A. salictorum* the postcercale + ovipositor sheaths are 0.5-0.7 as long as hind tibia and body with distinct olive-green to bluish to olive metallic tints (in *A. astragali* postcercale + ovipositor sheaths as long as hind tibia and body black); in *A. verutus* the postcercale is subequal in length to the exerted part of the ovipositor sheaths (in *A. astragali* postcercale 1.6× the exerted part of the ovipositor sheaths). Of the mentioned species the male is known only for *A. anodaphus*, *A. salictorum*, and *A. verutus*. In *A. astragali* the position and the length of the ventral plaque on the scape are more similar to that of *A. anodaphus*, but the ventral plaque is not completely in lower half of the ventral margin. All species show digitus with 2 teeth and other differences with *A. astragali*. In the *caudatus* -group only *A. zosimus* (Walker) has digitus with 3 teeth as *A. astragali*, but funicular segments F2-F4 are longer (3-4 times as long as broad) as the ventral plaque on the scape (0.65-0.75 length of scape).

After Graham (1987) about 160 new species of *Aprostocetus* were described worldwide; of them 41 from Europe (Noyes, 2021). Based on the comparison of their description, in several cases incomplete, none of this species fits with *A. astragali*. In fact, the new species is recognized mostly on the comparison with the two species of *Aprostocetus* known as gall inducer on Fabaceae in Europe: *A.gallicolus* Nieves-Aldrey & Askew (2011) and*A. monacoi* Viggiani, (2014); both differ markedly from *A. astragali* in having an ovate gaster. The female (male unknown) of *A. bakkendorfi* Graham, (1987), from Denmark, reared from galls on *Astragalus glycyphyllos*, differs mostly from *A. astragali* in having shorter flagellar segments of antennae and gaster.

Final-instar larva

Body whitish, with rather marked segmentation, without setae, length 1.5-1,8 mm (Figure 1D).

Head small with reduced sclerotization, only epistoma visible; mandible triangular (Figure 4C), sharply pointed. Respiratory system with 9 pairs of spiracles, 2 on thorax (segments II and III) and 7 on abdomen (segments I-VII); the pair on the abdominal segment I (Figure 4D) in some specimens with opening smaller than on the following segments (Figure 4E).

The gall

The galls are irregularly roundish to spindle-shaped (Figure 1B) and usually located on the lower part of the stem, just above ground. The walls are hard, surrounding a single cavity.

Biologal notes

In the first galls, collected in July 2019, gregarious larvae, up to 10 in the single gall cavity were found (Figure 1C). The presence of associated fungi was not observed. From the galls collected in September and kept outdoor until end of March, when they were brought indoor at room temperature for hatching, the adults of *A. astragali* start to emerge after 1-2 weeks. According to our observations *A. astragali* overwinters as larva in the galls caused in the spring of the previous year. The adult emergence takes place in

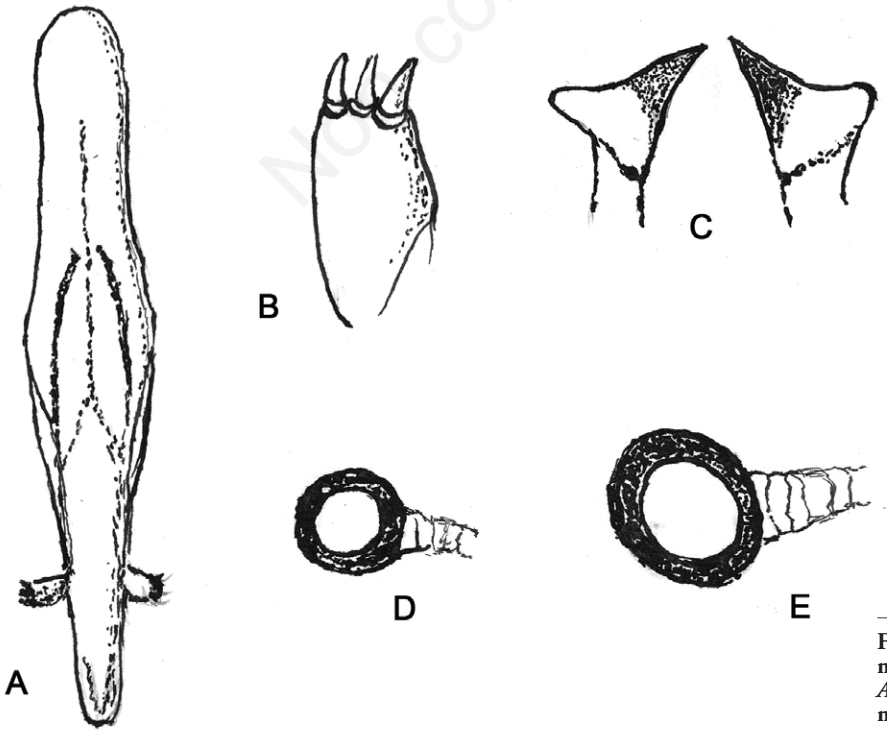

Figure 4. A) *Aprostocetus astragali* sp. n., male genitalia; B) volsellar digitus; C) *Aprostocetus astragali* sp. n., final-instar larva mandibles; D) 3° spiracle; E) 4° spiracle.

April-May, when the host plant is ready to be colonized. This biological correlation supports the gall inducer behaviour of the eulophid.

Parasitoids

Larvae of parasitoids were frequently found in the galls, sometimes killing all larvae of *Aprostocetus* in a single gall. They were characterized by a distinct head capsule and rather long setae on the body. Adults of *Macroneura vesicularis* (Retzius) (Eupelmidae) and *Eurytoma* sp. (Eurytomidae) emerged. The first species was recorded also as parasitoid of *Aprostocetus monacoi*, gall inducer on *Melilotus indicus* (Viggiani & Monaco, 2014).

Conclusions

Galls on *Astragalus* species are caused by several organisms, mostly by Diptera Cecidomyiidae, but the agent for several of them remains unknown (Roskam, 2020). The present paper reports for the first time that a species of *Aprostocetus* is gall inducer of stem gall on *Astragalus alpinus*. This behaviour is supported by the absence of other gall inducers (mostly Diptera Cecidomyiidae), larval abundance of the eulophid in the galls, in its single annual generation, and in the emergence of the adults in the spring, when the host plant phenology allows gall induction. The new species shows important morphological differences from the other known species of *Aprostocetus* causing stem galls on Fabaceae, namely the long gaster and ovipositor. The gall induced by *A. astragali* is unilocular, as that of *A. gallicolus*; on the contrary in *A. monacoi* it is plurilocular (Nieves-Aldrey & Askew, 2011; Viggiani & Monaco, 2014). The final-instar larva of *A. astragali* is very similar to that of *A. monacoi* Viggiani, 2015, the only known in some details The biology of the three known species gall inducers on Fabaceae in Europe is very similar; there is one generation per year and the adult emergence of the gall inducer eulophid gall takes place in spring.

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