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A review of the subfamily Charipinae (Hymenoptera: Cynipoidea: Figitidae), hyperparasitoids potentially affecting the biological control of aphids

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Summary. Charipinae is an important subfamily of aphid hyperparasitoids. As aphids are some of the most significant pests in the world, studying the insect community associated with them is of crucial importance. Here, we review the biological information available on Charipinae. The information has been grouped into different sections: (a) historical background; (b) morphological and ecological features; (c) phylogenetic relationships; (d) overview of aphid biology and biological control; (e) evolutionary history; and (f) future perspectives. After this review, it is evident that there are still some aspects of charipine taxonomy and biology that remain elusive: (a) reliability and improvement of host associations; (b) validity of the brachypterous species; (c) problems related with the paraphyly of the genus *Phaenoglyphis*; (d) taxonomic improvement of these morphologically difficult species by the combination of molecular and morphological data; and (e) phylogeny. Additionally, important information on morphological variations among taxa and the synapomorphies of the valid genera are given for the first time. Finally, we premiere the Interactive Charipinae worldwide database and highlight the importance of this tool for the scientific community in terms of interactive determination keys for agronomists, ecologists, taxonomists, and evolutionary biologists.

Résumé. Une revue de la sous-famille des Charipinae (Hymenoptera : Cynipoidea : Figitidae), hyperparasitoïdes affectant potentiellement la lutte biologique contre les pucerons. Les Charipinae sont une importante sous-famille d'hyménoptères hyperparasitoïdes de pucerons. Leurs hôtes étant des ravageurs majeurs des cultures dans le monde, il est important de mieux connaître leurs ennemis naturels. Nous avons compilé ici toutes les informations disponibles sur cette sous-famille en les regroupant dans différentes sections : (a) contexte historique ; (b) caractéristiques morphologiques et écologiques ; (c) relations phylogénétiques ; (d) aperçu de la biologie des pucerons et de la lutte biologique ; (e) histoire évolutive ; et (f) futures perspectives. Cette révision montre que beaucoup d'aspects de la taxonomie et de l'écologie des Charipinae doivent être approfondis, comme : (a) la fiabilité et l'étendue des associations hôtes-parasitoïdes ; (b) la validité des espèces brachyptères ; (c) les problèmes relatifs à la paraphylie du genre *Phaenoglyphis* ; (d) l'amélioration de la taxonomie de ces espèces difficiles à distinguer morphologiquement par la combinaison de données morphologiques et moléculaires ; et (e) la phylogénie des Charipinae. De plus, des informations importantes sur les variations morphologiques entre taxons et les synapomorphies des genres valides sont données pour la première fois. Enfin, nous présentons la base de données interactive mondiale sur les Charipinae et rappelons l'importance pour la communauté scientifique de cet outil qui fournit des clés de détermination interactives utiles aux agronomes, écologistes, phylogénéticiens et biologistes de l'évolution.

Keywords: aphid secondary parasitoids; host–parasitoid interaction; interactive identification keys

The Charipinae (Hymenoptera: Cynipoidea: Figitidae) is composed of very small wasps (0.8–2.0 mm) primarily characterised by their smooth and shiny bodies. The subfamily is subdivided into eight valid genera: *Alloxysta* Förster, 1869 (cosmopolitan), *Phaenoglyphis* Förster, 1869 (cosmopolitan), *Lytoxysta* Kieffer, 1909 (North America), *Lobopterocharips* Paretas-Martínez & Pujade-Villar, 2007 (Nepal), *Dilyta* Förster, 1869 (cosmopolitan except Australia), *Apocharips* Fergusson, 1986 (Eastern Palaearctic and Neotropics), *Dilapothor* Paretas-Martínez & Pujade-Villar, 2006 (Australia), and *Thoreauana* Girault, 1930 (Australia) (Paretas-Martínez et al. 2007).

Since the first description of *Alloxysta victrix* (Westwood, 1833), the number of charipine species described has increased to a total of 280, of which 167 are considered valid (Ferrer-Suay et al. 2012a). Early descriptions were very brief, mostly based on variation in coloration and not paying attention to the important characters used today to distinguish between species. In addition, descriptions of the species were not always

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disseminated globally, resulting in a large number of synonymies (Hartig 1840, 1841; Thomson 1862, 1877; Hellén 1963). The most important factor accounting for this problematic taxonomy is rooted in the morphological features of specimens, hence rendering identification very difficult: their small size, smooth, shiny and unsculptured cuticle, low levels of variability between species, and lack of diagnostic characteristics all contribute to a certain taxonomic chaos (Ferrer-Suay et al. 2012a).

Charipinae are hyperparasitoids of aphids via Aphidiinae (Hymenoptera: Ichneumonoidea: Braconidae) and Aphelininae (Hymenoptera: Chalcidoidea: Aphelinidae), and hyperparasitoids of psyllids via Encyrtidae (Hymenoptera: Chalcidoidea) (Menke & Evenhuis 1991). Since Charipinae often attack parasitoids released as biological control agents of pest aphids, their presence could potentially disrupt effective biological control in agroecosystems. According to Van Veen et al. (2001), hyperparasitoids can reduce the efficiency of primary parasitoids on their hosts in at least three ways: (i) primary parasitoid mortality; (ii) indirectly by the growth rate of the aphid population; and (iii) the propensity of primary parasitoids to disperse. Therefore, it is important to study charipine populations, distribution, and trophic interactions to plan effective biological control programs of aphid populations. Recently Tougeron & Tena (2019) have pointed out the importance of considering hyperparasitoids in biological control programs. In their study, they emphasize that hyperparasitoids may have huge potential to disrupt biological control in natural and agricultural settings, and this disruption may increase in frequency and magnitude in the near future due to global change. They finally propose that hyperparasitoids may become new targets in biological control and recommend different methods to control them or limit their impact.

The type material of all species described within this subfamily has been reviewed (Ferrer-Suay et al. 2012a, 2012b, 2012c, 2012d, 2013a, 2013b, 2013c, 2013d, 2013e, 2014a, 2014b, 2015) and keys are available to assist in identifications. The primary purpose of this study is to combine all the information currently available for Charipinae into one resource to facilitate the study of this subfamily by other researchers. In this study, synapomorphies of each of the valid genera are given for the first time and some characters usually considered as definitions in the bibliography are discussed; in addition, we summarize data on the morphology and its variation of the considered taxa. In fact, although the understanding of the Charipinae taxonomy has improved significantly, there are aspects that remain a challenge, and these are also critically commented on in this study.

Historical background of Charipinae

Origin of studies

The study of this subfamily began in the mid-nineteenth century with the description of *Allotria victrix* Westwood, 1833. Later, major works emerged in which many new species were described and depicted. Important examples of such works include Hartig (1840, 1841) and Thomson (1862, 1877), as well as Kieffer (1902, 1904) in the early twentieth century. In addition to these authors, many specialists can be found around the world, reflecting the cosmopolitan character of the Charipinae genera (Belizin 1962, 1966, 1968, 1973; Hellén 1963; Fergusson 1986). Towards the end of the twentieth century, Evenhuis published a number of studies devoted not only to the description of new species but also to their revision, examining many types of specimens and establishing many synonyms (Evenhuis 1972, 1973, 1974, 1976, 1978, 1982; Evenhuis & Barbotin 1977, 1987; Evenhuis & Kiriak 1985; Menke & Evenhuis 1991).

Charipinae

Charipinae were traditionally divided in two tribes, Charipini and Alloxystini. According to Fergusson (1986), the genera included in Alloxystini are hyperparasitoids of Aphididae by parasitising Aphidiinae and Aphelinidae, which in turn parasitize Aphididae. Menke & Evenhuis (1991) included in Charipini the hyperparasitoids of Psyllidae through Encyrtidae, which in turn parasitize Psyllidae. However, the validity of these tribes was recently tested by Paretas-Martínez et al. (2007), who concluded that the tribes cannot be maintained because the characteristics conventionally used for their separation continuously vary so that the formed groups are not natural. According to Paretas-Martínez et al. (2007), Charipini remain monophyletic, but Alloxystini are paraphyletic.

The position of Charipinae in Cynipoidea has changed throughout history; see the global review of Pujade-Villar (2019). In Weld (1952) and earlier studies, these aphid and psyllid parasitoids were viewed as a subfamily of Cynipidae. Riek (1971) considered them a subfamily of Figitidae, but Quinlan (1979) again moved them to Cynipidae. Rasnitsyn (1988) divided them into two groups: Alloxystinae (within the Figitidae), and Charipidae. Finally, Ronquist (1995) in a phylogenetic study concluded that Rasnitsyn's groups constitute a monophyletic grouping of Figitidae, the Charipinae.

