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Research article

Flagellar Sensillar Equipment of Two Morphologically Closely Related Aphid Hyperparasitoids (Hymenoptera: Figitidae: *Alloxysta*)

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Abstract

The antennal sensillar equipment in the parasitic wasp family Figitidae was analyzed to date only in few species, despite some are associated with crop pests and can have an economic importance. It is the case of the genus *Alloxysta*, which includes hyperparasitoids of aphids which can potentially reduce effectiveness of primary pest parasitoids. Here we analyzed, through scanning electron microscopy, the diversity, morphology, and distribution of the antennal sensilla in males and females of *Alloxysta consobrina* (Zetterstedt) and *Alloxysta victrix* (Westwood), two species with overall very similar morphology. In both species, antennae are filiform and cylindrical, and flagellum was longer in *A. victrix*. Eight sensillar types have been recognized: four types of sensilla trichoidea (ST-A, ST-B, ST-C, ST-D), sensilla coeloconica, sensilla placoidea, sensilla campaniformia, and sensilla basiconica. ST-A, ST-B, ST-C, and sensilla placoidea were the most abundant types on the antennae and often increased in number and decreased in size toward the tip of antenna. The two species seem to have several differences in their sensillar equipment, possibly in accordance with the different degree of host range. On the other hand, sexual dimorphism is probably due to the different stimuli that have to be correctly processed. The comparison with the other species of Figitidae studied by far showed, at subfamily-level, that variability in sensillar equipment and phylogeny do not agree. This suggests a complex series of morphological changes during evolution of this group. The taxonomic sample should be thus substantially enlarged to disclose possible trends in sensillar equipment evolution in the family.

Key words: antenna, sensilla, morphology, Charipinae, *Alloxysta*

The antennae are the most important sensory appendages of Hymenoptera, being involved in a wide range of behaviours including habitat selection, host location, and sexual communication, such functions being facilitated by specialized parts of the antennal epidermis, called sensilla (Bin et al. 1989; Isidoro et al. 1996, 2001; van Baaren et al. 2007). These sensilla present different morphologies and have different functions. For example, hair-like sensilla of different shape and length include both mechanoreceptors and chemoreceptors (Romani et al. 2010), pored plate-like sensilla are known to have an olfactory function (Ochieng et al. 2000), and pit-like sensilla seem to be involved in gathering information on temperature and humidity (Isidoro 1992).

For parasitoid Hymenoptera, previous studies have characterized in detail the antennal sensilla in species mainly from the superfamilies Ichneumonoidea and Chalcidoidea (van Baaren et al. 1996, Amornsak et al. 1998, Basibuyuk and Quicke 1999, Ochieng et al. 2000, Bourdais et al. 2006, Dweck and Gadallah 2008, Onagbola et al. 2009). Instead, information on the antennal sensillar equipment in another important parasitoid group, the Figitidae (Cynipoidea) (about 1,400 species worldwide), is still scarce (e.g., Butterfield and Anderson 1994, Tormos et al. 2013, Polidori and Nieves-Aldrey 2014). This contrasts to the many species (about 50) analyzed in the other large, primarily herbivorous (galler) cynipoid family (Cynipidae) (Polidori and Nieves-Aldrey 2014). In particular, as far as we know, the few studies carried out to date concern 11

species of Figitidae in seven subfamilies (Butterfield and Anderson 1994, Tormos et al. 2013, Polidori and Nieves-Aldrey 2014) and studies in which both sexes were considered are even rarer (Tormos et al. 2013).

Here, we contribute to the study of antennal sensilla diversity in the Figitidae analyzing two species from the subfamily Charipinae. For this subfamily, the sensillar equipment was studied by far only in females of one species in the genus *Apocharips* (Polidori and Nieves-Aldrey 2014). In females of this studied species, total of eight types of sensilla were observed on the antennae, i.e., in the upper limit of the range of types described overall for Figitidae (4–9 per species) (Butterfield and Anderson 1994, Tormos et al. 2013, Polidori and Nieves-Aldrey 2014).

