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The antenna cleaner in gall-inducers, inquilines and parasitic cynipoid wasps: a comparative study

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Abstract

Hymenoptera evolved structures on the legs which are able to remove particles from their antennae through grooming behaviour. The antenna cleaner (strigil) consists of an apical and modified protibial spur (calcar, composed of a trunk and a velum) and a modified basitarsus including a fine comb made up of setae and a notched inner surface. In "Terebrantia", the non-aculeate Apocrita, large comparative studies of strigil are scarce, especially within lineages. Here, we studied in detail this structure in Cynipoidea, a group of wasps including parasitoids (Ibaliidae, Liopteridae and Figitidae), gall-inducers (Cynipidae) and gall-inquilines (Cynipidae), through a SEM analysis. We found some traits quite conserved across species and lineages. For example, the shape of protibia is almost invariably broadening towards apex, and one single, straight rather than curved, dorso-apical socketed spur on apical margin of protibia occurs in almost all species. Other characters roughly differentiate families. For example, thick and long setae on the protibia are arranged in one row especially in Figitidae, Ibaliidae and Liopteridae, while they more often occur in more than one rows in Cynipidae, which was the family with the shortest calcar. Figitidae have shorter basitarsal notch, but had longer and denser setae on the notch, compared with Cynipidae and Ibaliidae. Further characters were extremely variable across and within lineages. The observed morphological variation did not seem to reflect the phylogeny of Cynipoidea, and a role of life-history traits on such variation was not suggested, at least with the methodological approach used here.

Key words: Hymenoptera Cynipoidea, Cynipidae, Figitidae, Liopteridae, Ibaliidae, legs, strigil, calcar.

Introduction

While interacting with their environment, insects accumulate and glue to the cuticle particles or detritus. These particles have a wide origin and not only include inorganic matter such as mineral dust, salt and ash, but also biological particles such as viruses, bacteria, spores and pollen. Since these particles also cover the antennae, the most important olfactory organs in insects, contamination of cuticular surfaces can negatively affect, among other physiological functions, chemical communication (Böröczky *et al*., 2013). Hence, insects evolved specific structures which are able to remove such dust. In the rich and diverse order Hymenoptera (sawflies, wasps, ants and bees), these structures are typically located on the anterior legs. Dust is removed from the antennae through grooming behaviour essentially consisting of scraping movements, i.e. unidirectional movements performed by the cleaning structure (Farish, 1972; Basibuyuk and Quicke, 1999; Hackmann *et al*., 2015).

In particular, Hymenoptera possess both one of the fore (pro) tibial spurs and the basitarsus highly modified for such antennal grooming (e.g. Schönitzer, 1986; Schönitzer and Lawitzky, 1987; Francoeur and Loiselle, 1988; Basibuyuk and Quicke, 1994; 1995; Schönitzer *et al*., 1996; Keller, 2011; Beutel *et al*., 2020). Previous studies reveal that such antenna cleaner is preserved throughout virtually all hymenopteran lineages in both "Symphyta" and Apocrita, and within Apocrita in both "Terebrantia" and Aculeata. Indeed, the foretibiae offer a range of evolutionary novelties that clearly support the order as a natural clade (Beutel and Gorb, 2001; Beutel *et al*., 2020). While a bifurcated apex calcar is probably in the hymenopteran ground plan, most groups present a single apex; the apical tibial notch is probably an additional apomorphy and the probasitarsus notch including the strigil is probably a synapomorphy of Orussidae and Apocrita (Basibuyuk and Quicke, 1994; 1995; Vilhelmsen *et al*., 2010; Karlsson and Ronquist, 2012). In "Terebrantia" (a non-monophyletic group also termed "Parasitica", since include primarily parasitoid wasps), the antenna cleaner (which is termed strigil) consists of two parts. The first is an apical and modified protibial spur (which is termed calcar and consists of a hard trunk and a transparent velum). The second is a modified basitarsus, which is in the form of a fine comb made up of setae and has the inner surface of its proximal part notched opposite the spur (Schönitzer and Lawitzky, 1987; Basibuyuk and Quicke, 1995; Vilhelmsen *et al*., 2010). Despite such general common structure and morphology, the antenna cleaner of parasitic wasps shows some variation among groups (Basibuyuk and Quicke, 1995). However, within groups such variability seems apparently weak, though this may be partially due to the relatively few morphological characters studied and/or the few number of species and lineages used in such analyses (Basibuyuk and Quicke, 1995). Here, we present the first detailed morphological study of the antenna cleaner in the superfamily Cynipoidea.

The superfamily Cynipoidea includes more than 3000 species (Ronquist, 1999) belonging to four families: the Ibaliidae and Liopteridae (both made up of parasitoid species), the Figitidae (parasitoids) and Cynipidae (secondarily herbivorous and known as gall wasps) (figure 1). In the most recent phylogenetic hypotheses (figure 1) Ibaliidae and Liopteridae were recovered either as basal in the

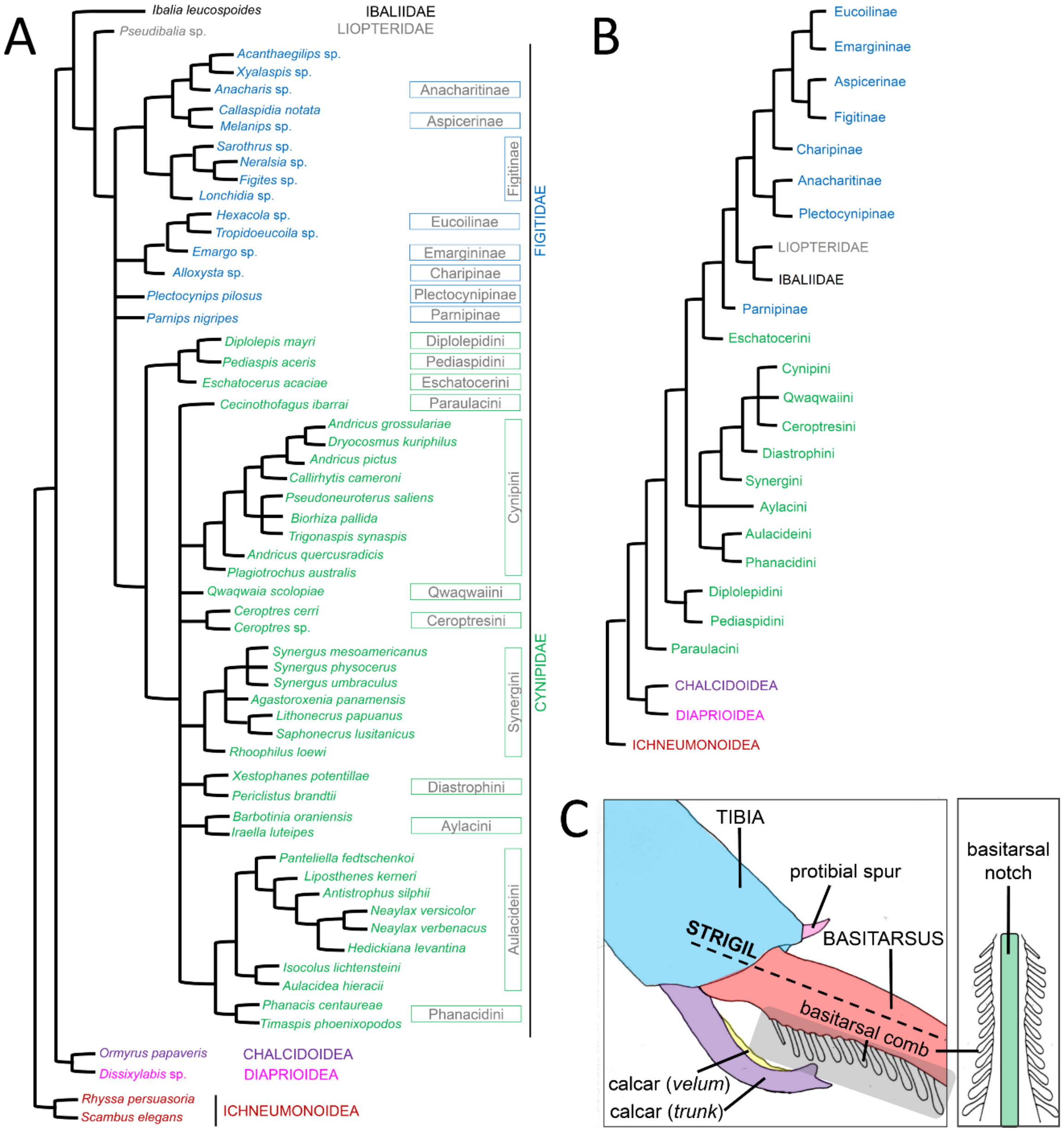

Figure 1. A-B: Phylogenetic relationships among the studied species of Cynipoidea, as depicted from recent studies and unpublished data (see text for details). **A**: hypothesis mainly following Ronquist *et al*. (2015). **B**: hypothesis mainly following Blaimer *et al*. (2020) (in this case only relationships among above-genus taxa are shown). Different colours of species names indicate different families. **C**: Schematic morphology of the typical antenna cleaner of Cynipoidea (left: lateral view of the whole organ, right: ventral view of the basitarsus).

superfamily (in terms of their phylogenetic position, Ronquist *et al.*, 2015) (figure 1A), or they fall within a clade including Figitidae, with Cynipidae being basal (Blaimer *et al.*, 2020) (figure 1B). Gall wasps got their common name due to their peculiar life-cycle that includes the capability to induce the formation, or in some cases the usurpation (inquilinism), of particular structures on plants (galls), in which the larvae feed and develop. Galls are nutritive substrates supplying food for developing wasps, help to reduce climatic fluctuation during larval development and limit the attack of brood by natural enemies (Price *et al.*, 1986; Nieves-Aldrey, 2001; Stone and Schönrogge, 2003; Csóka *et al.*, 2005; Gil-Tapetado *et al.*, 2021). The latter, in turn, evolved traits aiming to increase gall penetrations, such as metal-enriched ovipositor's cuticle (Polidori *et al.*, 2013). Gall-inducers cynipids represent the second largest radiation of galler insects after cecidomyiid midges (Diptera) (Ronquist and Liljeblad, 2001). Some species show alterning sexual and asexual generations (which reproduce via arrhenotokous and thelytokous parthenogenesis, respectively), particularly those in the lineage associated to Fagaceae plants (Nieves-Aldrey, 2001). The gall inquilines are phytophagous. They are not able to form gall on their own, but they oviposit in galls formed by other gall wasp species (Ronquist, 1994). Gall inquilines are essentially present in Cynipidae, while it is still not clear if few species of Figitidae are also inquilines or parasitoids (discussed in

Ronquist *et al*., 2018).

Recent works on a considerable number of species, lineages and both sexes showed that in Cynipoidea the antennae are equipped with a rich and diverse array of sensilla and gland products-spreading structures (Polidori and Nieves-Aldrey, 2014; Jorge *et al*., 2019; Polidori *et al*., 2020) whose cleaning is vital to properly maintain foraging, feeding and mating activities, but to date their antenna cleaner was not analysed in detail in large comparative studies (Basibuyuk and Quicke, 1994; 1995). Currently the antenna cleaner was studied in detail in 15 species of Cynipoidea, four in Cynipidae, seven in Figitidae, one in Ibaliidae and three in Liopteridae (Basybuyuk and Quicke, 1995). This previous study, based on 11 morphological characters, showed a relatively weak variability, with only one character (morphology and rows of setae anteriorly on basitarsus) polymorphic among the studied species. In general, cynipoids do not have a posterior apical spur, have an anterior pointed and sclerotized spur, have a curved and bifurcate calcar with a smooth velum and no apex, and have a basitarsus harbouring a weak but distinct notch and a comb of closely set, fine setae (Basybuyuk and Quicke, 1995). In this study we aimed to evaluate in more detail the morphological variability of antenna cleaner in Cynipoidea, through a scanning electron microscope (SEM) analysis, by using a larger dataset including both a higher number of morphological characters and a higher number species spanning most of extant families, subfamilies and tribes.