With regard to the position of the Charipinae within Figitidae, the Anacharitinae were proposed as the sister group to Charipinae [supported by two synapomorphies (Ronquist 1999)]. Vardal et al. (2003) found the same sister-group relationship between charipines and anacharitines based on egg structure. Buffington et al. (2007) found Charipinae monophyletic in all analyses, but they found sister-group relationships between charipines and other figitids unstable. The Anacharitinae+Charipinae relationship was not recovered in any analysis performed by

Buffington et al. (2007), independent of data type (molecules, morphology or both) or analytical method. Instead, the combined analyses placed Charipinae as the sister group to either Emargininae + Eucoilinae (parsimony) or to Figitinae + Aspicerinae (Bayesian). Partitioned Branch Support scores supporting (Charipinae (Emargininae + Eucoilinae)) were high, with most support coming from morphological data partition. Later in Buffington et al. (2012), Anacharitinae and Charipinae were only recovered as sister group in the parsimony analysis (sister group to Figitinae + Aspicerinae in Bayesian) where *Parnips* was excluded; the relationship was not recovered elsewhere. Relationships among figitid subfamilies are largely unresolved according to Ronquist et al. (2015), with two notable exceptions: First, the Charipinae and Eucoilinae form sister groups; second, the Aspicerinae and Figitinae together constitute a monophyletic group, with the Aspicerinae nested within a paraphyletic Figitinae. Most recently, Blaimer et al. (2020) found moderate support for Charipinae (Figitinae (Aspicerinae(Emargininae+Eucoilinae))).

The Charipinae differ biologically from all other figitids by being hyperparasitoids of Hemiptera (Aphididae and Psyllidae). Some of the morphological synapomorphies proposed for Charipinae, many of which are also summarized in Van Noort et al. (2015) and Buffington et al. (2020), are: (1) having a rounded scutellum without sculpture: no spine, plate, cup, or carinae are present on the scutellum dorsal area (at most, some have very small carinae at the apex); (2) very small size, 0.8–2 mm in length; (3) shiny, smooth bodies without sculpture (except for *Lytoxysta* spp., which has a very fine reticulated sculpture); (4) antennae with filiform/cylindrical segments, never globular; (5) forewing not bilobed (Ferrer-Suay et al. 2012a).

The subfamilies Eucoilinae, Emargininae and Thrasorinae (genus *Thrasorus*) also include small figitids with a smooth, shiny body. Eucoilinae differ from Charipinae in that they have a scutellar cup or plate, a feature unique in the Cynipoidea. Emargininae differ from Charipinae in that they have a deeply bilobed forewing and most species have two faint semi-parallel scutellar dorsal carinae which are slightly ovoid. *Thrasorus* spp. (Thrasorinae) differ from Charipinae in that the posterior dorsal surface of the scutellum is irregularly carinate, and they have a circumtorular impression and notauli.

Based on these morphological features, identification at the species level is an incredibly challenging task, and so the taxonomy of the group has been very chaotic. Until now, few diagnostic characteristics have been established and confirmed for species identification.

Records of Charipinae species richness

A total of 280 species of Charipinae have been described since the first species was identified by Westwood in 1833 (including two fossils, one of which was recently transferred to a new family, Protimaspidae, according to Liu et al. 2007). An updated world catalogue of the Charipinae was presented in Ferrer-Suay et al. (2012a), with 167 valid species: 111 *Alloxysta*, 31 *Phaenoglyphis*, 12 *Dilyta*, five *Apocharips*, four *Thoreauana*, and one *Dilapothor*, as well as single species classified as *Lobopterocharips, Lytoxysta* and †*Protocharips*.

Distribution

Charipinae are cosmopolitan, as they are widely distributed around the six biogeographical regions, including every continent and the oceanic island of Hawaii, as well as most of the Pacific Islands and throughout the Caribbean. They can be found in the Arctic Circle in Lapland and Alaska, all the way to 47°S in Argentina, and they have been collected at elevations above 2750 m in Arizona to below sea level in California. They are, of course, limited to places where aphids and their primary parasitoids occur, and they are therefore much less common in the tropics than in temperate regions (Andrews 1978). It is likely that some species are moved via anthropogenic means.

Charipinae are distributed worldwide, with different proportions of species and genera in each of the biogeographical regions (Figure 2); Africa: 19 species and four genera; Australia: 14 species and three genera (*Thoreuana* is endemic to this area); Neotropics: 34 species and four genera; Nearctic: 42 species and four genera; Palaearctic: Asia (Oriental region with the Western Palaearctic) 42 species and four genera; and Europe: 68 species and four genera. See Figure 2 for the global distribution of Charipinae specimens.

General morphology of Charipinae species

The reduction in size of the Charipinae over the course of evolution has resulted in significant structural reductions as is common in other micro-Hymenoptera. Morphologically, they share a number of general features such as shiny, smooth, glabrous (and in part pubescent) bodies that are not more than 3 mm in length. The taxonomic issues of the Charipinae are undoubtedly primarily due to this apparent lack of structures showing sufficient interspecific morphological variation, or whose presence or absence varies according to the species. The general morphological features of each charipine genus are grouped on different figures: *Alloxysta* (Figure 3), *Apocharips* (Figure 4), *Dilapothor* (Figure 5), *Dilyta* (Figure 6), *Lobopterocharips* (Figure 7), *Lytoxysta* (Figure 8), *Phaenoglyphis* (Figure 9) and *Thoreauna* (Figure 10).

Overview of phylogenetic relationships

The internal phylogeny of the Charipinae was studied by Paretas-Martínez et al. (2007). This study, based on external morphology, tested the validity of the current Charipinae tribes by considering species morphology. The Alloxystini were shown to be paraphyletic, while the monophyly of Charipini was strongly supported (98%). Both results are in line with the perspectives reported elsewhere (Menke & Evenhuis 1991; Ronquist 1999).

A characteristic that has been used to differentiate the Alloxystini and the Charipini is the position of the spiracles on the eighth tergum; if all species are considered, it seems that this characteristic varies continuously. Menke & Evenhuis (1991) suggested that if they were to try to delimitate this characteristic, the limits would be completely subjective and would fail to create groups with clearly distinct morphologies. In line with these arguments, the Charipini could be kept as a valid tribe, but this would leave the remaining Charipinae (the former Alloxystini) as a paraphyletic group with no possibility of grouping all the genera outside the Charipini into major monophyletic groups. For these reasons, Paretas-Martínez et al. (2007) argued against a tribal classification at all.

Charipinae genera: diagnosis and synapomorphies

For synonymous names, species descriptions and keys, consult www.charipinaedatabase.com.

The 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. The transfacial line is measured as the distance between the inner margins of the compound eyes, measured across the face through the antennal sockets divided by the height of the eye. The malar space is measured by the distance from the lower part of the gena from the mouthparts to the ventral margin of the compound eye, divided by the height of the eye. Females and males of the species described have the same characters, except where indicated.

Alloxysta Förster, 1869 (Figure 3)

Diagnosis. *Alloxysta* (Figure 3A) can be differentiated from other Charipinae genera based on the combination of certain characters. The characters that are unique in this genus are: (i) antennae filiform, last flagellomeres not broadly joined (shared with *Phaenoglyphis, Lobopterocharips* and *Lytoxysta*) (Figure 3E, F); (ii) metasoma with terga visible (Figure 3D) (shared with *Phaenoglyphis, Lobopterocharpis* and *Lytoxysta*). However, as Paretas-Martínez et al. (2007) have reminded us, it is better to define a genus according to its own synapomorphies and not based on a combination of characters, but rather also considering key and diagnostic characters.

Synapomorphies. Superior flange of petiole not extending out of T3 (Figure 3D) and with the presence of two short lateral rows of setae on the ventral spine, not close to the apex (figure 16b in Paretas-Martínez et al. 2007).

Taxonomy. Currently, this genus includes 107 species.

Apocharips Fergusson, 1986 (Figure 4)

Diagnosis. *Apocharips* has been characterized previously by an M-shaped carina on the apex of scutellum (Figure 4E); however, this character is very variable and sometimes difficult to distinguish (Paretas-Martínez & Pujade-Villar 2006; Paretas-Martínez et al. 2007; Ferrer-Suay et al. 2012a). *Apocharips* can be distinguished from the two most abundant genera of Charipinae, *Alloxysta* and *Phaenoglyphis*, as the last two flagellomeres of the female antenna are broadly joined and wider than previous flagellomeres, but this character is also variable and shared with other charipine genera (Figure 4B) (Ferrer-Suay et al. 2013a).

Synapomorphies. Small basal tergum in the metasoma, which ends just after the ring of setae at the base of the metasoma (Figure 4F).

Taxonomy. Currently, this genus includes six species.