The Charipinae are very small wasps (no more than about 2 mm in length) widely geographically distributed and biologically characterized by being secondary parasitoids (hyperparasitoids) of aphids via Aphidiinae (Ichneumonoidea: Braconidae) and Aphelininae (Chalcidoidea: Aphelinidae) and secondary parasitoids of psyllids via Encyrtidae (Chalcidoidea) (Menke and Evenhuis 1991). Because they attack primary parasitoids of crop pests, such wasps may have the potential to make ineffective the biological control plans based on the release of their hosts (Höller et al. 1993).

This study has been focused on two closely related species, *Alloxysta victrix* (Westwood) and *Alloxysta consobrina* (Zetterstedt), both being aphid hyperparasitoids and cosmopolitan. Because of their great morphologically similarity (Rakhshani et al. 2010, Ferrer-Suay et al. 2011), these species have been often confused in past studies, considered synonymous species, or population-varying morphs of a single species (Ferrer-Suay et al. 2013). The body size of these two species is very similar, body length ranging from 0.9 to 1.2 mm in *A. consobrina* and 0.9 to 1.5 in *A. victrix*. The few morphological differences between *A. consobrina* and *A. victrix* are mainly based on the coloration, proportion between flagellomeres, grade and distribution of propodeal pubescence, and size of radial cell (Ferrer-Suay et al. 2011). On the other side, despite both species are clearly generalist in both primary and secondary host use (Ferrer-Suay et al. 2014), they show different host range, *A. victrix* attacking about 30% more aphid species than *A. consobrina* and doubling the number of aphid parasitoid species attacked by *A. consobrina* (Ferrer-Suay et al. 2014).

In this study, we aimed 1) to provide the first data on the morphology, abundance and distribution of the antennal sensilla in both sexes of any Charipinae, 2) verify if some characters related to sensillar equipment can help in discriminating the two *Alloxysta* species, otherwise morphologically very similar, and 3) placed our results within the variability of antennal morphology and sensory equipment known for Figitidae, in the light of the most recent phylogenetic scenarios for the family. Within Hymenoptera, the use of sensillar characters as additional tools to discriminate closely related species was previously suggested by inter-specific differences found within genera such as *Bombus* (Apoidea: Apidae) (Shang et al. 2010), *Anaphes* (Chalcidoidea: Mymaridae) (van Baaren et al. 1999), and *Trichrogramma* (Chalcidoidea: Trichogrammatidae) (Voegelé et al. 1975, but see Ruschioni et al. 2012 for cases of great intra-specific variation). Within Figitidae, differences in size and numbers of certain sensillar types were found within the genus *Aganaspis* (Tormos et al. 2013).

Materials and Methods

Scanning Electron Microscopy

For the quantitative study of sensilla abundance and morphology, one antenna of six individuals per species and sex were observed (i.e., a total of 24 individuals). The antennae were dehydrated in 70% ethanol and then removed from the head and mounted on microscope sample holders ("stub") through a bio-adhesive disk conductive. Then they were covered by a thin layer of gold (Jeol JFC-1100). Antennae were inspected dorsally/dorsolaterally or ventrally/ventrolaterally, depending on their orientation on the stubs, with all these views represented for each species and sex. The scanning electron microscopy (SEM) pictures were taken using an environmental scanning electron microscope (FEI Quanta 200 ESEM, Hillsboro, Oregon, USA) at 12 or 15 kV.

Terminology

Antennae are composed of (proximally to distally) a scape, a pedicel, and a number of antennal segments (flagellomeres) jointly called the flagellum (Goulet and Huber 1993). For characterization of general antennal morphology, we used the established classification provided by Goulet and Huber (1993), based on antennal shape.