Materials and methods

Sample and scanning electron microscopy (SEM)

A total of 54 species of Cynipoidea were studied: 37 species of Cynipidae, 15 species of Figitidae, one species of Liopteridae and one species of Ibaliidae (table 1). Additionally, four "outgroup" species were analysed: two species of Ichneumonidae (Ichneumonoidea), one species of Diapriidae (Diaproidea) and one species of Ormyridae (Chalcidoidea). Samples were identified to species level, excepting some individuals which were identified to genus level, by JLN-A, with the help of published identification keys (Fegusson, 1986; Fontal-Cazalla *et al*., 2002; Forshage and Nordlander, 2008; Buffington, 2010; Van Noort *et al*., 2013; 2015; Ferrer-Suay *et al*., 2019; Buffington *et al*., 2020). Voucher specimens are deposited at Museo Nacional de Ciencias Naturales (MNCN) (CSIC, Madrid, Spain). We mostly analysed males, while in some species females or both sexes were studied. In case of females of Cynipini, for some species we analysed the sexual generation and of others we studied the asexual generation (table 1). Characters used in the morphological analysis were compiled pooling together males and females for those species for which we studied both sexes, with the exception of *Biorhiza pallida* (Olivier), for which males and asexual females were characterized separately due to the known great differences in phenotypes between asexual and sexual generations (Liljeblad *et al*., 2008). Most of the specimens were preserved in ethanol at 95% and they could be dissected, while some species were not dissected, since belong to museum collections of dry and pinned specimens. From specimens of the first group, the legs were gently separated from the body under a stereomicroscope and then gold-coated after mounting on adhesive carbon pads attached to aluminium stubs. Specimens of the second group were not gold-coated and directly observed in the SEM microscope.

The morphological study was based on images obtained using a SEM Inspect Scanning Electron Microscope (FEI Company, Oregon-USA) at the MNCN. High vacuum conditions [resolution: 3.0 nm at 30 kV (Secondary electrons-SE), 10 nm at 3 kV (SE), and 4.0 nm at 30 kV (Backscattered electrons-BSEs)] were used. The accelerating voltage was 26 kV, the high vacuum was 53.3-66.6 Pa, and the working distance was 10 mm.

All images were processed and assembled to build the final figures using the software Adobe Photoshop CS5® and Adobe illustrator CS5®.

Morphological characterization

The general aspect of the leg and strigil of Cynipoidea is schematized in figure 1C. The morphological component of this study includes coding of character states of 27 characters (table 2). Some of them were not applicable to all species and these were scored as missing data ("-"), while in few cases the state for the character was not clear and was indicated as "?".

Table 1. Classification, biology, sex of studied individuals, and collection site for the species included in the study. For species with a parthenogenetic generation, A indicate females of the asexual generation (*D. kuriphilus* has only parthenogenetic females); M indicate males and F indicate females of the sexual generation. Depository: JLNA = J.L. Nieves-Aldrey collection, Museo Nacional de Ciencias Naturales, Madrid.

Taxon	Sex	Biology	Collection country
Cynipidae: Aulacideini			
Antistrophus silphii Gillette 1891	M	Galler on *Silphium* (Asteraceae)	USA
Aulacidea hieracii (L. 1758)	M	Galler on *Hieracium* (Asteraceae)	Spain
Hedickiana levantina (Hedicke 1928)	M	Galler on *Salvia* (Lamiaceae)	Jordan
Isocolus lichtensteini (Mayr 1882)	M	Galler on *Centaurea* (Asteraceae)	Spain
Liposthenes kerneri (Wachtl 1891)	F	Galler on *Nepeta* (Lamiaceae)	Spain
Neaylax verbenacus (Nieves-Aldrey 1988)	M	Galler on *Salvia* (Lamiaceae)	Spain
Neaylax versicolor (Nieves-Aldrey 1985)	F	Galler on *Fumaria* (Papaveraceae)	Spain
Panteliella fedtschenkoi (Rubsaamen 1896)	M	Galler on *Phlomis* (Lamiaceae)	Romania
Cynipidae: Aylacini			
Barbotinia oraniensis (Barbotin 1964)	M	Galler on *Papaver* (Papaveraceae)	Spain

(Continued)

(Table1 continued)

Taxon	Sex	Biology	Collection country
Iraella luteipes (Thomson 1877)	M	Galler on *Papaver* (Papaveraceae)	Spain
Cynipidae: Ceroptresini			
Ceroptres cerri (Mayr 1872)	F	Gall-inquiline of Cynipini	Spain
Ceroptres sp.	M	Gall-inquiline of Cynipini	Mexico
Cynipidae: Cynipini			
Andricus grossulariae Giraud 1859	M	Galler on *Quercus* (Fagaceae)	Spain
Andricus quercusradicis (F. 1798)	M	Galler on *Quercus* (Fagaceae)	Spain
Andricus pictus (Hartig 1856)	F (A)	Galler on *Quercus* (Fagaceae)	Spain
Biorhiza pallida (Olivier 1791)	M, F (A)	Galler on *Quercus* (Fagaceae)	Spain
Dryocusmus kuriphilus Yasumatsu 1951	F (A)	Galler on *Castanea* (Fagaceae)	Spain
Melikaiella cameroni (Medianero et Nieves-Aldrey 2014)	M	Galler on *Quercus* (Fagaceae)	Panama
Plagiotrochus australis (Mayr 1881)	M	Galler on *Quercus* (Fagaceae)	Spain
Pseudoneuroterus saliens (Kollar 1857)	M	Galler on *Quercus* (Fagaceae)	Spain
Trigonaspis synaspis (Hartig 1841)	M	Galler on *Quercus* (Fagaceae)	Spain
Cynipidae: Diastrophini			
Periclistus brandtii (Ratzeburg 1832)	M	Gall-inquiline of *Diplolepis* (Diplolepidini)	Spain
Xestophanes potentillae (Retzius in De Geer 1773)	M	Galler on *Potentilla* (Rosaceae)	Spain
Cynipidae: Diplolepidini			
Diplolepis mayri (Schlechtendal 1877)	M	Galler on *Rosa* (Rosaceae)	Spain
Cynipidae: Eschatocerini			
Eschatocerus acaciae Mayr 1881	M	Galler on *Prosopis* (Fabaceae)	Argentina
Cynipidae: Paraulacini			
Cecinothofagus ibarrai Nieves-Aldrey et Liljeblad 2009	M	Gall-inquiline of *Aditrochus* (Pteromalidae)	Chile
Cynipidae: Pediaspidini			
Pediaspis aceris (Gmelin 1790)	M	Galler on *Acer* (Sapindaceae)	Spain
Cynipidae: Phanacidini			
Phanacis centaureae Förster 1860	M	Galler on *Centaurea* (Asteraceae)	Spain
Timaspis phoenixopodos Mayr 1882	M	Galler on *Lactuca* (Asteraceae)	Spain
Cynipidae: Qwaqwaiini			
Qwaqwaia scolopiae Liljeblad, Nieves-Aldrey et Melika 2011	F	Galler on Scolopia (Salicaceae)	South Africa
Cynipidae: Synergini			
Agastoroxenia panamensis Nieves-Aldrey et Medianero 2010	F	Gall-inquiline of Cynipini	Panama
Lithonecrus papuanus Nieves-Aldrey et Butterill 2014	M	Gall-inquiline of unknown host on *Lithocarpus*	Papua New Guinea
Rhoophilus loewi Mayr 1881	M, F	Gall-inquiline of *Scyrotis* (Lepidoptera)	South Africa
Saphonecrus gallaepomiformis (Boyer de Fonscolombe 1832)	M	Gall-inquiline of *Andricus* + *Plagiotrochus*	Spain
Synergus mesoamericanus Ritchie et Shorthouse 1987	M	Gall-inquiline of Cynipini	Panama
Synergus physocerus Hartig 1843	M, F	Gall-inquiline of *Trigonaspis*	Spain
Synergus umbraculus (Olivier 1791)	M, F	Gall-inquiline of *Andricus*	Spain
Figitidae: Anacharitinae			
Acanthaegilips sp.	M	Endoparasitoid of Neuroptera	Colombia
Anacharis sp.	M	Endoparasitoid of Neuroptera	Spain
Xyalaspis sp.	M	Endoparasitoid of Neuroptera	Spain
Figitidae: Aspicerinae			
Callaspidia notata (Boyer de Fonscolombe 1832)	M	Endoparasitoid of Diptera: Cyclorrhapha	Spain
Melanips sp.	M	Endoparasitoid of Diptera: Cyclorrhapha	Spain
Figitidae: Charipinae			
Alloxysta sp.	M	Endoparasitoid of Hymenoptera	Spain
Figitidae: Emargininae			
Emargo sp.	F	Parasitoid of myrmecophilous Diptera larvae	Colombia
Figitidae: Eucoilinae			
Hexacola sp.	M	Endoparasitoid of Diptera	Spain
Tropidoeucoila sp.	M	Endoparasitoid of Diptera	Panama
Figitidae: Figitinae			
Figites sp.	M	Endoparasitoid of Diptera	Spain
Lonchidia sp.	M	Endoparasitoid of Diptera	Spain
Neralsia sp.	M	Endoparasitoid of Diptera	Colombia
Sarothrus sp.	M	Endoparasitoid of Diptera	Spain
Figitidae: Parnipinae			
Parnips nigripes (Barbotin 1963)	M	Gall-parasitoid of *Barbotinia* (Aylacini)	Spain
Figitidae: Plectocynipinae			
Plectocynips pilosus (Ros-Farre 2002)	M	Gall-parasitoid or gall-inquiline of *Aditrochus* (Pteromalidae)	Chile
Ibaliidae			
Ibalia leucospoides (Hochenwarth 1785)	M	Endoparasitoid of Hymenoptera	Spain
Liopteridae			
Pseudibalia sp.	F	Parasitoid of Coleoptera larvaa in wood	Colombia
Ichneumonoidea: Ichneumonidae			
Scambus elegans (Woldstedt 1877)	M	Parasitoid of Lepidoptera Tortricidae	Spain
Rhyssa persuasoria (L. 1758)	M	Parasitoid of Ibalia (Ibaliidae)	Spain
Diaprioidea: Diapriidae			
Dissixylabis sp.	M	Parasitoid of Diptera	Chile
Chalcidoidea: Ormyridae			
Ormyrus papaveris (Perris 1840)	M	Ectoparasitoid of Aylacini (Cynipidae)	Spain

Table 2. Data matrix based on the characters listed in the Materials and methods. “-” was used to denote cases where a character is not applicable to that species. “?” indicates unclear state for the character. For species with a parthenogenetic generation, A indicate females of the asexual generation (*D. kuriphilus* has only parthenogenetic females); M indicate males and F indicate females of the sexual generation.