Dilapothor Paretas-Martínez & Pujade-Villar, 2006 (Figure 5)

Diagnosis. *Dilapothor–Thoreauana* form a clade that is well separated from *Dilyta* based on the number of flagellomeres of the female antenna and the shape of carinae at the apex of the scutellum (Figure 5C) (Paretas-Martínez & Pujade-Villar 2006). According to Paretas-Martínez et al. (2007), *Dilapothor* was the only member of the Charipinae with F1 shorter than F2 (Figure 5B). This character, however, was not coded in this study because after measuring the F1 of all the species studied no groups showing significantly different character states could be delimited. Moreover, after the revisions of *Alloxysta* by Ferrer-Suay et al., it has been documented that this character (F1 shorter than F2) also appears in some other species (*A. alpina* Ferrer-Suay & Pujade-Villar, 2014; *A. proxima* Belizin, 1962; *A. vandenboschi* Andrews, 1978). Thus, this short F1 may simply be a state in a continuous transformation series involving all Charipinae (Paretas-Martínez et al. 2007).

Synapomorphies. Female antenna 10-segmented and head elongated (Figure 5B).

Taxonomy. Only a single species is included: *Dilapothor carverae* Paretas-Martínez & Pujade-Villar, 2006

Dilyta Förster, 1869 (Figure 6)

Diagnosis. *Dilyta* can be determined by a combination of characters (Paretas-Martínez & Pujade-Villar 2006): female antenna with last two flagellomeres broadly joined (character shared with *Apocharips*); metasoma with only one large tergite visible (shared with *Thoreauana* and *Dilapothor*); radial cell small and open, R1 not reaching wing margin (Figure 6A) (shared with *Thoreauana*). The genera *Dilyta* and *Thoreauana* form a clade strongly supported (Paretas-Martínez et al. 2007) by the presence of two synapomorphies, a pedicel globular much bigger than F1 and the fusion of T3 and T4.

Synapomorphies. According to Paretas-Martínez et al. (2007) *Dilyta* does not have any unique or distinctive characters and can only be defined by a combination of characters. Historically, one of the diagnostic characters assigned to *Dilyta* was the ∩-shaped carina (Figure 6E) on the apex of the scutellum (Menke & Evenhuis 1991), but Paretas-Martínez et al. (2009) showed that this character is lacking in the Afrotropical species, which instead have only two small lateral symmetrical carinae (Figure 6F) (Paretas-Martínez et al. 2011).

Taxonomy. This genus currently includes 13 species.

Lobopterocharips Paretas-Martínez & Pujade-Villar, 2007 (Figure 7)

Diagnosis. *Lobopterocharips* is very interesting because it possesses two characters (Figure 7C) previously only known for *Phaenoglyphis*: pronotum sculpture on metascutellum defined by one longitudinal medial carina, and propodeal carinae narrow. However, *Lobopterocharips* lacks the mesopleural sulcus.

Synapomorphies. *Lobopterocharips* has two autapomorphies that are unique to the Charipinae: a cup-shaped pedicel and a forewing with an undulation/curvature in the apical posterior margin (Figure 7A, D).

Taxonomy. Only a single species is included: *Lobopterocharips arreplegata* Paretas-Martínez et al. (2007).

Lytoxysta Kieffer, 1909 (Figure 8)

Diagnosis. *Lytoxysta* possesses a fine reticulate sculpture unique among Charipinae (Figure 8C) which is present on the head, pronotum, mesoscutum and mesopleuron, while in *Phaenoglyphis ruficornis, P. pubicollis* and *P. evenhuisi* the sculpture is only present on the mesoscutum (limited only to certain parts of the mesoscutum in *P. ruficornis*) and pronotum. In these three *Phaenoglyphis* species this sculpture forms an imbricate sculpturing quite similar to that of other species including *Barbotinia oraniensis* (Cynipidae) and *Parnips nigripes* (Figitidae), which are used as outgroups in the analysis of Paretas-Martínez et al. (2007).

Synapomorphies. Symmetric patches of setae at both sides of occipital foramen, mesopleural triangle absent (Figure 8G) and propodeal carinae incomplete (Figure 8D). Other synapomorphies of *Lytoxysta* are: a distinctly shaped head with eyes smaller than in other Charipinae, a radial cell only partially indicated (only the beginning of R1 and Rs are present) (Figure 8A), and brachypterous males.

Taxonomy. Only a single species is included: *Lytoxysta brevipalpis* Kieffer, 1909.

Phaenoglyphis Förster, 1869 (Figure 9)

Diagnosis. *Phaenoglyphis* is the sister group to the remaining Charipinae, but it appears here as a paraphyletic genus described by plesiomorphic characters (Paretas-Martínez et al. 2007). Historically, all the Charipinae specimens with a mesopleural sulcus (Figure 9D) have been included in *Phaenoglyphis*. The other characters previously considered diagnostic have been recently questioned, and the mesopleural sulcus remains as the only character defining this genus (Pujade-Villar & Paretas-Martínez 2006). The phylogenetic analyses done by Pujade-Villar & Paretas-Martínez (2006) confirm that the presence of a mesopleural sulcus is plesiomorphic, and as such cannot define a clade, while the other Charipinae that form a monophyletic group (Figure 1) all lack the mesopleural sulcus.

Synapomorphies. This paraphyletic group is easy to recognize because all species have a distinct metapleural sulcus (Figure 9D).

Taxonomy. This genus currently includes 35 species.

Thoreauana Girault, 1930 (Figure 10)

Diagnosis. *Thoreauana* + *Dilyta* form a clade strongly supported (Paretas-Martínez et al. 2007) by the presence of two synapomorphies: pedicel globular and larger than F1 (Figure 10C), T3 and T4 fused (Figure 10F).

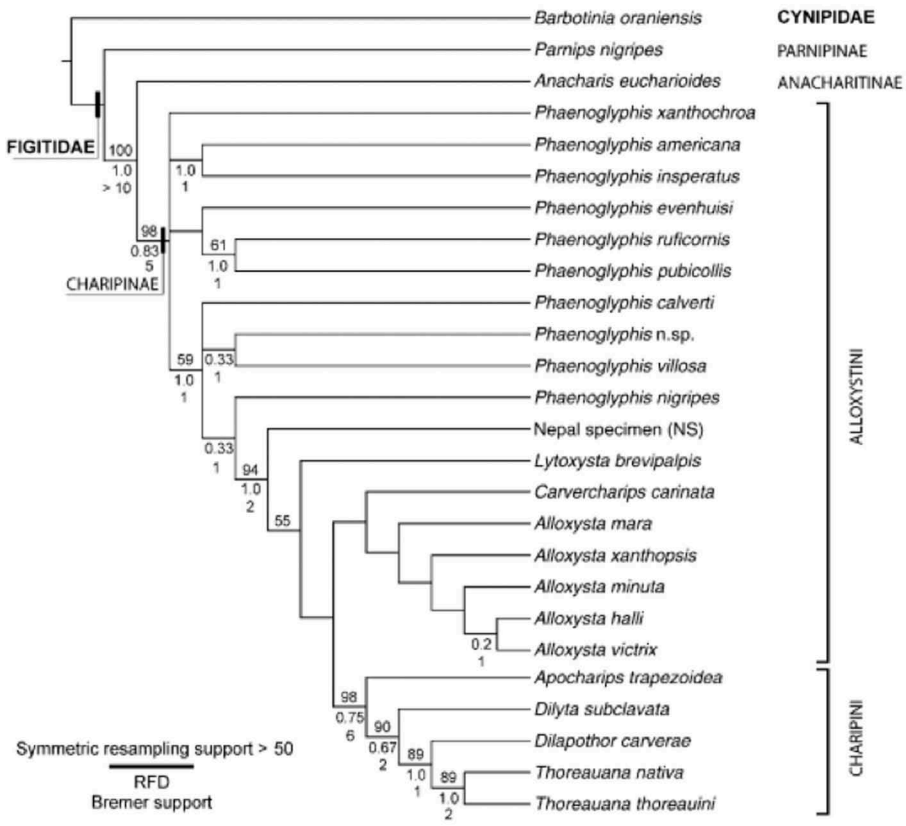

Figure 1. The most parsimonious tree under equal weights also recovered in the implied weight analyses (L = 218; CI = 0.60; RI = 0.77). This was considered as the best supported hypothesis of phylogenetic relationships of Charipinae (Paretas-Martínez et al. 2007).

