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New trophic associations for Charipinae from Spain

(Hymenoptera, Cynipoidea, Figitidae)

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Trophic associations where Charipinae are involved are still very unknown. It has been evidenced that the presence of the Charipinae affects the abundance of the primary parasitoids and thus the abundance of the aphids on crops, which are one of the most important pests. For this reason, working on these trophic relations is so important. Here, we give information of new trophic associations for the species *Alloxysta arcuata* (Kieffer, 1902), *A. mullensis* (Cameron, 1883) and *A. pleuralis* (Cameron, 1879). These species have a wide distribution, in this case they have been collected in different provinces of Spain. This is the first time that *A. arcuata* is recorded on *Liosomaphis berberidis* (Kaltenbach, 1843) through *Aphidius hortensis* Marshall, 1896 and on *Therioaphis trifolii* (Monell, 1882) through *Trioxys complanatus* Quilis Pérez, 1931 and *Praon exsoletum* (Nees, 1811). *A. mullensis* is recorded on *Aphis gossypii* Glover, 1877 through *Trioxys angelicae* (Haliday, 1833) and *Lysiphlebus testaceipes* Cresson, 1880 and *A. pleuralis* is recorded on *Tinocallis saltans* (Nevsky, 1929) through *Trioxys tenuicaudus* Stary, 1978. DNA information of these species are given to facilitate future studies.

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Introduction

The Charipinae (Hymenoptera: Cynipoidea: Figitidae) are very small wasps (0.8–2.0 mm). They are mainly characterized by their smooth and shiny body, only one radial cell present in forewings and antennae filiform. Members of the subfamily Charipinae are widely distributed around the world (Ferrer-Suay et al. 2012a). They are biologically characterized being hyperparasitoids of aphids via Aphidiinae (Hymenoptera: Ichneumonoidea: Braconidae) and Aphelininae (Hymenoptera: Chalcidoidea: Aphelinidae) and hyperparasitoids of psyllids via Encyrtidae (Hymenoptera: Chalcidoidea) (Menke & Evenhuis 1991). *Alloxysta* Förster, 1869 and *Phaenoglyphis* Förster, 1869 are the most species rich, chaotic and widely distributed genera.

The impact of the Charipinae on the aphid biological control programs is very important, especially members of the *Alloxysta* Förster, 1869 and *Phaenoglyphis* Förster, 1869 genera. It has been evidenced that the presence of the Charipinae affects the abundance of the primary parasitoids and thus the abundance of the aphids on crops, which are one of the most important pests. Höller et al. (1993) and Mackauer & Völkl (1993) state that hyperparasitoids can also influence biological control of herbivores indirectly by modifying the behaviour of primary parasitoids. It was demonstrated that when hyperparasitoids are present, primary parasitoid females could abandon patches of their herbivore host without having exploited the resource completely, to minimize the mortality risk of their progeny (Ayal & Green 1993, Höller et al. 1993, 1994, Mackauer & Völkl 1993, Weisser et al. 1994, Petersen 2000).

The host specificity of these hyperparasitoids is still under debate. One view is that polyphagia in the Charipinae is limited to a few species, whereas most species are specialized to variable extent, with a specialization on a certain aphid/primary parasitoid combination at the extreme. The opposite view considers the Charipinae to contain relatively few species that are morphologically variable and that are fairly polyphagous. DNA analysis of one species group indicated that the latter point of view is incorrect and Charipinae are more diverse and more specialized (van Veen et al. 2003). In order to get a better idea of the grade of *Alloxysta* species-specificity, Ferrer-Suay et al. (2014) used the Jaccard index to determine the host range dissimilarity; the results of this index are given using a Cluster analysis. A routine was applied to analyse if the host range dissimilarity is significantly different from what is expected by chance. The level of specificity of each *Alloxysta* species was also evaluated with different indices and additional information for the matrices was also given: mean number of links, mean number of shared partners, niche overlap and extinction slope. As a result of this study, Ferrer-Suay et al. (2014) stated that primary parasitoid genera *Aphidius* Nees, 1818, *Lysiphlebus* Förster, 1862, *Praon* Haliday, 1833 and *Trioxys* Haliday, 1833 are the most common hosts for *Alloxysta* species, while among the aphids are *Aphis* Linnaeus, 1758, *Uroleucon* Mordvilko, 1914, *Myzus* Passerini, 1860 and *Sitobion* Mordvilko, 1914. The most specialized *Alloxysta* species based on specialization indices are *A. citripes* (Thomson, 1862), *A. halterata* (Thomson, 1862), *A. leunisii* (Hartig, 1841) and *A. ramulifera* (Thomson, 1862). *A. arcuata* (Kieffer, 1902), *A. brevis* (Thomson, 1862), *A. fuscicornis* (Hartig, 1841) and *A. victrix* (Westwood, 1833) are recognized as generalist species sharing most of their hosts.