Taxon	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
Acanthaegilips sp.	0	2	1	1	1	1	1	-	-	-	1	1	0	0	1	0	0	0	0	2	1	2	1	1	2	0	1
Agastoroxenia panamensis	0	1	2	1	1	1	0	0	0	1	0	-	0	2	0	0	0	0	0	1	1	1	1	1	0	0	0
Alloxysta sp.	0	2	2	1	1	0	0	0	0	1	0	-	0	1	1	0	1	0	0	2	0	1	1	1	1	0	0
Anacharis sp.	0	1	1	1	1	1	1	-	-	-	1	1	0	1	1	0	1	0	0	1	1	2	1	1	2	0	1
Andricus grossulariae	0	1	1	1	2	1	0	0	0	2	0	-	0	0	0	0	1	0	0	1	1	1	2	1	0	0	0
Andricus pictus (A)	0	2	2	1	1	1	0	0	0	2	0	-	0	1	0	0	1	0	0	1	1	1	1	1	0	0	0
Andricus quercusradicis	0	1	2	1	1	1	0	0	0	1	1	0	0	2	1	0	1	0	0	0	0	1	2	1	1	0	0
Antistrophus silphii	0	0	1	1	2	1	0	1	1	1	1	0	0	0	0	0	0	0	0	1	1	1	0	1	0	0	0
Aulacidea hieracii	0	0	0	1	1	0	0	0	1	1	0	-	0	1	0	0	0	0	0	1	0	1	0	1	0	0	0
Barbotinia oraniensis	0	1	1	1	1	1	0	0	0	1	1	0	0	0	2	0	0	0	0	1	0	1	0	1	1	0	0
Biorhiza pallida (M)	0	2	2	0	-	-	0	0	0	1	0	-	0	2	0	0	0	0	0	0	1	1	2	1	0	0	0
Biorhiza pallida (A)	0	2	2	0	-	-	0	0	0	2	0	-	0	1	0	0	0	0	0	0	1	1	1	1	0	0	0
Callaspidia notata	0	1	0	1	1	1	0	0	0	1	1	2	0	1	1	0	0	0	0	1	1	2	1	1	1	0	1
Cecinothofagus ibarrai	0	2	1	0	-	-	0	0	1	1	0	-	0	1	1	0	0	0	0	0	1	1	1	1	1	0	0
Ceroptres cerri	0	1	1	1	1	1	0	0	0	2	0	-	0	2	2	0	0	0	0	1	1	1	2	1	1	0	0
Ceroptres sp.	0	1	0	1	1	1	0	0	0	2	0	-	0	1	2	0	0	0	0	1	1	1	1	1	1	0	0
Diplolepis mayri	0	2	2	1	1	1	0	0	1	1	0	-	0	1	1	0	1	0	0	2	1	1	1	1	1	0	0
Dissixylabis sp.	1	2	1	0	-	-	0	0	0	2	0	-	0	2	2	0	1	0	0	2	1	2	1	1	1	0	1
Dryocosmus kuriphilus	0	0	1	1	1	1	0	0	0	1	0	-	0	1	1	0	1	0	0	0	1	1	1	1	1	0	0
Emargo sp.	1	1	2	1	1	0	0	0	0	0	0	-	0	2	1	0	2	0	1	-	0	2	3	1	2	0	1
Eschatocerus acaciae	0	0	0	0	-	-	0	0	0	2	0	-	1	0	1	1	-	0	1	-	1	0	-	0	-	-	-
Figites sp.	0	1	1	1	1	0	0	0	0	1	1	2	0	2	2	0	0	0	0	1	1	2	1	1	2	0	1
Hedickiana levantina	0	1	1	1	1	1	0	2	0	1	1	0	0	1	1	0	0	0	0	1	1	1	1	1	0	0	0
Hexacola sp.	0	0	1	1	1	0	0	0	0	1	1	0	0	1	1	0	1	2	0	1	0	2	1	1	2	0	1
Ibalia leucospoides	0	1	0	0	-	-	0	2	0	2	1	1	0	0	1	0	0	1	0	1	0	1	2	1	0	0	1
Iraella luteipes	0	1	0	1	1	1	0	0	1	0	1	0	0	0	1	0	1	0	0	1	1	1	1	1	1	0	0
Isocolus lichtensteini	0	2	1	1	2	1	0	0	1	1	1	1	0	0	0	0	0	0	0	1	1	1	0	1	0	0	0
Liposthenes kerneri	0	1	1	1	1	1	0	0	0	1	1	2	0	1	0	0	1	0	0	1	1	1	2	1	0	0	0
Lithonecrus papuanus	0	2	1	1	1	0	0	0	0	1	0	-	0	2	1	0	0	0	0	1	1	1	2	1	1	0	0
Lonchidia sp.	0	1	1	1	1	0	0	0	0	1	1	1	0	1	2	0	1	0	0	1	1	2	1	1	2	0	0
Melanips sp.	0	1	1	1	1	0	0	0	0	1	1	1	0	1	1	0	0	0	0	1	1	1	1	1	1	0	1
Melikaiella cameroni	0	1	1	1	2	1	0	1	0	2	0	-	0	1	1	0	0	0	0	0	1	1	2	1	0	0	0
Neaylax verbenacus	0	2	1	1	2	1	0	0	1	1	0	-	0	0	1	0	0	0	0	1	1	1	0	1	0	0	0
Neaylax versicolor	0	1	1	1	1	1	0	1	0	0	1	1	0	1	1	0	1	0	0	1	1	1	1	1	1	0	0
Neralsia sp.	0	1	1	1	1	0	0	0	0	0	1	2	0	2	2	0	0	0	0	2	1	2	2	1	2	0	1
Ormyrus papaveris	0	2	3	0	-	-	1	-	-	-	1	2	0	2	1	0	1	0	0	1	0	0	3	1	0	?	1
Panteliella fedtschenkoi	0	0	0	1	2	0	0	0	1	1	1	?	0	0	0	0	0	0	0	1	0	1	2	1	0	0	0
Parnips nigripes	0	1	0	1	0	0	0	0	0	1	1	1	0	1	2	0	1	0	0	1	0	1	1	1	1	0	0
Pediaspis aceris	0	2	1	0	-	-	0	0	1	2	1	2	0	2	2	0	0	0	0	0	0	1	1	1	1	0	0
Periclistus brandtii	0	1	1	1	1	1	0	0	1	1	0	-	0	1	1	0	1	0	0	1	1	1	1	1	1	0	0
Phanacis centaureae	0	1	1	1	1	1	0	0	1	1	0	-	0	0	0	0	1	0	0	1	0	1	1	1	0	0	0
Plagiotrochus australis	0	1	1	1	1	1	0	0	0	2	0	-	0	0	1	0	1	0	0	0	1	1	0	1	0	0	0
Plectocynips pilosus	0	1	1	1	0	0	0	0	0	1	0	-	0	2	2	0	0	0	0	1	1	1	2	1	1	0	1
Pseudibalia sp.	0	-	-	0	-	-	0	0	0	2	1	1	0	-	1	0	0	0	0	1	-	1	-	1	1	0	1
Pseudoneuroterus saliens	0	1	0	1	2	1	0	1	0	1	1	1	0	0	1	0	0	0	0	0	1	1	1	1	0	0	0
Qwaqwaia scolopiae	0	2	2	1	1	1	?			-	0	-	0	-	-	0	0	0	0	0	1	1	1	1	0	0	0
Rhoophilus loewi	0	1	1	1	2	1	0	0	0	1	0	-	0	0	0	0	0	0	0	1	0	1	1	1	1	0	0
Rhyssa persuasoria	0	0	0	1	0	0	1	-	-	-	1	2	0	0	0	1	-	0	2	2	1	1	0	1	0	1	1
Saphonecrus gallaepomiformis	0	1	1	1	1	1	0	0	0	2	0	-	0	0	0	0	0	0	0	1	1	1	1	1	0	0	0
Sarothrus sp.	0	1	1	1	1	0	1	-	-	-	1	2	0	0	2	0	0	0	0	1	2	1	0	1	2	0	1
Scambus elegans	0	2	2	1	0	0	1	-	-	-	1	1	0	2	1	1	-	0	0	2	0	1	1	1	0	1	1
Synergus mesoamericanus	0	1	2	1	1	1	0	0	0	1	0	-	0	1	0	0	0	0	0	1	1	1	1	1	0	0	0
Synergus physocerus	0	1	1	1	1	1	0	0	0	1	1	1	0	0	0	1	0	0	1	1	1	0	1	1	0	0	
Synergus umbraculus	0	2	2	1	1	1	0	0	0	2	1	2	0	0	0	0	1	0	0	1	1	1	1	1	1	0	0
Timaspis phoenixopodos	0	1	0	0	-	-	0	0	1	1	0	-	0	0	1	0	1	0	0	0	1	1	1	1	0	0	0
Trigonaspis synaspis	0	2	1	0	-	-	0	0	0	1	0	-	0	0	1	0	0	0	0	1	1	1	0	1	1	0	0
Tropidoeucoila sp.	0	1	2	1	1	0	0	0	0	1	1	0	0	2	1	0	1	2	0	1	0	2	2	1	2	0	1
Xestophanes potentillae	0	1	1	1	1	0	0	1	1	2	1	2	0	1	1	0	1	0	0	1	0	1	2	1	0	0	0
Xyalaspis sp.	0	1	1	1	1	0	1	-	-	-	1	0	0	1	1	0	1	0	0	1	1	2	1	1	2	0	1

The characters and character states are described below and are exemplified in a series of plates in figures 2-3.

1. *Shape of protibia*: (0) gradually and slightly broadening towards apex; (1) strongly expanded towards apex (not illustrated).

2. *Protibial/profemur length ratio*: (0) very short (protibia/profemur ratio = 0.6-0.8) (not illustrated); (1) a little shorter than profemur (0.81-0.9); (2) almost equal than profemur (0.91-1).

3. *Protibial/probasitarsus length ratio*: (0) equal or a little longer than probasitarsus (protibia/probasitarsus ratio = 1-1.5); (1) clearly longer than probasitarsus (1.51-2); (2) much longer than probasitarsus (2.1-2.7); (3) more than three times the probasitarsus length (3.5).

4. *Modified thicker and longer setae on distal half of protibia (in lateral and ventral view of protibia)*: (0) absent; (1) present.

5. *Shape of the modified setae of protibia*: (0) conical, pointed (not illustrated); (1) more or less flat, not expanded distally; (2) paddle shaped, expanded apically.

6. *Number of rows of modified setae of protibial*: (0) 1 row; (1) arranged irregularly in more than one row.

7. *Dorso-apical socketed spur on apical margin of protibia (in lateral view opposite to calcar)*: (0) present; (1) absent.

8. *Number of spurs (when present)*: (0) 1; (1) 2; (2) 3 or more (not illustrated).

9. *Shape of spurs on apical margin of protibia*: (0) straight; (1) slightly curved at apex.

10. *Spur width/length on protibial apical margin (ratio)*: (0) extremely narrow and long (ratio = 0.2-0.3); (1) intermediate length (0.31-0.5); (2) short and wide, width at least one half of length (0.51-0.8).

11. *Spines (or stout long flat spines, or setae) of apical margin of protibia*: (0) absent; (1) present.

12. *Number of these modified setae*: (0) 1; (1) 2-4; (2) 5 or more.

13. *Insertion and shape of protibial long spur (calcar)*: (0) inserted on a recess of apical margin of protibia, curved and slightly twisted to one side, base not on an elevation; (1) inserted slightly before apical margin of protibia on an elevated base, uniformly curved inward.

14. *Calcar/basitarsus length ratio*: (0) very short (calcar/basitarsus ratio = 0.2-0.4); (1) about the half of basitarsus length (0.41-0.5); (2) more than half of basitarsus length (0.51-1).

15. *Calcar length/apical margin of protibia width (ratio)*: (0) almost equal or a little longer than the protibia margin width (ratio calcar/protibial = 1.1-1.5); (1) from 1.5 × to the double of the protibia margin width (1-51-2); (2) more than the double of the protibia margin width (2.1-2.5).

16. *Apex of protibial spur (calcar)*: (0) cleft tip; (1) single tip.

17. *Shape and size of bifurcated tip of calcar*: (0) acute tip and apical lobe of velum almost of equal size, or the former only slightly longer; (1) acute spine longer than apical lobe; (2) acute spine much more long than apical lobe of velum.

18. *Ventrolateral margin of velum* (0) straight or uniformly curved, without a projected lobe; (1) slightly projected basally; (2) with a broad projected lobe medially.

19. *Shape and extension of the micro-sculpture on dorsal area of calcar*: (0) spine-like, with lying or erect setae; (1) smooth, without setae; (2) mixed scale-like and spine-like.

20. *Shape and density of setae on dorsal area of calcar*: (0) short, lying, widely spaced; (1) longer, slightly erect at apex and more closely spaced; (2) very long, erect and dense.

21. *Probasitarsus/protarsus length ratio*: (0) very short (probasitarsus/protarsus ratio = 0.2-0.4); (1) about half of the protarsus length (0.41-0.54); (2) more than half of the protarsus length (0.8).