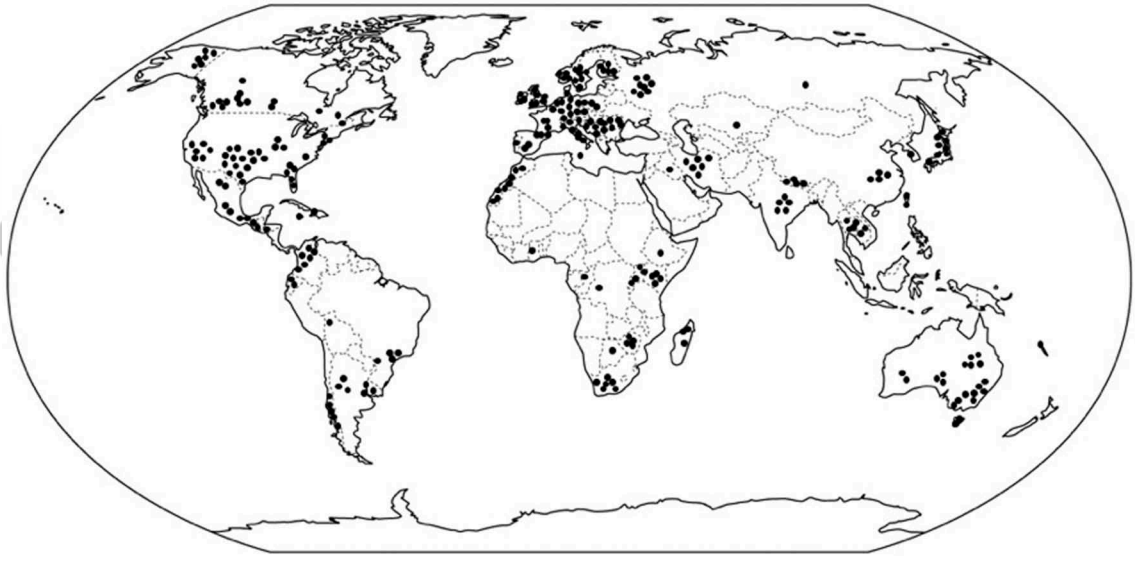

Figure 2. Distribution patterns of Charipinae species at a worldwide level.

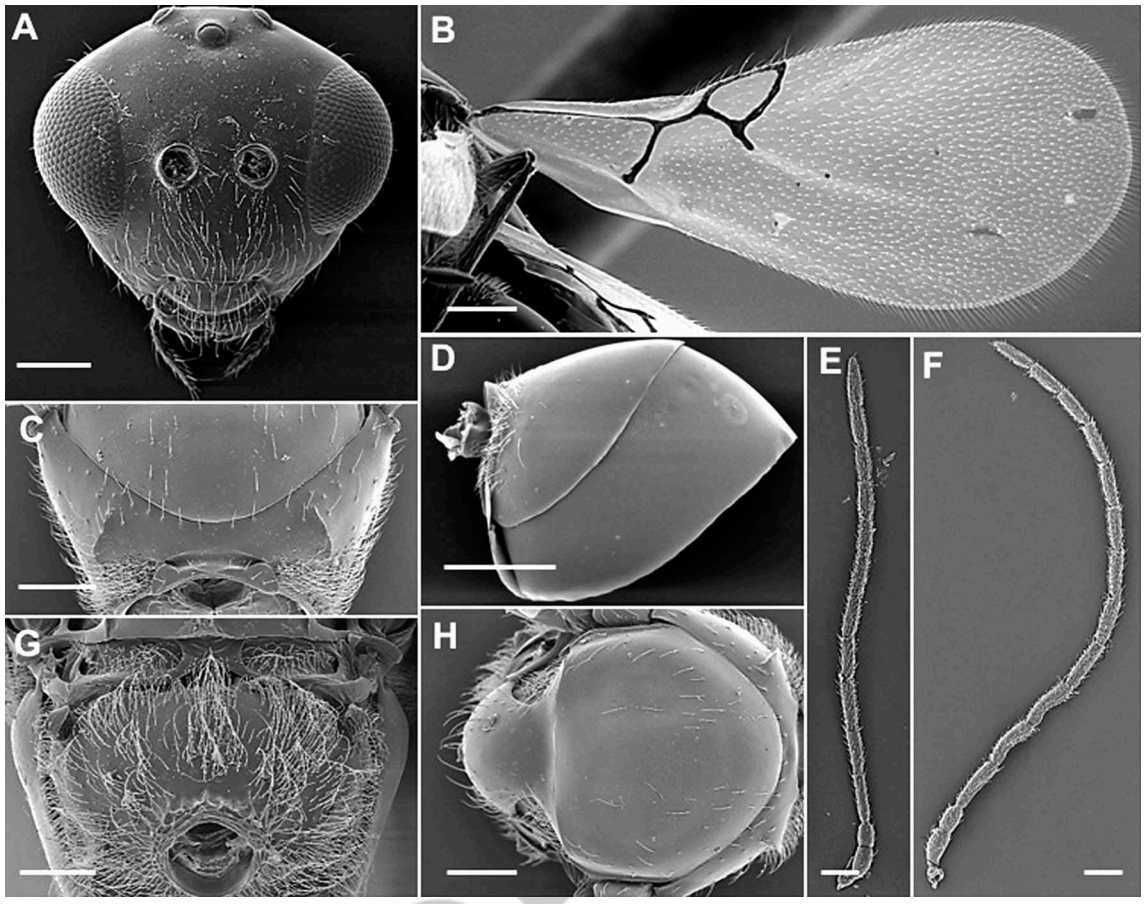

Figure 3. Diagnostic morphological features of *Alloxysta*: **A**, head; **B**, forewing; **C**, pronotum; **D**, metasoma; **E**, female antenna; **F**, male antenna; **G**, propodeum; **H**, mesoscutum (scale bar = 0.05 mm).

Synapomorphies. *Thoreauana* is well supported as monophyletic by the female antenna only having nine flagellomeres and an unsegmented apical club (Figure 10C).

Taxonomy. This genus currently includes four species.

Interactive Charipinae worldwide database

The website www.charipinaedatabase.com contains all the information currently known about the Charipinae: generic key, species keys, complete description of each valid species, bibliography, and an application to check the specific names and verify their validity. About 1000 figures (optical and SEM) are included to illustrate the diagnostic characters. Although the website and database are very user-friendly, Ferrer-Suay & Garrido-Salas (2014) can be consulted to get helpful information on how to navigate it. Moreover, it is intended to complete and improve the data of other databases providing information on this subfamily. The database is periodically updated with all the available information and include the most recent published results concerning this group of Hymenoptera. It is an important tool which can help in ecological, biological, and phylogenetic research in both the present and future.

Role of Charipinae in aphid biological control

The Charipinae affect the effectiveness of the primary parasitoids by decreasing their abundance and modifying their behaviour (Sampaio et al. 2017). As a result, an increase in aphid host populations can cause severe yield losses in some of the most important crops (Zapata et al. 2016; Vázquez-Navarro et al. 2016; Anonymous 2017). The activities of Charipinae hyperparasitoids can modify the efficiency of these biological control agents in at least three increasingly significant ways: (i) mortality of the primary parasitoid; (ii) indirect growth rate of the aphid population; and (iii) propensity for primary parasitoids to disperse (Van Veen et al. 2001). For example, the impact of *Diaretiella rapae* (M'Intosh 1855) may be constrained by secondary parasitoids, or hyperparasitoids, which may also significantly affect the biotic regulation of aphid populations (Mackauer & Völkl 1993; Sullivan & Völkl 1999).