The main purpose of this study is to provide information about the new relations recorded for some *Alloxysta* species collected in Spain. Barcodes of these species are also given to facilitate future studies.

Material and methods

The aphid colonies were collected in the context of different samplings in natural areas and in urban parks carried out by the second author throughout the Spanish territory. The mummies were conserved and the parasitoids and hyperparasitoids were fixed in 100 % ethanol as they emerged. The aphids were identified with the keys of Nieto Nafría and Mier Durante (1998) and Blackman and Eastop (2018) and the parasitoids with the key of Starý (1976) and Davidian (2005). Both, aphids and parasitoids are deposited in the Entomology Collection of the "Institut Cavanilles de Biodiversitat i Biologia Evolutiva" (ICBiBE).

Specimens of Charipinae were studied using stereomicroscopy (Leica MZ6). The field-emission gun environmental scanning electron microscope (FEI Quanta 200 ESEM) was used for high-resolution imaging without gold-coating of the specimens.

Morphological terms used are taken from Paretas-Martínez et al. (2007). Measurements and abbreviations include F1–F12, first and subsequent flagellomeres. The width of the forewing radial cell is measured from the margin of the wing to the beginning of the Rs vein. Females and males have the same morphology unless where indicated.

Host data follows: HP: host plant; HA: host aphid; HW: primary host parasitoid (wasp); when any of these categories is not known, "unknown" is inserted into the corresponding trophic level.

Total DNA was extracted separately from each individual that had been preserved in 100 % ethanol since the collection date. DNA was extracted following the HotSHOT (Hot Sodium HidrOxide and Tris) method (Truett et al. 2000) using 60 µl of both alkaline lysis and neutralizing reagents. A 710 bp fragment of the 5' region

of the mitochondrial gene coding the cytochrome c oxidase subunit 1 (COI) was amplified using primers LCO1490 and HCO2198 described by Folmer et al. (1994). For the PCR reactions, 0.5 µl of the extracted DNA were used in a total reaction volume of 50 µl. Each PCR reaction also contained one unit of Taq polymerase (VWR), 1X buffer, 0.2 mM of each dNTP and 0.2 µM of each primer. PCR conditions for COI amplification were as follows: 94 °C for 1 min; 40 cycles of 94 °C for 30 s, 48 °C for 30 s and 68 °C for 30 s; a final extension step of 10 min at 68 °C was included after cycling.

PCR products were purified by ammonium acetate-ethanol precipitation and reconstituted in 10 µl of LTE buffer (10 mM Tris, 0.1 mM EDTA). Direct sequencing of amplified fragments was done on both DNA strands using PCR primers. Sequencing was conducted using the Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) following manufacturer's instructions, and samples were loaded onto an ABI 3730XL automated sequencer.

Chromatograms were revised, primers trimmed and sequences corresponding to each individual assembled using the Staden package v1.6.0 (Staden et al. 2000). Multiple alignments and phylogenetic analysis of COI sequences were done using MEGA 6 (Tamura et al. 2013).

The sequences were deposited in GenBank (accession numbers: MH291272, MH291273, MH291274).

Results

Alloxysta arcuata (Kieffer, 1902)

Alloxysta minuta (Hartig, 1840) det. Cameron (misidentification). Type: BMNH (Ferrer-Suay et al. 2012b: 238).
Allotria (*Allotria*) *arcuata* Kieffer, 1902: 12.
Charips (*Charips*) *arcuatus* (Kieffer) Dalla Torre & Kieffer, 1910: 277.
Alloxysta arcuata (Kieffer) Evenhuis & Barbotin, 1977: 189.

Material studied: 2♀♀: "Orzonaga (León), 11–6–2017", "*Alloxysta arcuata* (Kieffer, 1902) det. M. Ferrer-Suay, 2017": 1♀; "Valdelinares (Teruel), 10–10–2017", "*Alloxysta arcuata* (Kieffer, 1902) det. M. Ferrer-Suay, 2017": 1♀.