The flagellomeres were designated F_1 to $F_{11/12}$ (the number depending on the sex, see Results), in a proximal to distal direction. For the sensilla inventory, we primarily followed the classification of sensilla by Polidori and Nieves-Aldrey (2014), who rely in their large study on Cynipidae on recognized sources for Hymenoptera sensory system terminology (e.g., Callahan 1975, Romani et al. 2010), based on external morphological characters. We also referred to definitions found in The Hymenoptera Anatomy Ontology (HAO) project portal (Yoder et al. 2010, Hymenoptera Anatomy Consortium [http://glossary.hymao.org]). The classification of sensillar types here used should be considered, for some sensilla types, as preliminary because the internal structure and function of different types of sensilla are poorly known (Altner et al. 1977).

Morphological Analysis

The number of sensilla belonging to each morphological type has been counted along the antennae in each flagellomere (see Results for sensillar classification). The length, width, height, and diameter of the sensilla types were obtained from the micrographs where the sensilla were well visible in adequate orientation. Linear measurements were taken by importing the SEM images into the software ImageJ (National Institutes of Health, USA).

Means and standard deviations (SD) related with the antennal morphology (flagellum length, flagellomere length, and width) and to the number of all types of sensilla and their sizes were then calculated for each species, sex, and for the different flagellomeres across the flagellum. Scape and pedicel, the more proximal segments of the antennae, were not studied in detail since they harbor only few sensilla in both sexes and species. Multiple linear regression models were employed to test for dependence of sensilla number (all types) and size (except those types with very insufficient sample size) on species, sexes, and flagellar segments. To avoid problems related with pseudoreplication, we applied statistics to the mean values per flagellomere calculated across the six individuals analyzed per species and sex. Untransformed data were used because they were normally distributed (Jarque-Bera test not significant). Data used for the regression models are available as Supplementary Data (Supp Table 1 [online only]).

To qualitatively compare our results with those available for the 11 previously studied species of Figitidae, we both referred to the morphological descriptions of antennae and sensilla provided and to the characters coded in the recent study of Polidori and Nieves-Aldrey (2014) on Cynipoidea sensillar equipment. Females only were considered in such a comparison, because most of previously studied species (9 out of 11) regarded only this sex. Inter-sexual