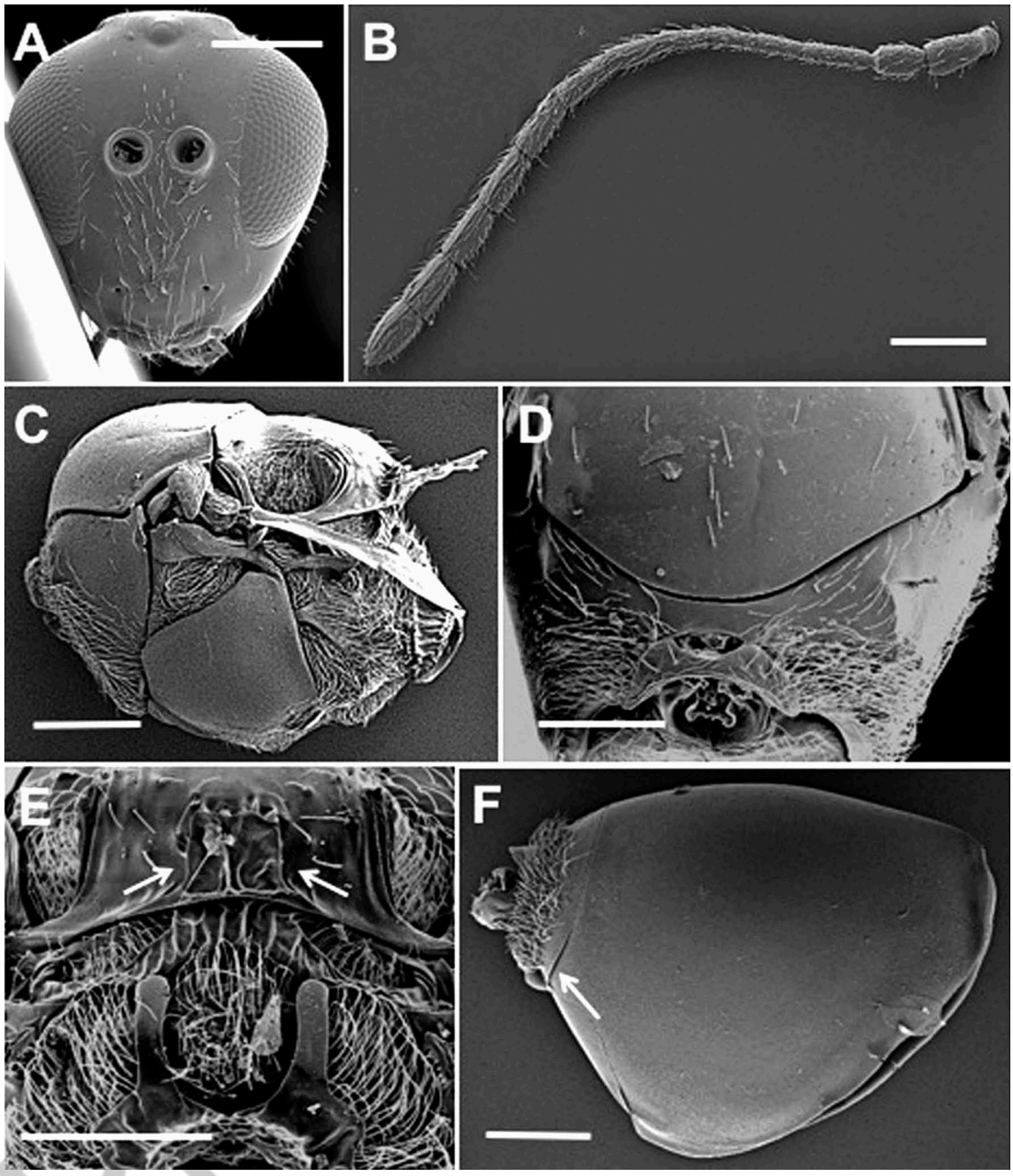

Figure 4. Diagnostic morphological features of *Apocharips*. **A**, head; **B**, female antenna; **C**, mesosoma; **D**, pronotum; **E**, propodeum (arrows mark the carinae present in the apex of propodeum); **F**, metasoma (arrow marks the first small tergite in this genus) (scale bar = 0.05 mm).

Aspects of Charipinae behaviour

The behaviour of *Phaenoglyphis americana* Baker, 1896 was observed on numerous occasions in the foothills around Riverside, California, on the *Encelia farinosa* Torr. & Gray (Asteraceae)/*Uroleucon katonkae* (Hottes, 1933) (Aphidae)/*Lysiphlebus confusus* (Tremblay & Eady, 1978) (Braconidae) complex Tremblay 1984; see Tomanović et al. 2018). The hyperparasitoids could be seen flying from plant to plant in short flights consisting of a series of short bounces. They always land on sunlit leaves where they spend several minutes preening before moving to a leaf-shaded area to hunt for prey. *Uroleucon katonkae* usually occur in dense colonies on the terminal stems and leaves, but it could also be found in less dense colonies on older growth

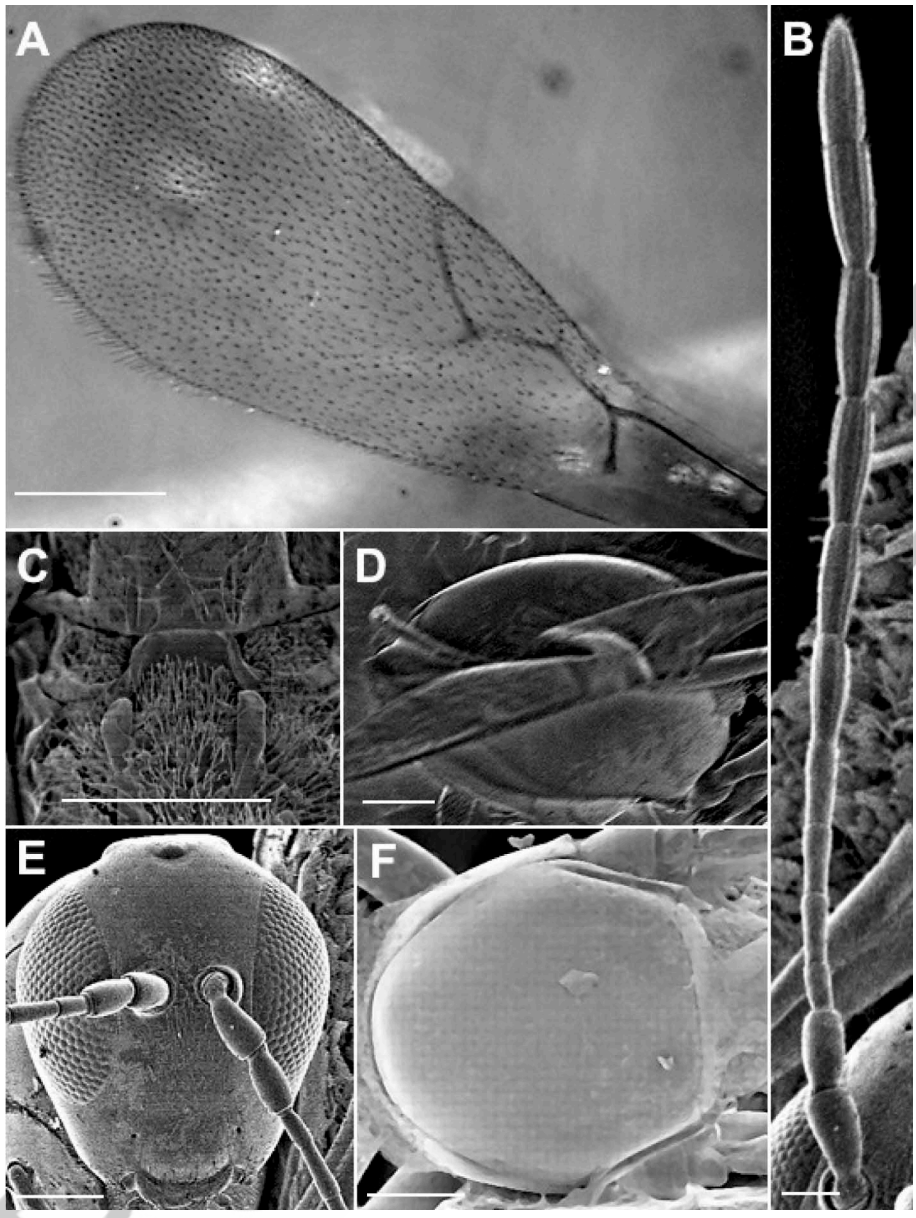

Figure 5. Diagnostic morphological features of *Dilapothor*. **A**, forewing; **B**, female antenna; **C**, propodeum; **D**, metasoma; **E**, head; **F**, mesoscutum (scale bar = 0.05 mm).

and towards the base of leaves. When approached by a hyperparasitoid, aphids usually make jerky movements from side to side and extend their hind legs, causing the parasitoid to either take flight or interrupt its foraging and oviposition behaviour. Perhaps for this reason, the searching *Phaenoglyphis* is rarely seen approaching densely packed aphid colonies, but rather they searched for single or sparsely congregated individuals. Those individuals seldom react as described above when approached by the parasitoid. The parasitoid approaches the aphid from any angle, palpates it with the antennae for several seconds, and either leaves or rises on it and palpates it again while turning on top of its dorsum. If the aphid is accepted, the parasitoid orients itself, head-to-head with its host and inserts its ovipositor into the abdominal dorsum. It may insert its ovipositor several times into the same aphid host and, in some occasions, the insertion lasts for as long as 5 minutes.