22. *Shape of basitarsal notch (lateral view)*: (0) straight; (1) slightly curved; (2) extremely curved in lateral view, with basal area of basitarsus broadened and sharply narrowed medially.

23. *Basitarsal notch/basitarsus length ratio (in ventro apical view)*: (0) very short (notch/basitarsus ratio = 0.2-0.4); (1) about half the basitarsus length (0.41-0.6); (2) clearly longer than half of the basitarsus length (0.61-0.8); (3) equal to basitarsus length (1-1.1).

24. *Basitarsal comb*: (0) present; (1) absent.

25. *Size and arrangement of setae of the basitarsal comb (compared with width at apical margin of protibia in lateral view)*: (0) very short (setae/protibial margin ratio = 0.1-0.3); (1) short (0.31-0.5); (2) more than half the protibial margin width (0.51-0.8).

26. *Number of rows of setae of the basitarsal comb*: (0) 1; (1) 2.

27. *Density and separation of setae of basitarsal comb*: (0) slightly separated; (1) closely spaced, almost contiguous.

Data analysis

To preliminary hypothesize evolutionary patterns of the antenna cleaner in the superfamily, two hand-made phylogenetic trees of the studied species was generated following the recent phylogenetic hypotheses available in Ronquist *et al.* (2015) (based on molecular + morphological + life-history data) and in Blaimer *et al.* (2020) (based on genomic data). We kept these two trees as separated and did not try to build a consensus tree, since these two phylogenetic hypotheses substantially differ in the proposed relationships among key lineages. Since these published studies did not include few of our analysed species and/or lineages, we completed our trees by adding information on their phylogenetic position as hypothesized in few further studies. In particular, position of some Figitidae were retrieved from Buffington *et al.* (2007) and few unpublished data (J.L. Nieves-Aldrey) (figure 1A-B). To explore if antenna cleaner's morphology may reflect the phylogeny of Cynipoidea (as depicted in the built trees mentioned above), the morphological relationships among the studied species were analysed using PAUP*v4.b10 (Swofford, 2002). In particular, a UPGMA (unweighted pair group method analysis) cluster analysis was performed to obtain an overall similarity phenogram. This method was also used to explore if variation in morphology of antenna cleaner among species reflects the variation on life-history traits.

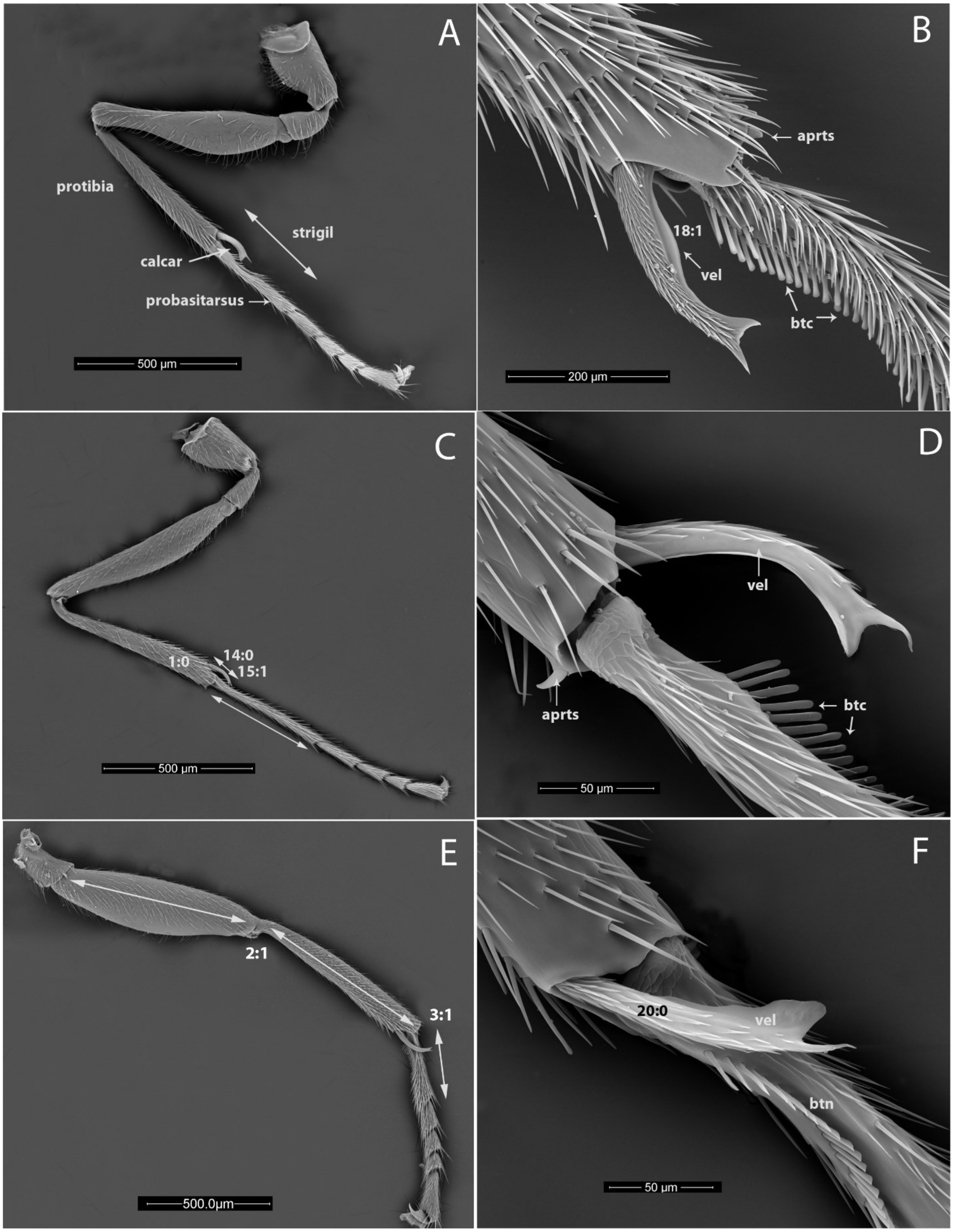

Figure 2. Morphology of the antenna cleaner (strigil) in Cynipoidea. **A**: Foreleg, and details of the strigil in ventral view (**D**) and lateral view (**F**) of *Cecinothofagus ibarrai* (Cynipidae Paraulacini). **B**: Lateral view of the strigil of *Ibalia leocospoides* (Ibaliidae); for comparison of protibial shape, an inset picture of the protibial of *Emargo* sp. (Figitidae: Emargininae) is shown. **C**: Foreleg of *Plagiotrochus australis* (Cynipidae: Cynipini). **E**: Foreleg of *Qwaqwaia scolopiae* (Cynipidae Qwaqwaiini). aprts: dorso-apical socketed protibial projection (spur or horn); btc: basitarsal comb; btn: basitarsal notch; vel: velum. **B**, **C**, **E** and **F** show the characters and character states (as character: state, see text) evidenced by the figure.

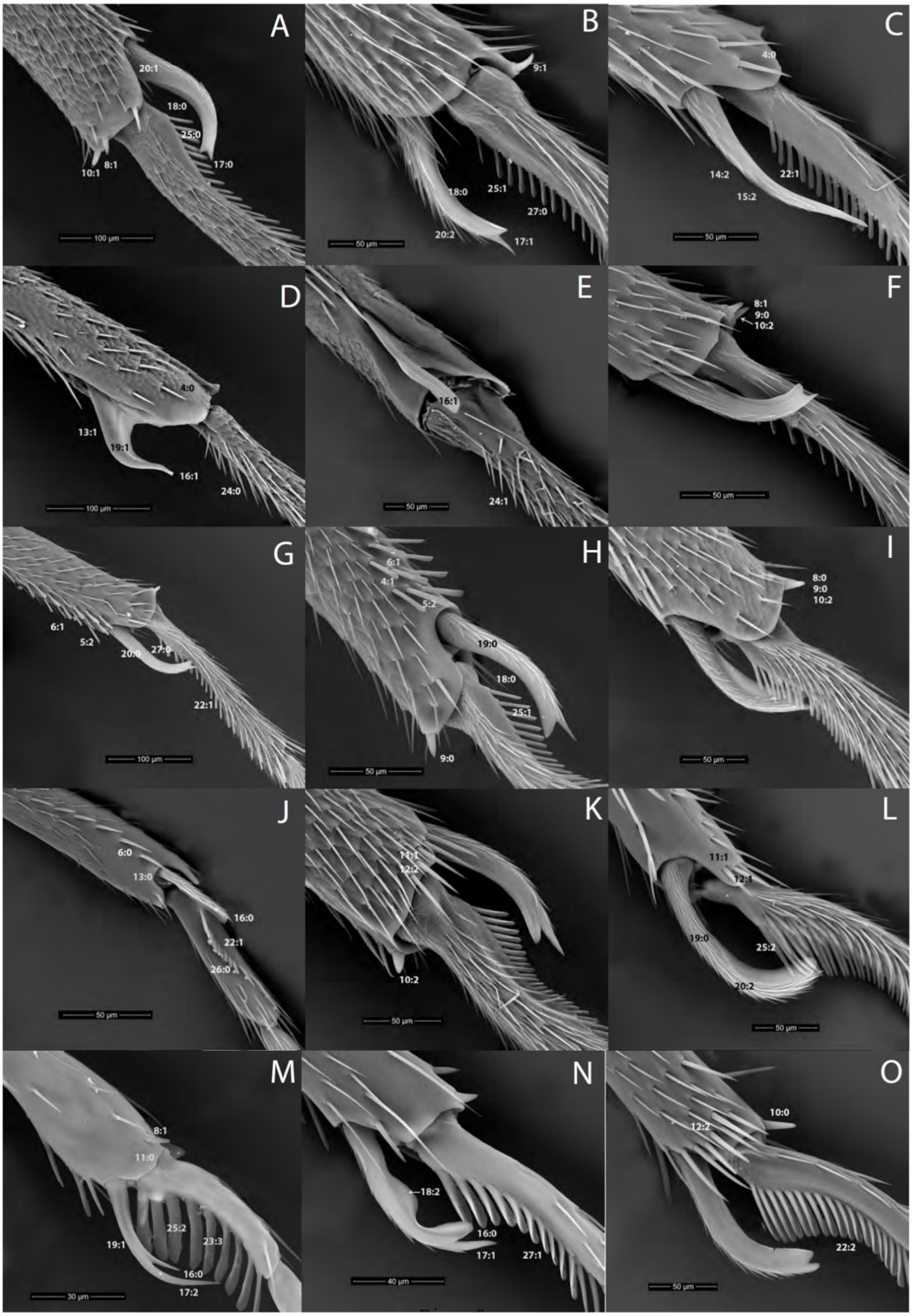

Figure 3. Variability of characters and character states (shown as character: state, see text) in the strigil of selected Cynipidae (Aulacideini, Diplolepidini, Pediaspidini, Eschatocerini, Cynipini, Synergini) and Figitidae (Emargininae, Anacharitinae, Eucoilinae, Figitinae). **A**: *Antistrophus silphii*; **B**: *Diplolepis mayri*; **C**: *Pediaspis aceris*; **D-E**: *Eschatocerus acaciae*; **F-G**: *Melikaiella cameroni*; **H**: *Rhoophilus loewi*; **I**: *Agastoroxenia panamensis*; **J**: *Lithonecrus papuanus*; **K**: *Synergus umbraculus*; **L**: *Acanthaegilips* sp.; **M**: *Emargo* sp.; **N**: *Hexacola* sp.; **O**: *Neralsia* sp..

Results

Our analysis showed that overall the antenna cleaner of Cynipoidea (figures 1C, 2B, 2D, 2F) possess the typical and general morphology as already described (Basybuyuk and Quicke, 1995), but it also shows that this organ is variable both among lineages and within lineages in many characters.