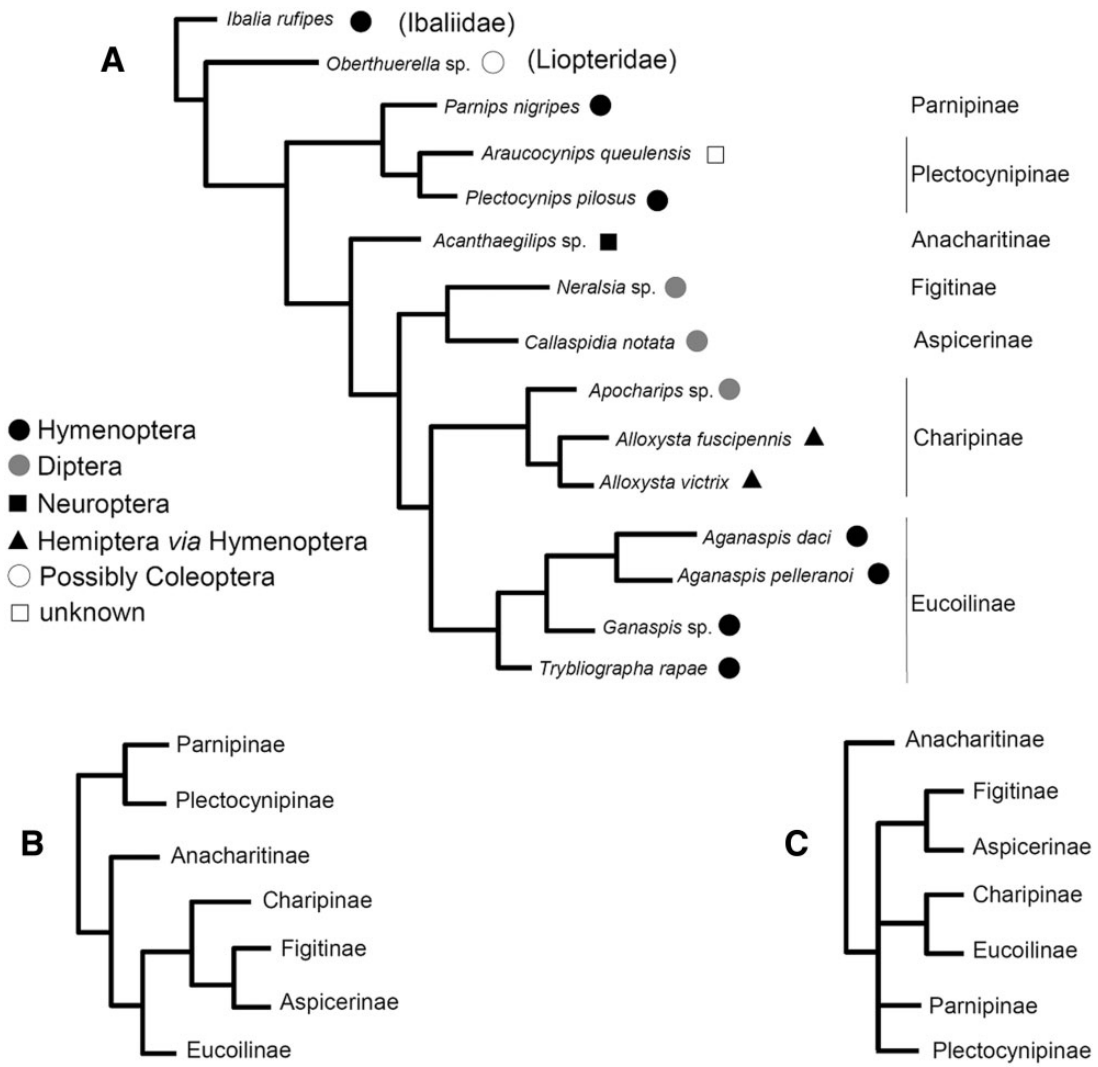

Fig. 1. Recent phylogenetic scenarios for the relationships among the 13 species of Figitidae, plus Ibaliidae and Liopteridae (ancestors), for which data on antennal sensillar equipment are available (including those from this study). The trees are based on the figitid subfamily-level relationships as depicted by Buffington et al. (2007) (based on combined analysis (28S D2 + D3, 18S, COI and morphology), Buffington et al. (2012) (for the position of Plectocynipinae), and Ronquist et al. (2015) (based one COI, 28S, LWRh, EF1alpha F1, and EF1alpha F2, morphology and life-history data). (A) Parsimony results in Buffington et al. (2007). (B) Bayesian inference result in Buffington et al. (2007). (C) Combined analysis in Ronquist et al. (2015). The host relationships for the 15 species are also shown, if information is available.

differences found in our study were only compared with those reported in the sole paper in which figitid males were studied (two species of a single genus) (Tormos et al. 2013) and with results of studies on non-figitid parasitoids (see Discussion). Two species from the basal families of Cynipoidea (Liopteridae and Ibaliidae), whose sensillar morphology was also provided in Polidori and Nieves-Aldrey (2014), were also incorporated as ancestors in the comparison. Known hosts for these 15 species range from Hymenoptera, Diptera, Hemiptera (via Hymenoptera), and Neuroptera, with an uncertain case of Coleoptera hosts (for Liopteridae) and one species for which the host is still unknown (belonging to Plectocynipinae) (Fig. 1). We performed a Hierarchical Cluster Analysis (Jaccard coefficient of dissimilarity) to study how these 15 species (13 Figitidae and 2 basal Cynipoidea) are morphologically related based on presence/absence of the different types of sensilla (Polidori and Nieves-Aldrey 2014). The data matrix used for the cluster analysis is available in Supp Table 2 (online only).