The ovipositional behaviour of *Alloxysta victrix* (Charipinae) in the *Medicago sativa* L. (Fabaceae)/*Acyrthosiphon pisum* (Harris, 1778) (Aphidae)/*Aphidius smithi* Sharma & Subba Rao, 1959 (Braconidae) complex was thoroughly studied by Gutiérrez & Van den Bosch (1970). They found that the hyperparasitoid prefers second or third instar aphids and is able to oviposit in all

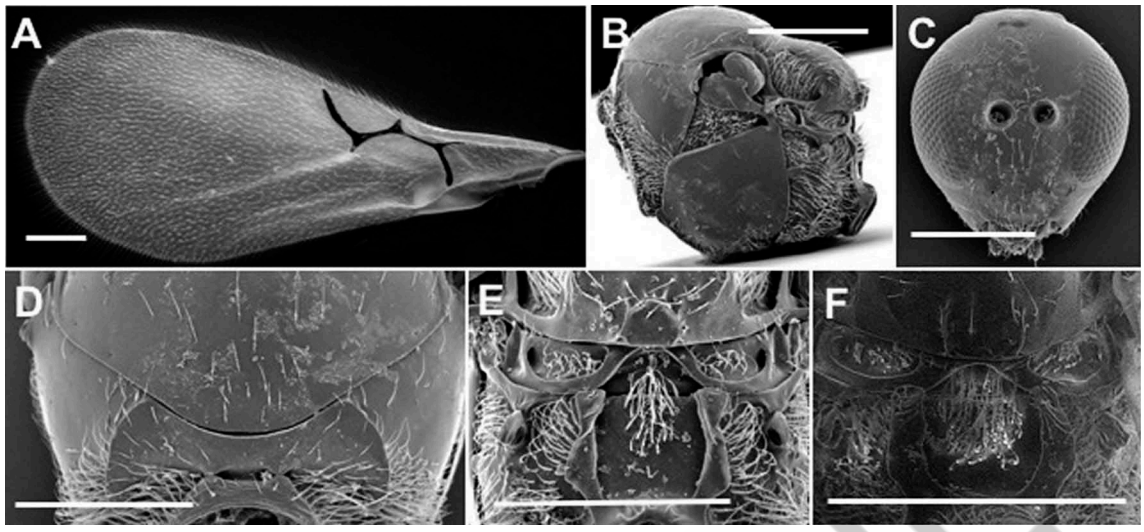

Figure 6. Diagnostic morphological features of *Dilyta*. **A**, forewing; **B**, mesosoma; **C**, head; **D**, pronotum; **E**, propodeum; **F**, propodeum (African species) (scale bar = 0.05 mm).

developmental instars of the primary parasitoid found in the haemocoel of living parasitized aphids, including the embryoned eggs. An aphid may be probed numerous times and supernumerary eggs may be deposited, of which only one will complete development. Hyperparasitoids commonly probe unparasitized aphids but never oviposit in their body. The hyperparasitoid has quite active foraging and probing behaviour; however, when a host is found and hyperparasitation is in progress, the parasite becomes motionless.

The development of *Alloxysta victrix* was elucidated by Gutiérrez & Van den Bosch (1970). It begins with egg hatching in the hemocoel of the primary parasitoid, which typically occurs soon after the primary parasitoid has mummified the aphid. The exact number of larval instars is not yet known. The hyperparasitoid develops within the hemocoel of the *A. smithi* larva until it reaches about two-thirds of its ultimate size, at which point it pierces the integument of its host and consumes it entirely: a process that takes several days. The total period of development ranges from 20 days in *A. victrix* to as much as four months in diapausing *P. americana*. The larva does not spin a cocoon but uses the one spun by the primary parasitoid prior to its death. The duration of the pupal stage in *Alloxysta victrix* varies from 8–11 days in California (Gutiérrez & Van den Bosch 1970) to 22–26 days in England (Haviland 1921).

Aspects related to the trophic role of hyperparasitoids

Theoretical studies of host–parasitoid–secondary parasitoid interactions show that secondary parasitoids can easily establish and increase host equilibrium (Beddington & Hammond 1977; May & Hassell 1981; Hassell & Waage 1984).

Other aspects of aphid–primary parasitoid–secondary parasitoid interactions that have been studied include: (i) the spatial heterogeneity of the risk of secondary parasitoidism (Schooler et al. 1996; Müller & Godfray 1998); (ii) the consequences of secondary parasitoidism for primary parasitoid foraging behaviour (Ayal & Green 1993; Weisser et al. 1994); (iii) the interactions of hyperparasitoids with ants (Völkl et al. 1994); (iv) and the behavioural responses by primary parasitoids (Micha et al. 1993; Höller et al. 1993, 1994; Völkl et al. 1995) and aphids (Boenisch & Jürgens 1994; Boenisch et al. 1997).

Traditionally, hyperparasitoids have been thought to have a negative effect on primary parasitoid populations. There are several ways in which hyperparasitoids can influence primary parasitoid populations, directly through mortality or indirectly by changing the behaviour of parasitoids or host herbivores.

Theoretically, if a large fraction of a parasitoid population is attacked by hyperparasitoids, an increase in the herbivore's density should be expected. When that fraction becomes larger, the herbivore population may entirely escape control by the primary parasitoid (Luck et al. 1981). On the other hand, Beddington & Hammond (1977) predicted that in a stable host–primary parasitoid–hyperparasitoid system, hyperparasitoidism weakens biological control efficiency, but when the system is unstable, the presence of a hyperparasitoid may dampen the oscillations and enable a stable three-species equilibrium to be attained.

Tritrophic interactions (aphid/primary parasitoid/Charipinae)

The impact of the Charipinae on biological control programs against aphids is very important, especially for

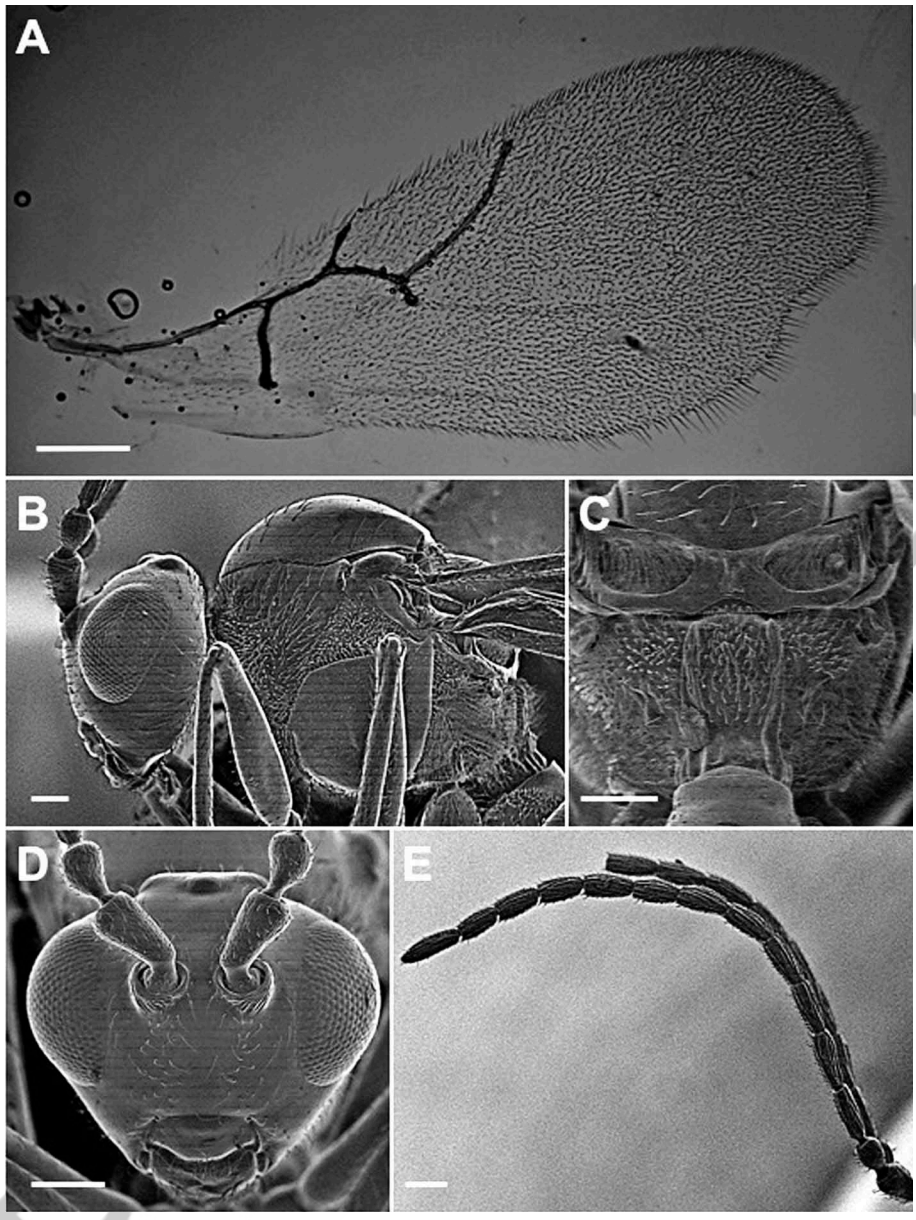

Figure 7. Diagnostic morphological features of *Lobopterocharips*. **A**, forewing; **B**, mesosoma; **C**, propodeum; **D**, head; **E**, male antenna (scale bar = 0.05 mm).