The shape of protibia (character 1) is almost invariably gradually and slightly broadening towards apex (figures 2A, 2C, 2E), being the only exception *Emargo* sp. (Figitidae: Emargininae), in which the protibia is strongly expanded towards apex. This typical weakly expanded protibia seems also the rule outside Cynipoidea, given our observation of the outgroup species, but is more expanded in Diapriidae. The protibial/profemur length ratio (character 2) was quite variable within Cynipoidea and even within Cynipidae (figures 4-8). However, one notes that relatively long protibia (i.e. state: 1, figure 2E and state: 2) essentially occurs in some groups of Cynipidae (Aulacideini, Aylacini, Cynipini and Ceroptresini) (figures 4-6), being very short (state: 0) in all the other Cynipidae, all Figitidae (except *Emargo* sp.) and all outgroups (except Diapriidae) (figures 4-9). On the other hand, the protibial/probasitarsus length ratio (character 3), seems extremely variable between and within lineages, even within tribes or subfamilies (figures 4-9). Among Cynipoidea, Cynipini and Synergini had most often very long protibia (more than three times the probasitarsus length, state: 2) (figure 6), while protibia which are equal or a little longer than probasitarsus (state: 0) were observed in different, even distantly related, lineages, but in general with a minor occurrence than the other situations (states: 1-2) (figures 2E, 4-9).

In some species, the distal half of protibia possess modified thicker and longer setae (character 4). This trait was common in Cynipidae, with the exception of Eschatocerini, Paraulacini and Pediaspidini (figures 4-6), and in Figitidae (all species) (figures 3H, 7-8), but seems to lack in Ibaliidae and Liopteridae (figures 3C, 4). The outgroups closer to Cynipoidea (Diapriidae and Ormyridae) also lacked this trait, while such setae were observed in Ichneumonidae (figure 9). When present, these modified setae can have different shape (character 5). They were very rarely conical and pointed (2 species of Figitidae), being more often roughly flat and not expanded distally (state: 1, 44 species) (figures 4-9); only eight species, essentially concentrated in Aulacideini and Cynipini, have paddle shaped, expanded apically setae on the protibia (state: 2, figure 3G) (figures 3, 6). Furthermore, the protibia's modified setae can occur in one or more rows (character 6). One row of setae (state: 0, figure 3J) was observed especially in Figitidae, Ibaliidae and Liopteridae (as well as in the outgroups) (figures 4, 7-8), while more than one, irregularly arranged, rows (state: 1, figure 3G), were observed in Cynipidae (figures 4-6).

A dorso-apical socketed spur on apical margin of protibia (in lateral view opposite to calcar) (character 7) occurs only in almost all species of Cynipoidea, but notably not in four species of Figitidae (including all three studied species of Anacharitinae) (figures 7-8). In the outgroups, the spur was only observed in Diapriidae. When present (character 8), a single spur was observed in most cases (state: 0) (figures 4-9), being the only exception six species of Cynipidae and the only studied species of Ibaliidae (figures 4-6). All of these exceptions showed two spurs (state: 1, figure 3A), but Ibaliidae and one species of Aulacideini showed three or more spurs (state: 2). Also the shape of spurs (character 9) varies: it was straight in most species, including all Figitidae, Ibaliidae and Liopteridae (as well in Diapriidae) (state: 0, figure 3F) (figures 4, 7-8), while it was slightly curved at the apex in most Aulacideini, in one species of Aylacini and in Diastrophini, Diplolepidini, Paraulacini, Phanacidini and Pediaspidini (state: 1, figure 3B) (figures 4-6). Furthermore, width/length ratios (character 10) show that the spurs are extremely narrow and long (state: 0) in only four species across Cynipidae and Figitidae, having an intermediate length (state: 1, figure 3A) in most species, essentially in Aulacideini, Phanacidini, Synergini and most Figitidae (figures 4-8), and being short and wide (state: 2, figure 3F) in 15 species, essentially in Cynipini, Ceroptresini, Eschatocerini, Pediaspidini and in the Ibaliidae and Liopteridae (figures 4-6).

The apical margin of the protibia also bears spines (or stout long flat spines, or setae) (character 11) in about half of the studied species, including most Ibaliidae, Liopteridae, most Figitidae and, within Cynipidae, especially in Aulacideini and Aylacini (figures 3K, 3M, 4-9). When present, these setae range in number (character 12) from 1 (state: 0) to 2-4 (state: 1) to more than 5 (state: 2) (figures 3K, 3O, 4-9). The number of setae does not seem to follow a taxonomically-organized pattern, with lineages within families often showing large differences [e.g. Figitinae (mostly state: 2) vs. Eucoiliinae (mostly state: 0)] (figures 7-8). Even within cynipid tribes, variation is important. For example, within Aulacideini, all three character states are represented. Ibaliidae and Liopteridae, however, have both 2-4 setae. The outgroups have all > 2 setae (figure 4).

The protibial calcar is typically inserted on a recess of apical margin of protibia, curved and slightly twisted to one side and its base is not on an elevation (character 13) (figures 3D, 3J, 4-9). The only exception is *Eschatocerus acaciae* Mayr, in which the calcar is inserted slightly before apical margin of protibia on an elevated base, uniformly curved inward (figure 4). Compared with basitarsus, the calcar (character 14) varies from very short (state: 0, figure 2C) to moderately long (about the half of basitarsus length) (state: 1), to very long (more than half of basitarsus length) (state: 2, figure 3C). States for this character are distributed across almost all main clades and even within clades (figures 4-9). Ibaliidae and Liopteridae (figure 4), however, have a very short calcar, while most of the outgroups (figure 9) have a very long calcar. Compared with the width of the apical margin of protibia (character 15), the calcar length also shows great variability among and within groups (figures 4-9). However, at least one notes that Ibaliidae, Liopteridae and Figitidae all have from moderately long (1.5-2 × protibia margin width) to very long (> 2 × protibia margin width) calcar (figures 2C, 3C, 4, 7-8), while shorter values were only observed in Cynipidae (16 species, especially in Synergini and Aulacideini) (figures 4-6).

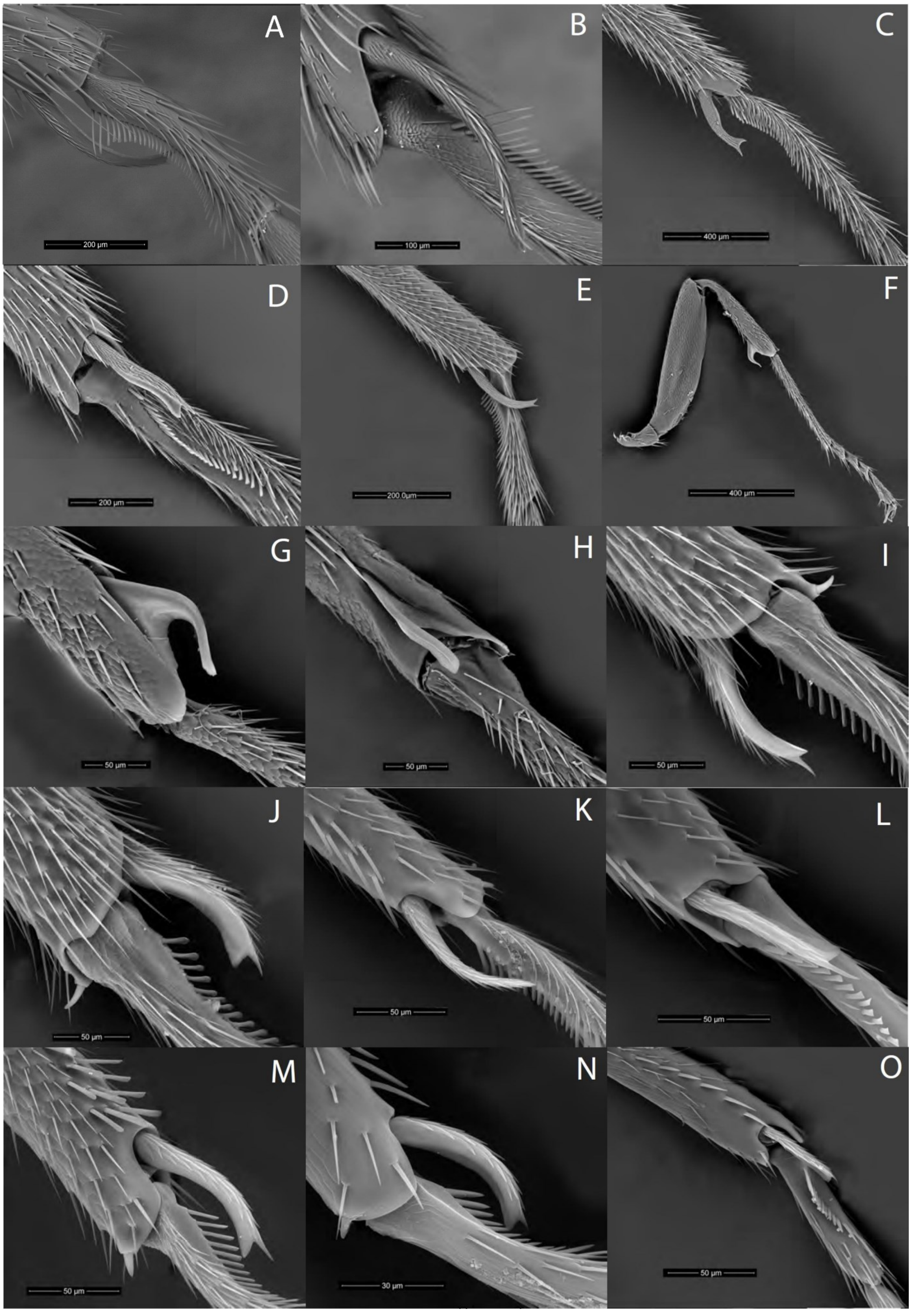

Figure 4. Variability in the strigil morphology of selected Cynipidae (Diplolepidini, Eschatocerini, Qwaqwaiini, Ceroptresini, Synergini), Ibaliidae and Liopteridae. **A-B**: *Pseudibalia*; **C-D**: *Ibalia leucospoides*; **E**: *Qwaqwaia scolopiae*; **F-H**: *Eschatocerus acaciae*; **I-J**: *Diplolepis mayri*; **K**: *Ceroptres* sp.; **L-M**: *Rhoophilus loewi*; **N-O**: *Lithonecrus papuanus*.