The morphological variability observed was qualitatively "mapped" on the most recent scenarios of figitid evolution (Buffington et al. 2007, 2012; Ronquist et al. 2015) (Fig. 1). Basically, we generated an intuitive, hand-drawn phylogenetic tree based on phylogenies based on molecular, morphological, and life-history data analyses available in these recent works. Within Figitidae, however, there is still weak consensus between results obtained through different methods (parsimony or Bayesian inference) on the relationships between certain subfamilies (Buffington et al. 2007). Furthermore, the most recent analysis of Ronquist et al. (2015) showed the relationships among figitid subfamilies as largely unresolved, except for Charipinae + Eucoilinae which form sister groups and Aspicerinae and Figitinae constituting together a monophyletic group, with the Aspicerinae nested within a paraphyletic Figitinae. Thus, we depicted here three scenarios (Fig. 1A–C), which correspond to the two proposed by Buffington et al. (2007) and that proposed by Ronquist et al. (2015).

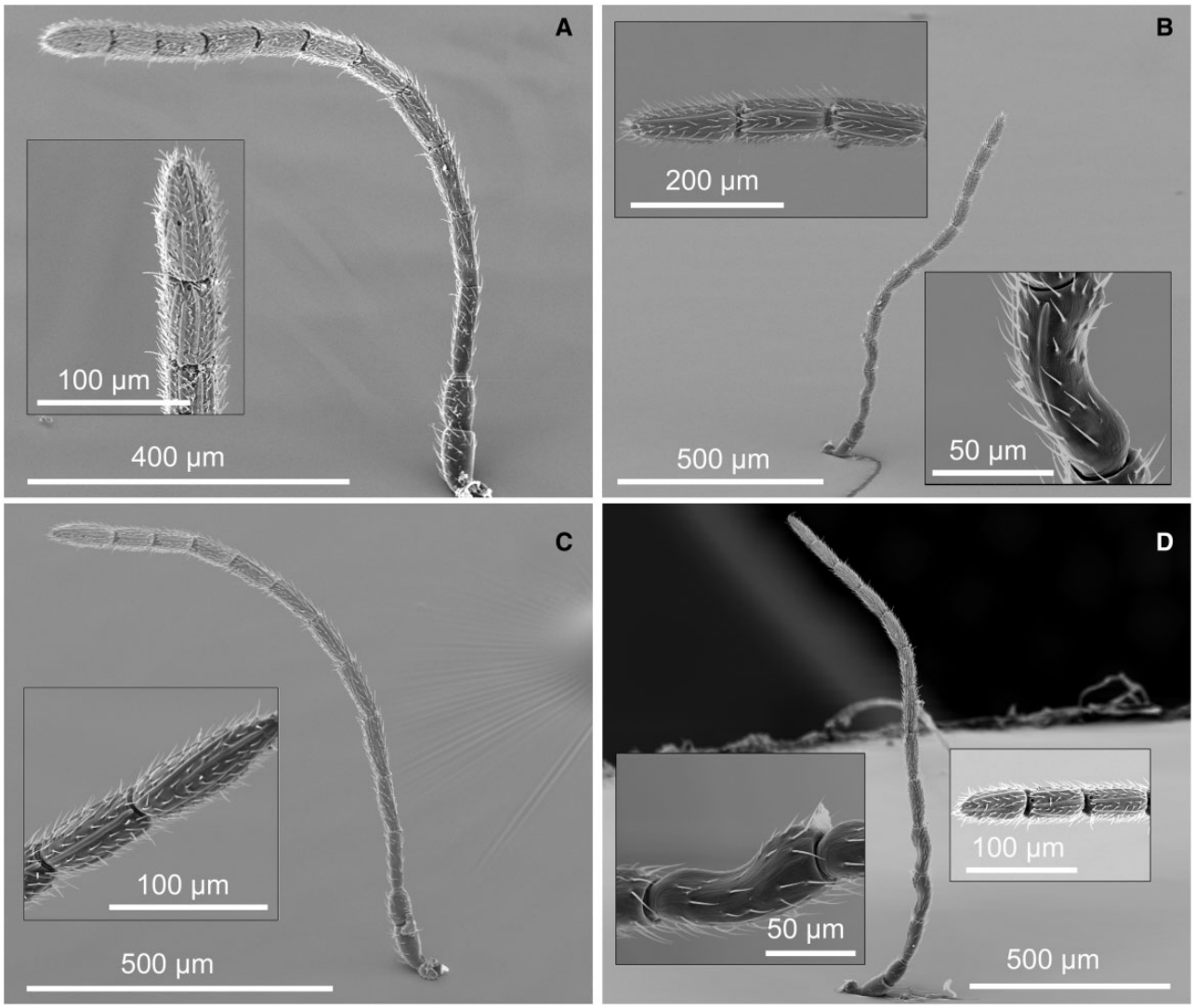

Fig. 2. Antennal morphology of *Alloxysta* spp. (A) *A. consobrina* female. (B) *A. consobrina* male. (C) *A. victrix* female. (D) *A. victrix* male. Note in B and D, the modified F3 containing the "release and spread structure."

All statistics were carried out in the software XLSTAT (Addisnoft).

Results

Antennae

In both *Alloxysta* species, males have 12 antennal flagellomeres and the females 11 flagellomeres (Fig. 2). In females, F_1 was 1–1.1 longer than F_2 (Figs. 2A and C). Female and male antennae belong to the filiform type and do not present distally a distinct club (i.e., a greatly enlarged apical flagellomere or flagellomeres of an antenna) (Fig. 2). Antennae are filiform (i.e., linear and slender), not moniliform (i.e., like a string of beads) (Fig. 2). *A. victrix* possesses a longer flagellum than *A. consobrina* (Fig. 2A and C and Tables 1 and 2), and females possess a wider flagellum than males (Tables 1 and 2). The mean length of a flagellomere was also greater in *A. victrix* and overall decreased distally along the antenna (Fig. 2A and C and Tables 1 and 2).