Diagnosis. *Alloxysta arcuata* is mainly characterized having a small closed radial cell being 2.3 times as long as wide, pronotal carinae present, propodeal carinae forming a plate, female antennae with beginning of rhinaria in F3; F1 subequal to pedicel and longer to F2, F2 subequal to F3, male antennae with beginning of rhinaria in F2, F2 slightly curved, F1 longer than pedicel, F1 subequal to F2, F2 shorter than F3. It is similar to *Alloxysta ramulifera* but they can be differentiated by the beginning of rhinaria: in F3 in *A. arcuata* while in F4 in *A. ramulifera*; shape of pronotal carinae: well defined and visible in *A. arcuata* but small and sometimes difficult to see under the pubescence in *A. ramulifera*; size of radial cell: 2.3 times as long as wide in *A. arcuata* but 2.0 in *A. ramulifera* and shape of propodeal carinae: with curved sides in *A. arcuata* and with straight sides in *A. ramulifera*.

Images available at www.charipinaedatabase.com

Distribution and hosts. Consult Charipinae worldwide catalogue (Ferrer-Suay et al. 2012a).

New hosts records: HP: *Medicago sativa* L./ HA: *Therioaphis trifolii* (Monell, 1882)/ HW: *Trioxys complanatus* Quilis Pérez, 1931, *Praon exsoletum* (Nees, 1811); HP: *Berberis* sp. L./ HA: *Liosomaphis berberidis* (Kaltenbach, 1843)/ HW: *Aphidius hortensis* Marshall, 1896.

Alloxysta mullensis (Cameron, 1883)

Allotria mullensis Cameron, 1883: 366. Type: BMNH. (Ferrer-Suay et al. 2012b: 244).
Allotria (*Allotria*) *mullensis* Cameron: Dalla Torre & Kieffer, 1902: 40.
Charips (*Charips*) *mullensis* (Cameron) Dalla Torre & Kieffer, 1910: 284.
Alloxysta mullensis (Cameron) Quinlan, 1974: 8.

Material studied: 5♂♂: "Guadalajara, 23–4–2017", "*Alloxysta mullensis* (Cameron, 1883) det. M. Ferrer-Suay, 2017".

Diagnosis. *Alloxysta mullensis* is mainly characterized having closed radial cell being 2.2 times as long as wide, pronotal carinae absent, propodeal carinae present forming a plate, male and female with the beginning of rhinaria in F4, F1 longer than F2, F2 subequal to F3, F3 shorter than F4. It is similar to *A. fracticornis* (Thomson, 1862) but they can be differentiated by the relation between F1 and pedicel: F1 subequal to pedicel in *A. mullensis* while F1 longer than pedicel in *A. fracticornis*; proportion between flagellomeres: F1 longer than F2 and F2 subequal to F3 in *A. mullensis* female but F1–F3 subequal in length in *A. fracticornis* female; without any flagellomere curved in *A. mullensis* male but F3 curved in *A. fracticornis* male.

Images available at www.charipinaedatabase.com

Distribution and hosts. Consult Charipinae worldwide catalogue (Ferrer-Suay et al. 2012a).

New hosts records: HP: *Catalpa* sp. Scopoli/ HA: *Aphis gossypii* Glover, 1877/ HW: *Lysiphlebus testaceipes* (Cresson, 1880) and *Trioxys angelicae* (Haliday, 1833).

Alloxysta pleuralis (Cameron, 1879)

Allotria pleuralis Cameron, 1879: 113. Type: BMNH (Ferrer-Suay et al., 2013: 16).
Allotria (*Allotria*) *pleuralis* Cameron: Dalla Torre & Kieffer, 1902: 40.
Charips (*Charips*) *pleuralis* (Cameron) Dalla Torre & Kieffer, 1910: 279.
Alloxysta pleuralis (Cameron) Andrews, 1978: 88.

Material studied: 1♀: "León, 24–9–2017", "*Alloxysta pleuralis* (Cameron, 1879) det. M. Ferrer-Suay, 2017": 1♀.