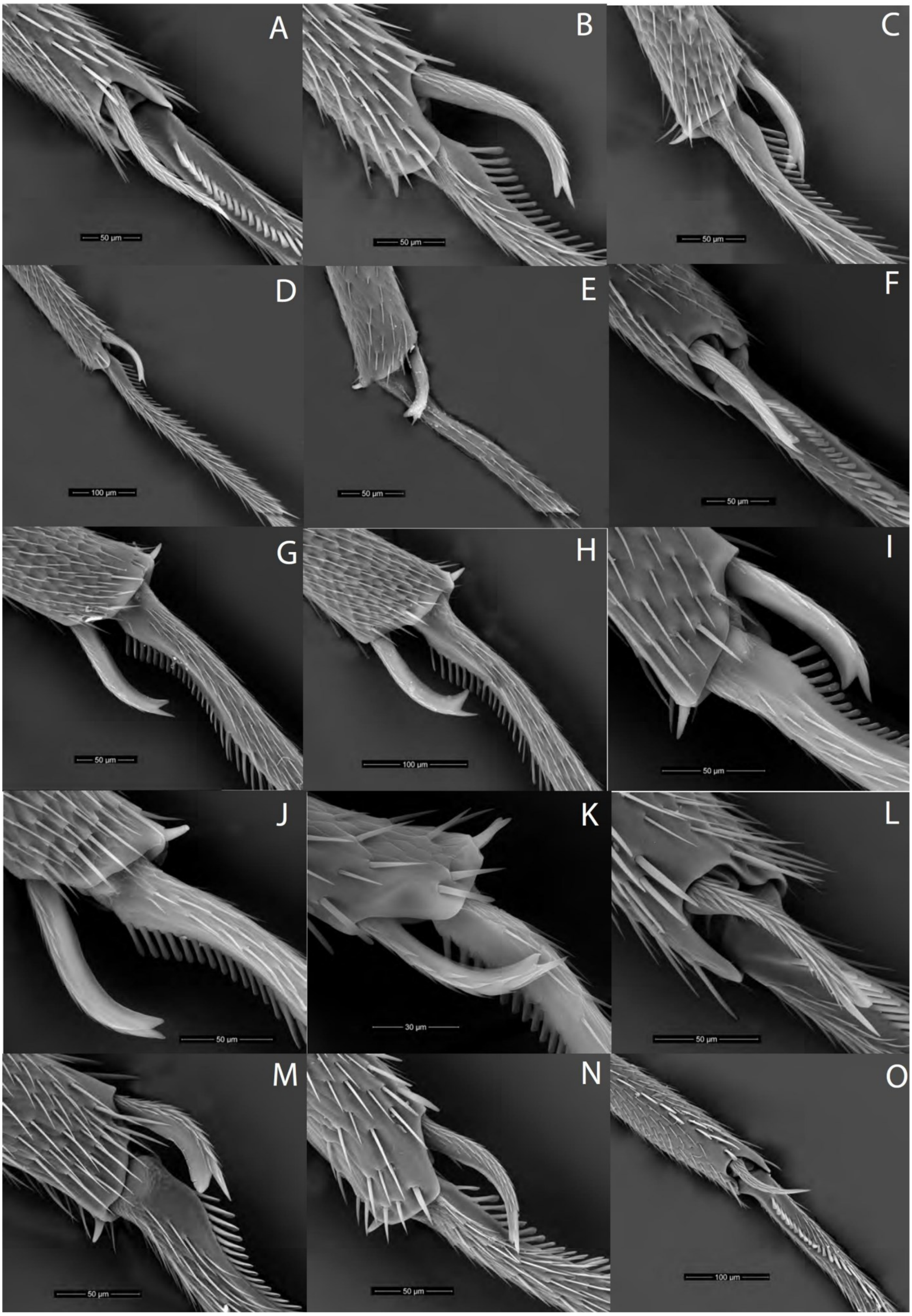

Figure 5. Variability in the strigil morphology of selected Cynipidae (Aylacini, Aulacideini, and Diastrophini). **A-B**: *Barbotinia oraniensis*; **C-D**: *Iraella luteipes*; **E**: *Panteliella fedtschenkoi*; **F-G**: *Aulacidea hieracii*; **H**: *Isocolus lichtensteini*; **I**: *Liposthenes kerneri*; **J**: *Neaylax verbenacus*; **K**: *Neaylax versicolor*; **L-M**: *Periclistus brandtii*; **N-O**: *Xestophanes potentillae*.

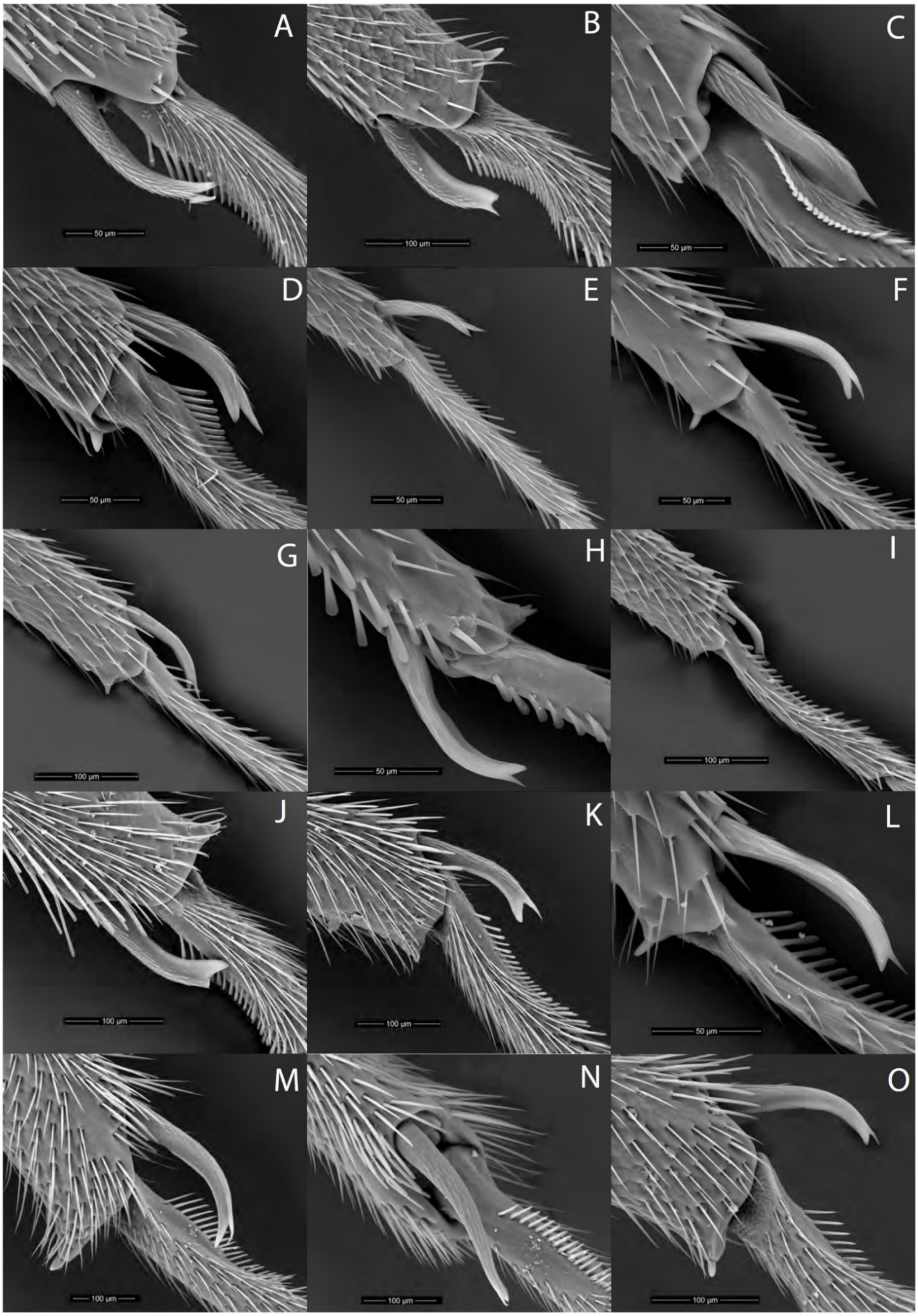

Figure 6. Variability in the strigil morphology of selected Cynipidae (Synergini and Cynipini). **A**: *Saphonecrus gallaepomiformis*; **B-C**: *Synergus mesoamericanus*; **D**: *Synergus umbraculus*; **E**: *Synergus physocerus*; **F**: *Dryocosmus kuriphilus* (asexual female); **G**: *Plagiotrochus australis*; **H**: *Pseudoneuroterus saliens*; **I**: *Andricus grossulariae*; **J-K**: *Andricus pictus* (asexual female); **L**: *Andricus quercusradicis*; **M-N**: *Biorhiza pallida* (asexual female); **O**: *Biorhiza pallida*.

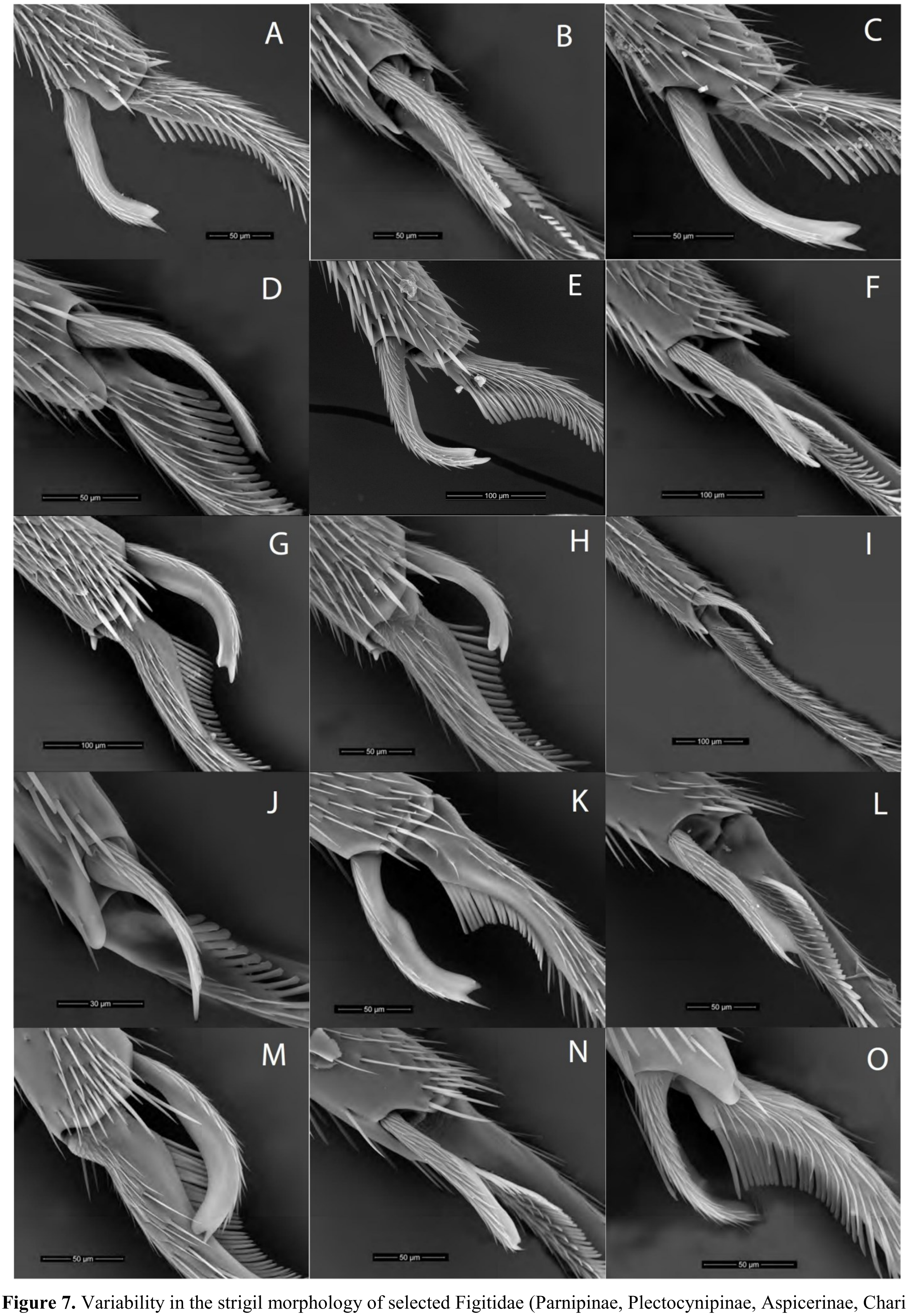

Figure 7. Variability in the strigil morphology of selected Figitidae (Parnipinae, Plectocynipinae, Aspicerinae, Charipinae and Anacharitinae). **A-B**: *Parnips nigripes*; **C-D**: *Plectocynips pilosus*; **E-G**: *Callaspidia notata*; **H-I**: *Melanips* sp.; **J**: *Alloxysta* sp.; **K-L**: *Anacharis* sp.; **M-N**: *Acanthaegilips* sp.; **O**: *Xyalaspis* sp..