Alloxysta and *Phaenoglyphis*. It has been demonstrated that the presence of Charipinae affects the abundance of the primary parasitoids and thus the abundance of aphids (one of the most common pests) on crops. We are going to focus on *Alloxysta* because it represents the most abundant genus within the Charipinae and its species are always collected in a greater number than those of other genera.

Little information exists regarding the trophic relationships in which this genus is involved. Most of the samples studied come from field collections of living specimens (malaise trap, sweeping, entomological rearing sleeve), which are methods that cannot provide information about their hosts. However, fortunately, some researchers take aphids or mummies from the field and wait for the emergence of the parasitoids, which often turn out to be Charipinae. These researchers are primarily interested in the primary parasitoids and their effects in controlling aphid pests. Once the Charipinae are identified, all of the data can be obtained from the trophic relationships: plant–aphid–primary parasitoid–secondary parasitoid. It is worth noting that some researchers are aware of how important this information is and sometimes Charipinae specimens are deposited in institutions, such as the National Museum of Natural History, Smithsonian Institution (Washington,

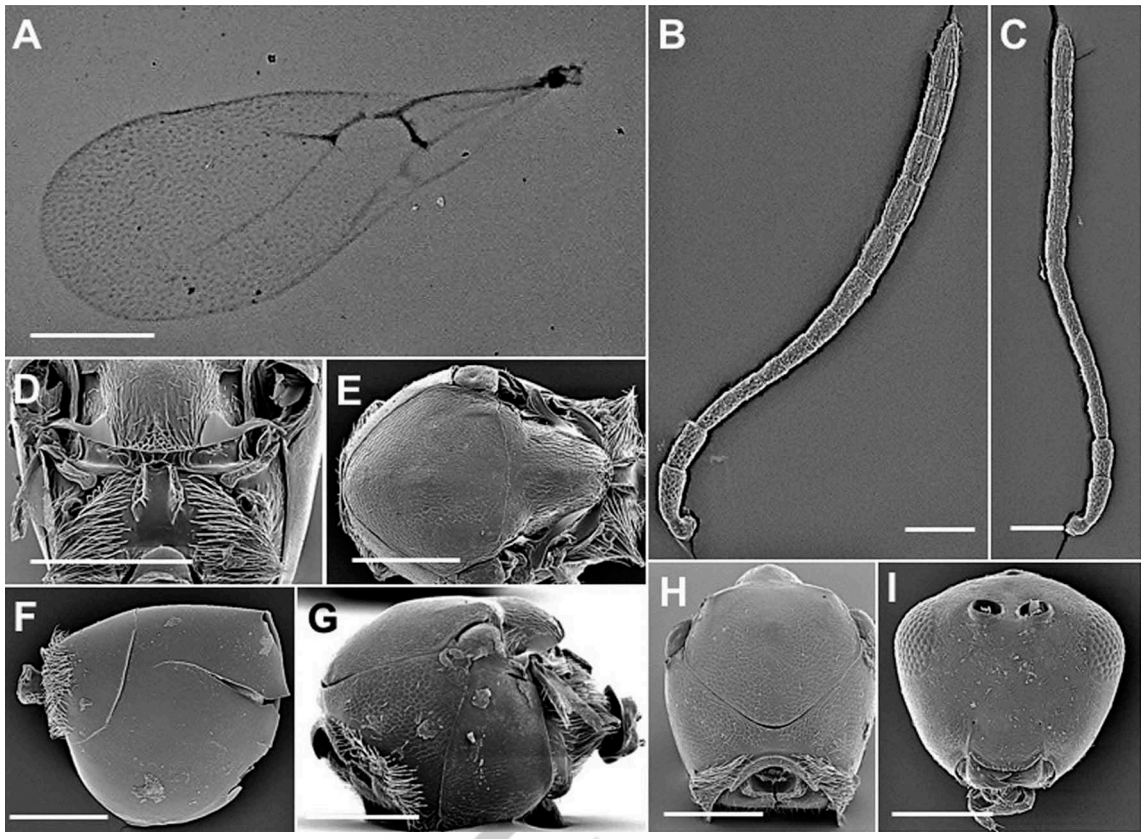

Figure 8. Diagnostic morphological features of *Lytoxysta*: **A**, forewing; **B**, female antenna; **C**, male antenna; **D**, propodeum; **E**, mesoscutum; **F**, metasoma; **G**, mesosoma; **H**, pronotum; **I**, head (scale bar = 0.05 mm).

DC, USA) or The Natural History Museum (London, UK), and labelled with the names of their hosts. The most conscientious researcher in this regard has been H.H. Evenhuis. As a result, a great number of trophic associations involving members of the genus *Alloxysta* have been collected (Ferrer-Suay et al. 2014c). In this study, we have built host–hyperparasitoid networks based on Charipinae interactions with aphids or primary parasitoids.

The host specificity of these hyperparasitoids is still an ongoing debate. One perspective is that polyphagy in the Charipinae is limited to a few species, whereas most species are specialised to varying extents, i.e. with a specialisation to a given aphid/primary parasitoid combination at one extreme (Evenhuis 1976; Sullivan & Völkl 1999). The opposite view considers the Charipinae to contain relatively few species that are morphologically variable and fairly polyphagous. DNA analysis of one species group indicated that the latter viewpoint is incorrect and that the Charipinae are both more diverse and specialised (Van Veen et al. 2003). To achieve a stronger understanding of the range of *Alloxysta* species specificity, Ferrer-Suay et al. (2014c) used the Jaccard index (vegan package, Oksanen 2013) to determine host range dissimilarity, the results of which were provided using a cluster analysis. A routine was applied to analyse if host range dissimilarity is significantly different from what is expected by chance. The level of specificity of each *Alloxysta* species was also evaluated using different indices, and additional information on the matrices was also provided: mean number of links, mean number of shared partners, niche overlap, and extinction slope. As a result of this study (Ferrer-Suay et al. 2014c), they found that the primary parasitoid genera *Aphidius* Nees, 1818, *Lysiphlebus* Förster, 1862, *Praon* Haliday, 1833, and *Trioxys* Haliday, 1833 represent the most common hosts for *Alloxysta* species, whereas they are also the most common Aphidiinae genera; while on the other hand this role is played among aphids by *Aphis* Linnaeus, 1758, *Uroleucon* Mordvilko, 1914, *Myzus* Passerini, 1860 and *Sitobion* Mordvilko, 1914. The most specialised *Alloxysta* species, based on specialisation indices, are *A. citripes* (Thomson, 1862), *A. halterata* (Thomson, 1862), *A. leunisii* (Hartig, 1841), and *A. ramulifera* (Thomson, 1862). Meanwhile, *Alloxysta arcuata* (Kieffer, 1902), *A. brevis* (Thomson, 1862), *A. fuscicornis* (Hartig, 1841), and *A. victrix* (Westwood, 1833) are recognised as generalist species sharing most of the same hosts.