Diagnosis. This species is easily differentiated from other *Alloxysta* species by the following combination of features: partially open radial cell; pronotal carinae present; two propodeal carinae well defined reaching the base independently; female antennae: F1 longer than F2, F2 shorter than F3 and F3 shorter than F4; male antennae: F1–F3 subequal in length and slightly curved.

Images available at www.charipinaedatabase.com

Distribution and hosts. Consult Charipinae worldwide catalogue (Ferrer-Suay et al. 2012a).

New hosts records: HP: *Ulmus pumila* L./ HA: *Tinocallis saltans* (Nevsky, 1929)/ HW: *Trioxys tenuicaudus* Stary, 1978.

Discussion

As has been already stated, Charipinae hyperparasitoids affect effectiveness of the primary parasitoids of aphids by decreasing their abundance and modifying their behaviour. As a result, increase of aphid populations can cause severe yield losses in some crops. Therefore, ecological studies on the subfamily Charipinae have a great economical and biological importance. Host specificity of these hyperparasitoids is still under debate and for many Charipinae species very little is known about their trophic relations.

Alloxysta arcuata, *A. mullensis* and *A. pleuralis* are species with Holarctic distribution, usually collected in field works. *Alloxysta arcuata* is considered one of the most generalist species within this genus, both based on its aphid and primary parasitoids hosts (Ferrer-Suay et al. 2014). It has been recorded on a wide variety of hosts, mainly on members of the genera *Aphis* Linnaeus, *Myzus* Passerini and *Rhopalosiphum* Koch, and regarding primary parasitoids their main hosts are species of *Aphidius* and *Ephedrus* (Ferrer-Suay et al. 2014). Here, a new trophic relationship has been found for *A. arcuata* in Spain. It has been collected from mummies of *L. berberidis*, the parasitoid here collected was *A. hortensis* and from mummies of *T. trifolii*, in this case two primary parasitoid species were identified, *T. complanatus* and *P. exsoletum*.

Alloxysta mullensis has been recently recorded on different hosts from Europe and Iran (Ferrer-Suay et al. 2013, 2015). *Aphis* is the main genus of aphids where this species has been found, whereas regarding primary parasitoids it has been recorded in species of *Aphidius* Nees, *Ephedrus* Haliday, *Binodoxys* Mackauer, and *Lysiphlebus* Förster (Ferrer-Suay et al. 2014). There is still not enough data to establish if this species is specialist or generalist. Here, a new trophic relationship has been found for *A. mullensis* in Spain. It has been collected from mummies of *A. gossypii*, and the parasitoid also collected is *T. angelicae*. Taking into account the previous hosts records, it is the first time this relation has been recorded.

Alloxysta pleuralis was established as one of the most generalist species based on its primary parasitoids records (Ferrer-Suay et al. 2014). The main aphid genus which it has been recorded is *Aphis* and related to primary parasitoids are *Aphidius*, *Ephedrus*, *Binodoxys* and *Lysiphlebus* (Ferrer-Suay et al. 2014). And also here, a new trophic relationship has been found for *A. pleuralis* in Spain. It has been collected from mummies of *T. saltans*, the parasitoid here collected was *T. tenuicaudus*.

It is important to notice that the relations here established are reflected in the emergence of Charipinae and Aphidiinae from aphid mummies collected in the field. From one mummy or charipine or aphidiine come out, thus we establish the relation between these two levels, but the reliability cannot be always complete. This is an issue we have to face in this type of works.

DNA barcode of these species has been obtained to characterize them and this information has been uploaded to GenBank.

A. arcuata (GenBank accession number: MH 291274)

A. mullensis (GenBank accession number: MH 291272)

A. pleuralis (GenBank accession number: MH 291273)

The information about trophic relations where Charipinae are involved is in general still very scarce. However, there are species which are generalists and there have been many cites in the references about their hosts, for example: *Phaenoglyphis villosa* (Hartig, 1841) and *Alloxysta victrix*. Thus, it is necessary to work on obtaining these data for most of the Charipinae species in order to improve the knowledge of their trophic associations. This

information will be very useful for aphid biological control implementations.

In addition, the use of new molecular tools to establish trophic relationships between aphids, parasitoids and hyperparasitoids should be highlighted (Smith et al. 2008, Ye et al. 2017). It is difficult to obtain adults from aphid mummies, so the DNA sequence allows parasites to be identified without problems, as long as there is a sequence deposited and perfectly identify in the databases used for comparison.

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