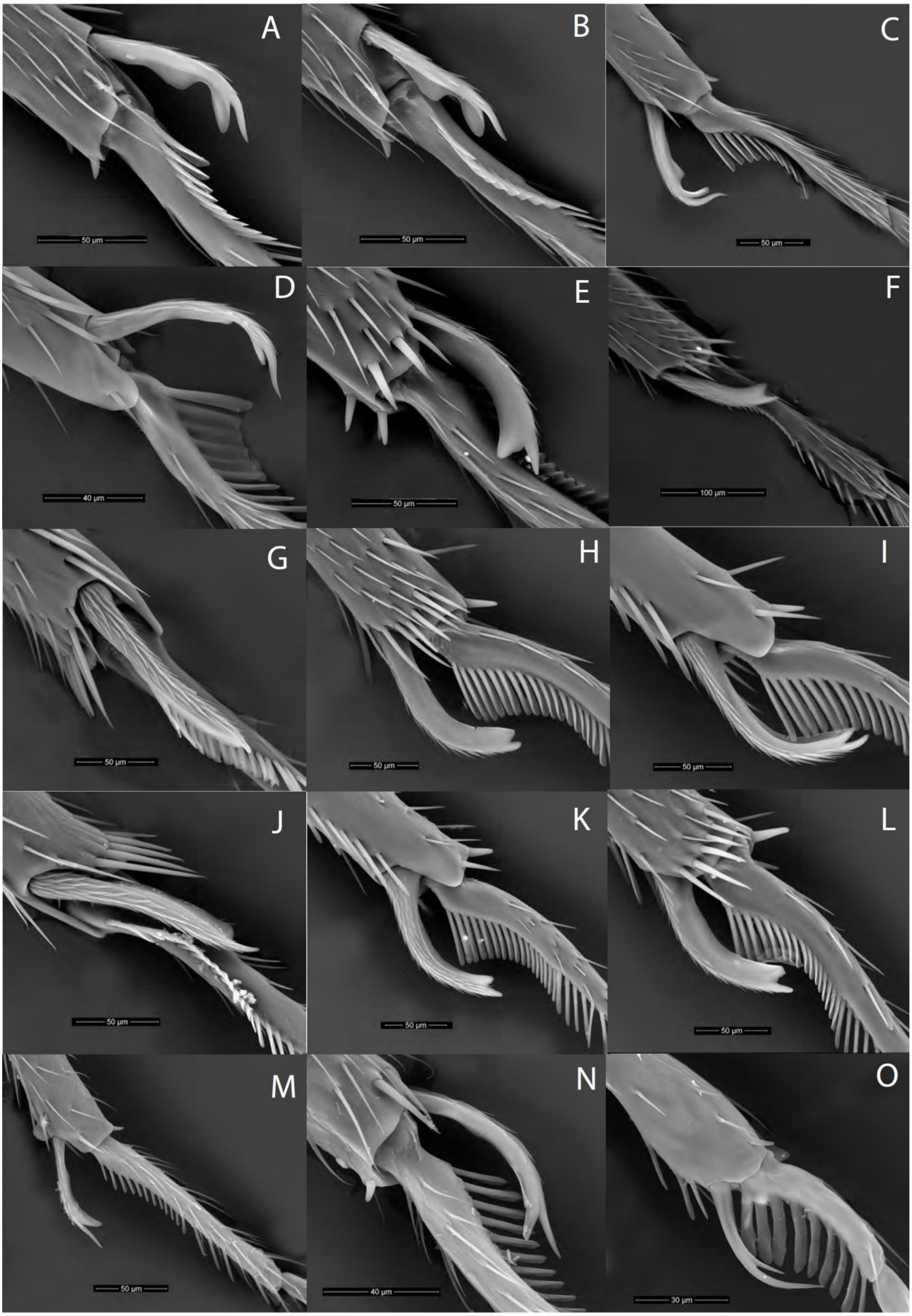

Figure 8. Variability in the strigil morphology of selected Figitidae (Figitinae, Eucoilinae and Emargininae). **A-B**: *Hexacola* sp.; **C-D**: *Tropidoeucoila* sp.; **E-F**: *Sarothrus* sp.; **G-I**: *Neralsia* sp.; **J-L**: *Figites* sp.; **M-N**: *Lonchidia* sp.; **O**: *Emargo* sp.

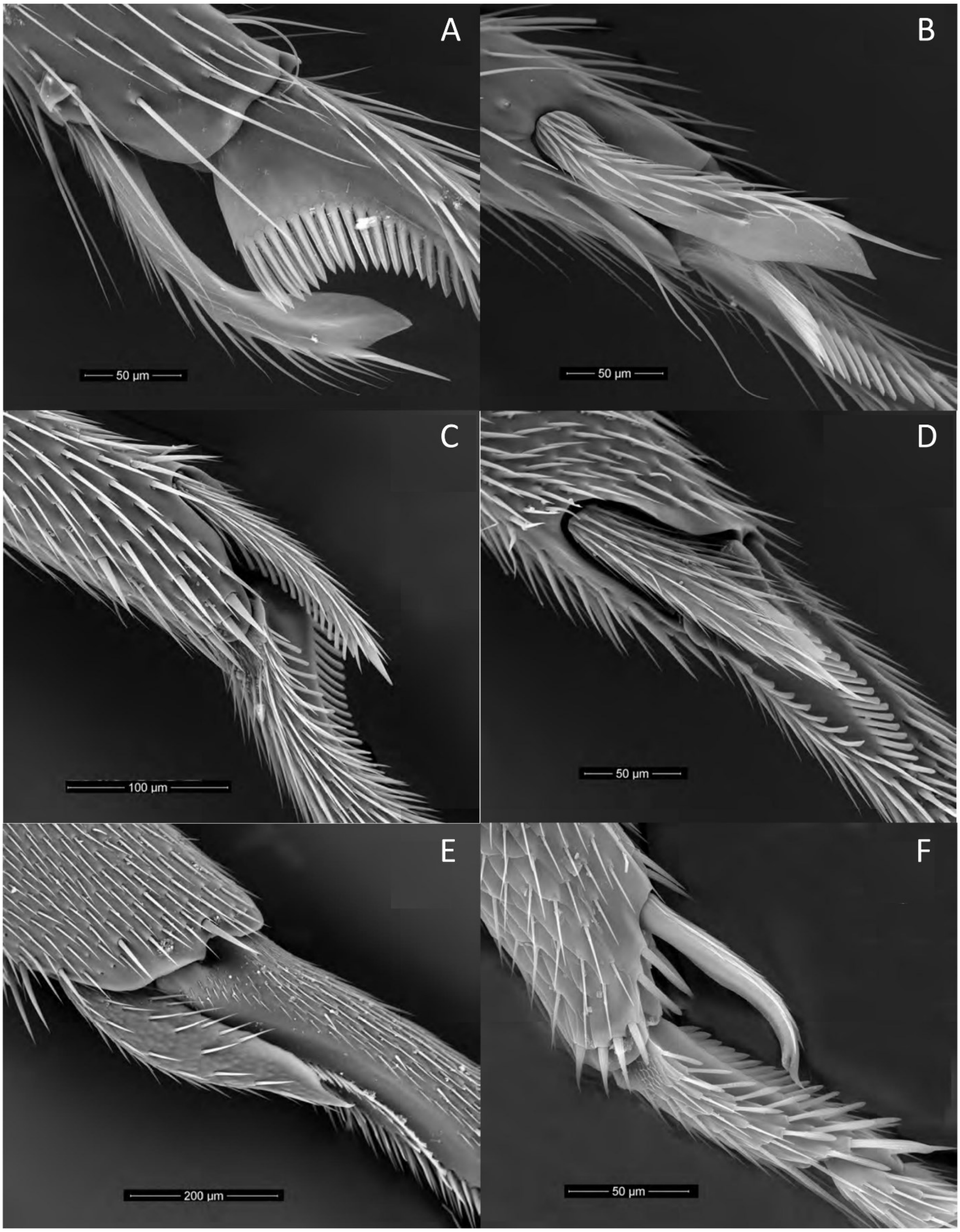

Figure 9. Variability in the strigil morphology of Hymenoptera outgroups analysed in this study (Diapriidae, Ichneumonidae and Ormyridae). **A-B**: *Dissixylabis* sp.; **C-D**: *Scambus elegans*; **E**: *Rhyssa persuasoria*; **F**: *Ormyrus papaveris*.

In almost all species, the calcar has a cleft tip-apex (i.e. bifurcated) (figures 4-9), with only *E. acaciae* (figure 4) and the two studied species of Ichneumonidae (figure 9) having a calcar with a single tip-apex (character 16, figures 3D, 3J). When the calcar has a bifurcated apex, the latter has either an acute tip and an apical lobe of velum almost of equal size (or the former only slightly longer) (character 17, state: 0, figure 3A) or an acute spine longer than apical lobe of velum (states: 1-2) (figures 3B, 3M, 3N, 4-9); variations in characters' states are found among and within most lineages. On the other hand, the ventrolateral margin of velum is essentially always straight or uniformly curved, without a projected lobe (character 18, state: 0) (figures 3A, 4-9). The very few excepions are Ibaliidae and Eucoiliinae, which have either a velum slightly projected basally (the former, figure 4) (state: 1, figure 2B) or a velum with a broad projected lobe medially (the latter, figure 8) (state: 2, figure 3N). The microsculpture on dorsal area of calcar (character 19) was also quite uniform across Cynipoidea, being almost invariably spine-like, with lying or erect setae (state: 0) (figures 4-9). Only *E. acaciae* (figure 4) and *Emargo* sp. (figure 8) presents a smooth microsculpture without setae (state: 1, figures 3D, 3H). A further modification, i.e. mixed scale-like and spine-like microsculpture (state: 2), was only found in the ichneumonid *Rhyssa persuasoria* (L.) (figure 9). The shape and density of setae on dorsal area of calcar (character 20) is variable but seems to follow a taxonomic pattern. Indeed, some lineages (e.g. Cynipini, Paraulacini, Pediaspidini) essentially have short, lying, widely spaced setae (state: 0, figures 2F, 3G) (figures 4-6), while others (e.g. Aulacideini, Aylacini, Ceroptresini, Synergini) have longer, slightly erect at apex and more closely spaced setae (state: 1) (figures 3A, 4-6). A further morphology (very long, erect and dense setae, state: 2, figure 3B) is rare and only occurs in 1 species of Cynipidae and 3 species of Figitidae (figures 7-8). Most outgroups also show this latter morphology (figure 9).

Compared with the protarsus, the probasitarsus (character 21) ranges from very short (Ibaliidae, four figitid subfamilies, Pediaspidini and some species spanning other clades) (state: 0), to moderately long (about half of the protarsus length) (state: 1) (e.g. Anaharitinae, Aspicerinae, most Figitinae, most Synergini, most Cynipini and most Aulacideini), to very long [only in *Sarothrus* sp. (Figitinae)] (state: 2) (figures 4-9). In lateral view, the basitarsal notch appears as slightly curved (character 22, state: 1, figure 3C) in most species, including Ibaliidae, Liopteridae and all Cynipidae except *E. acaciae* (figures 4-9). The latter is the only species having a straight notch, together with the outgroup species *Ormyrus papaveris* (Perris) (state: 0). In Figitidae, on the other hand, many species have an extremely curved notch, with the basal area of basitarsus broadened and sharply narrowed medially (state: 2, figure 3O), though also the state: 1 is well represented in this family (figures 7-8). The basitarsal notch (character 23) ranges from very short (very few cases, mostly in Cynipidae) (state: 0) (figures 4-6) to about half the basitarsus in length (more often in Figitidae) (state: 1) (figures 7-8), to clearly longer than half of the basitarsus in length (more often in Cynipidae, and also found in Ibaliidae) (figures 4-6) (state: 2), to equal to basitarsus in length (only in *Emargo* sp. and *O. papaveris*) (state: 3, figure 3M) (figures 8-9).

With the exception of *E. acaciae* (figures 3D, 4), all the studied species possess a basitarsal comb (character 24) (figures 4-9). In Cynipidae and Ibaliidae, the setae of the comb are more often very short (character 25, state: 0, figure 3A) (figures 4-6), while in Figitidae they are more often very long (state: 2) (figures 3M, 7-8). Intermediate length (state: 1, figure 3B) was observed in Liopteridae (figure 4) and other 21 species within Cynipoidea scattered across lineages. Very short or small setae on the comb are also observed in the studied outgroups. The setae are arranged on the comb almost universally in one row (character 26, state: 0, figure 3J) (figures 4-9), with the only exceptions of the two studied species of Ichneumonidae, in which setae are arranged in two rows (state: 1) (figure 9). All Cynipidae have slightly separated setae on the comb (character 27, state: 0) (figures 3B, 4-6), while most of Figitidae (figures 7-8), as well as Ibaliidae, Liopteridae (figure 4) and all the outgroups (figure 9) have closely spaced, almost contiguous setae on the comb (state: 1, figure 3N).