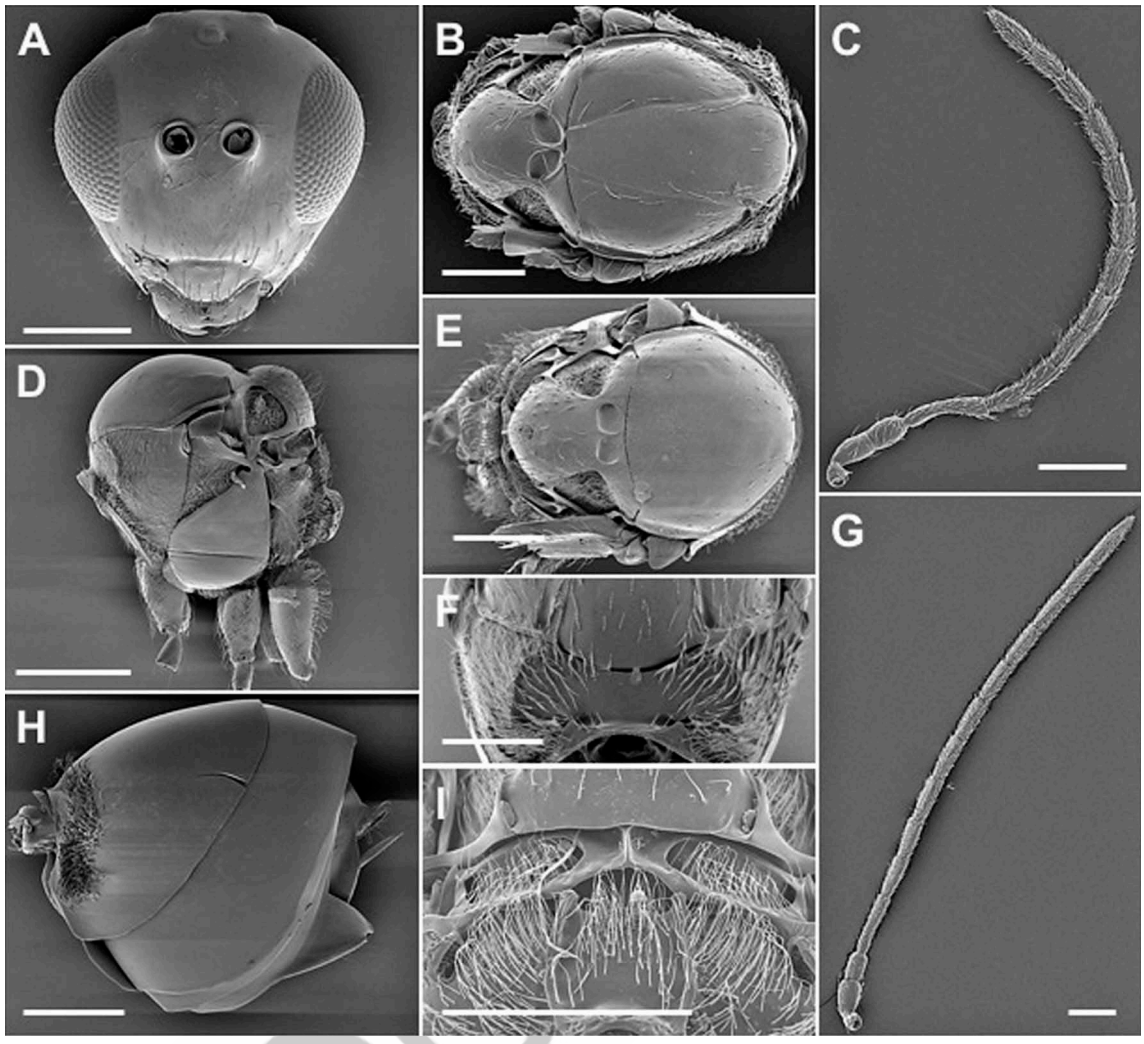

Figure 9. Diagnostic morphological features of *Phaenoglyphis*. **A**, head; **B**, mesoscutum, with notauli present; **C**, male antennae; **D**, mesosoma; **E**, mesoscutum, without notauli; **F**, pronotum; **G**, female antenna; **H**, metasoma; **I**, propodeum (scale bar = 0.05 mm).

However, these are only preliminary results. Further studies are needed to have a better idea of the degree of host specificity for *Alloxysta* species (where this is possible), with the imperative that the validity of host and parasitoid species identity has been established with certainty, thanks in particular to molecular analyses.

The cluster analysis made from these data can be compared with the phylogenetic analysis of the studied group to visualise their similarity. Unfortunately, the phylogeny of *Alloxysta* is still unknown. However, within the most generalist species, the group formed by *A. arcuata* and *A. brevis* seems to be well supported when considering their main diagnostic morphological features; in addition, the synonymy between the two species has recently been invalidated. The group including *A. fuscicornis* and *A. victrix* is also plausible because these two very similar species have been synonymised in the past (Ferrer-Suay et al. 2013f).

Quantitative analyses show more appropriately the properties of interaction networks compared to qualitative studies, and they are robust against variation in sampling intensity, network size, and symmetry (Blüthgen et al. 2006). Given the binary nature of these data (presence or absence) (Ferrer-Suay et al. 2014c), we could only perform qualitative analysis, the limits of which are well known. It is necessary to continue with sample collections all over the world to obtain new host data so that we can better understand host specificity within the Charipinae subfamily.

New perspectives

Although our collective knowledge of the taxonomy of Charipinae has greatly improved, some points are still under debate and require special attention.

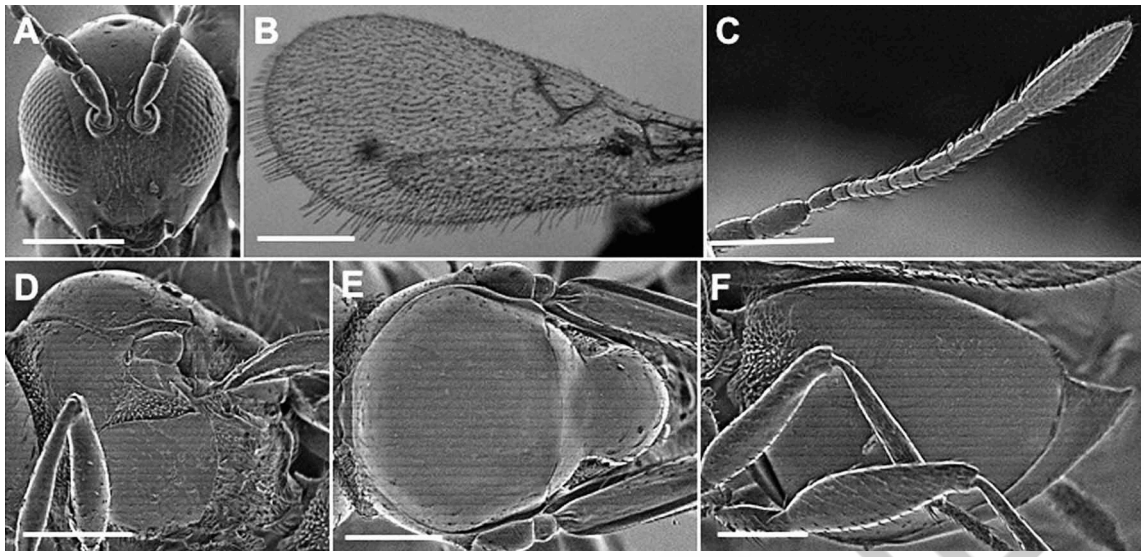

Figure 10. Diagnostic morphological features of *Thoreauana*: **A**, head; **B**, forewing; **C**, antenna; **D**, mesosoma; **E**, mesoscutum; **F**, metasoma (scale bar = 0.05 mm).

Many host association records were known in the past, but their accuracy remains dubious. Considering the problematic taxonomy of this group in the past, we cannot be completely sure about the reliability of identifications, and therefore the established associations remain questionable. For this reason, new studies based on the trophic relationships of the Charipinae using updated identification keys are needed to be completely sure of species identifications. It would also be interesting to re-examine the old material to check out these associations and eventually correct them. Consequently, the real associations between plant, aphid, primary parasitoid and Charipinae could be established.

Thus, major revisions are still needed. Furthermore, it is critical with a difficult group like Charipinae to link molecular sequence data with type specimens and modern taxonomy. Charipines need an integrated taxonomic solution, or they will remain in chaos. Lastly, a consistently applied species-limit approach is required in this group to make comparisons across the diversity of the subfamily possible.

Sometimes it is difficult to associate the Charipinae with their hosts, especially when the specimens have been collected in flight. The best way to proceed is to collect the mummies and wait for the emergence of the adults. By knowing the plant where they were collected and the aphid species, it will be possible to establish the real trophic associations (plant–aphid–primary parasitoid–hyperparasitoid).

Within *Alloxysta* some brachypterous species have been described. These species were until recently only known from the Palaearctic region. Brachypterous specimens have usually been treated as new species. However, in many cases, there is evidence that the same species can have two morphotypes: short and long winged (Ferrer-Suay et al. 2017). Frank Van Veen's experiments (pers. com.) focused on *Alloxysta halterata* (Thomson, 1862), and according to his studies, males of this species are always short-winged while the females occur as both short-winged and long-winged individuals. Based on morphological features, some hypotheses about the relationship between brachypterous and fully winged species have been proposed by Ferrer-Suay et al. (2017), but an in-depth molecular study is required to solve this issue. According to F. Van Veen (pers. comm.), the maintenance of a wing dimorphism is generally thought to depend upon the heterogeneity of the habitat and the trade-off between wing morphology and life-history traits.

Another important point is the paraphyly of the *Phaenoglyphis*. This genus is diagnosed from other charipines by its mesopleural sulcus and the presence of notauli and scutellar foveae. It is necessary to further study the Charipinae subfamily from different points of view, notably by using molecular markers combined with morphological characters, to clarify whether *Phaenoglyphis* is really a paraphyletic genus and, if so, how can this paraphyly be solved. It is important to remember that collecting *Phaenoglyphis* specimens is not always easy: we currently have no explanation for it given that we know on the contrary that *Alloxysta* specimens are always collected in great numbers.

Our knowledge and understanding of this subfamily at a genetic and molecular level is still in its infancy. Only some sequences of common species are known and available in BOLD, but most of them lack vouchers or credible taxonomy. Future Charipinae research should focus on improving our molecular knowledge and identification.

Once the morphological and molecular data of this subfamily has matured, the next step will be to solve the internal phylogeny of Charipinae. This is an important step that will help us determine the limits between species and establish whether the morphological limits currently recognized are correct and consistent. Through this new study, we will be able to solve potential problems for groups showing minimal morphological interspecific variations such as cryptic species or species complexes.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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