The similarity phenogram of the UPGMA (cluster analysis, unweighted pair group method analysis) based on the coded characters of antenna cleaner did not point towards the morphology of antenna cleaner morphology as a useful taxonomic or phylogenetic suite of characters (figure 10). However, at least the relative position in the tree of most Figitidae and most Cynipidae somehow reflects one of the available molecular phylogenies (figure 1A). Again, some species belonging to the same tribe or subfamily (Anacharitinae, Aulacideini) appeared reasonably closer in the same cluster, but this trend was not valid for most clades. Interestingly, while in general the life-history did not seem to influence the output of the cluster analysis, most parasitoids of Diptera in this tree are placed reasonably close to each other (figure 10), despite belonging to different clades (Figitinae and Eucoiliinae, which did not seem phylogenetically very close, following the recent molecular recosntructions) (figure 1A-B). Also gall-inquilines seem to fall all in the same (though large) cluster of the dendrogram (figure 10), despite spanning three different cynipid tribes.

Discussion and conclusions

The 'typical' antenna cleaner of Hymenoptera consists of an anterior apical spur on the foretibia, often with a velum, and a modified basitarsus bearing modified setae; this general structure seems to be an autopomorphy for the order (Basibuyuk and Quicke, 1995), though secondary simplifications can be seen in some lineages (Vilhelmsen, 2001). Although their fundamental role in maintaining clean and functional antennal surfaces (Hackmann *et al.*, 2015), the morphology of strigil was not studied in detail in most hymenopteran lineages.

Our study analysed the morphology of the antenna cleaner in a rich, biologically diverse group of wasps, the Cynipoidea, to a detail much deeper than any previous study on this as well as on other hymenopteran lineages. In particular, using a much larger taxonomic spectrum of

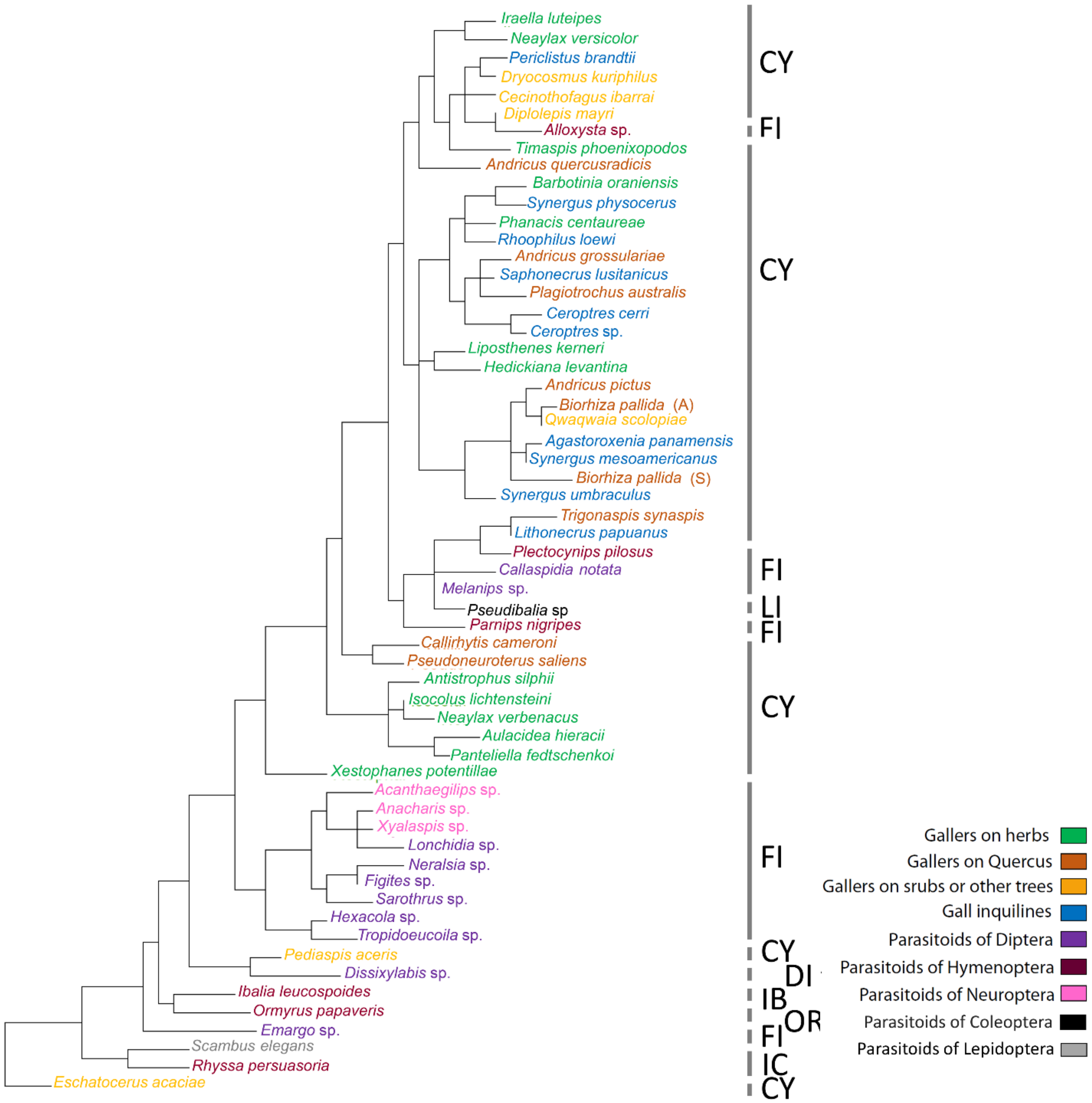

Figure 10. Overall similarity phenogram of a UPGMA unrooted tree (cluster analysis, unweighted pair group method analysis) of the studied species of Cynipoidea, based on the coded characters of antenna cleaner. IB: Ibaliidae, LI: Liopteridae, FI: Figitidae, CY: Cynipidae, DI: Diapriidae, IC: Ichneumonidae, OR: Ormyridae. Different colours represent different life-histories.

species and a much greater number of morphological characters than in previous investigations (Basibuyuk and Quicke, 1994; 1995), we were able to highlight a larger variation both within and between lineages. This allowed us to suggest that the morphology of antenna cleaner carries relatively weak information on phylogenetic relationships. A doubtful effect of biological cycle (gallers, inquilines or parasitoids) on the variation of this organ also emerged: for example, while gall inquilines (spanning three cynipid tribes) and Diptera-parasitoids (spanning two figitid lineages) seem to have some roughly convergent traits, herb-gallers (also spanning three cynipid tribes) seem much more variable in morphology. Certainly, such conclusion is dependent on the type of morphological analysis employed here, and different types of analyses may in the future evidence some kind of association between morphology and biology and/or phylogenetic history. For example, a lot of the variation of the antenna cleaner found here seems to consist of continuous “shape” differences, so that using a different methodological approach more adequate in case of continuous variation, such as geometric morphometrics, has great potential to unveil new patterns (reviewed in Tatsuta *et al*., 2018).

Results from Basibuyuk and Quicke (1995) indicated some patterns, that we can compare with our own observations. Such comparison evidenced similarities in distribution of main character states among taxa between both studies. For example, both observed a bifurcate calcar, with a smooth velum and a weak basitarsal notch, in Ibaliidae, Figitidae and Liopteridae. Within the Cynipidae, all the species studied by Basibuyuk and Quicke (1995) have the calcar with bifurcate apex, which was also the case in our study (with the only exception of Eschatocerini, not analysed previously). However, some new variable traits within lineages emerged in our study through the analysis of much more species and characters. For example, the protibia is strongly expanded towards apex only in Emargininae, while the protibia's modified setae occur in one row in some subfamilies (e.g. Charipinae, Figitinae) and in more than one rows in other subfamilies (Anacharitinae). Anacharitinae also differentiated from the rest of Figitidae by lacking a dorso-apical socketed spur on apical margin of protibia. Basibuyuk and Quicke (1995) also observed that the antenna cleaner of the Charipinae is similar in structure to that of Eucoilinae, but our analysis (see figure 10) did not show such similarity when many species are analysed. Additionally, Basibuyuk and Quicke (1995) found no paddle-shaped setae in Cynipidae, while we have found this character state in eight cynipid species, spanning several tribes, none of them previously analysed.

We found some morphological traits in the antenna cleaner quite conserved across species and lineages of Cynipoidea. For example, the shape of protibia is almost invariably broadening towards apex, and almost all species possess one single, straight rather than curved, dorso-apical socketed spur on apical margin of protibia. Another character which is quite invariable across species is the insertion of the protibial calcar on a recess of apical margin of protibia, curved and slightly twisted to one side. Such characters may be part of the groundplan of the Cynipoidea. On the other hand, other characters seem to be used to roughly differentiate cynipoid families. Hence, Cynipidae are almost the only ones in having a relatively long protibia, while Figitidae, Ibaliidae and Liopteridae have typically thick and long protibial setae which are arranged in one row (more than one rows is more typical of Cynipidae), and these families have also a longer calcar compared with Cynipidae. This observation in interesting, since it may support the phylogenetic hypothesis of Blaimer *et al.* (2020), in which Figitidae, Ibaliidae and Liopteridae as a whole form a large cluster. Furthermore, Figitidae have shorter basitarsal notch compared with Cynipidae and Ibaliidae, which may suggest either a reduction of the notch in Figitidae -following the phylogeny depicted in Ronquist *et al.* (2015)- or a size increase of the notch in Ibaliidae and Cynipidae -following the phylogeny depicted in Blaimer *et al.* (2020)-. Additionally, in Cynipidae, the setae of the basitarsal comb are more often short and slightly separated, while in Figitidae they are more often long and closely spaced. Further characters were extremely variables across and within lineages.

Also interesting is that Eschatocerini are placed within the cluster of Figitidae + Ibaliidae + Liopteridae in the hypothesis of Blaimer *et al.* (2020), and indeed this cynipid tribe seems to possess a very particular antenna cleaner with many morphological traits shared with these parasitoid families, and different from Cynipidae. This highlights the complicated position of Eschatocerini in Cynipoidea. Blaimer *et al.* (2020), depending on the analysis, recovered Eschatocerini either as sister-group to Figitidae, or as sister-group to Cynipidae s.s., or nested near Paraulacini and Diplolepidini (which also fall, together with Pediaspidini, outside Cynipidae s.s. in the phylogeny, making the family not monophyletic), with none of these hypotheses particularly robust. Apart from Eschatocerini, among the other tribes grouping outside of the family in the study of Blaimer *et al.* (2020), Pediaspidini also showed a quite particular antenna cleaner which resembled more that of *Dissixylabis* (Diapriidae) than that of Cynipoidea, while Paraulacini and Diplolepidini showed a similar antenna cleaner which did not differ so much from the other Cynipidae.

In any case, overall, the similarity phenogram did not reveal a clear role of the studied morphological characters in depicting phylogenetic relationships in Cynipoidea. Moreover, the morphological variation of antenna cleaner could not be associated with life-history traits (at least those here considered and through the used methodological approach), which may suggest that all species need an essentially similar cleaning device independently if they form galls on plants, search for galls to invade, or search for concealed or unconcealed hosts to parasitize. Indeed, while previous studies unveiled an important variation in antennal sensillar equipment (Polidori *et al.*, 2014; Jorge *et al.*, 2019), the overall shape and general morphology of antennae themselves, with their filiform, rarely clavate flagellum, did not greatly varied across cynipoid linegaes. Hence, a roughly similar strigil will probably supply a good cleaning service for all cynipoid antennae.

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