In the males, F_1, F_2, and F_3 are modified, curved, and slightly widened at the apex and base; being the F_1 less modified (Fig. 2B and D). This widened area present pores, with the number of pores apparently slightly greater in *A. victrix* (~30) than in *A. consobrina* (~20). Because their close morphological affinity to structures found in other Cynipoidea (Isidoro et al. 1999), this pored area is likely to be connected to an internal gland which produces pheromones, which males place on the female antenna during courtship ("release and spread" structure) (Isidoro et al. 1999). The rest of male flagellomeres (F_4–F_{12}) and all female flagellomeres (F_1–F_{11}) are cylindrical (Fig. 2). Females of both species have F_{10} clearly longer than wide (Fig. 2A and C).

Sensilla

Eight sensillar types have been detected in both species: four types of sensilla trichoidea (ST-A, ST-B, ST-C, ST-D), sensilla coeloconica (SCo), sensilla placoidea (SP), sensilla campaniformia (SCa), and sensilla basiconica (SB) (Fig. 3).

The hierarchical cluster analysis based on the presence/absence of the 10 different types of sensilla found on the whole in Figitidae reveals that the phylogenetic relationships among subfamilies (as suggested by the recent scenarios of Fig. 1) do not have a strong link with occurrence of the different sensillar types (Fig. 4). For example, Plectocynipinae and Parnipinae, phylogenetically closely related

Table 1. Variables related with size and number of the different types of sensilla in the antennae of *Alloxysta* spp.

Variable	*A. consobrina* (females)	*A. consobrina* (males)	*A. victrix* (females)	*A. victrix* (males)
Flagellum length (μm)	1015 ± 136 ($n = 6$)	1028 ± 78 ($n = 6$)	1124 ± 127 ($n = 6$)	1185 ± 98 ($n = 6$)
Flagellomere length (μm)	92 ± 16 ($n = 66$)	86 ± 11 ($n = 72$)	102 ± 23 ($n = 66$)	100 ± 17 ($n = 72$)
Flagellomere width (μm)	33 ± 8 ($n = 66$)	30 ± 3 ($n = 72$)	33 ± 5 ($n = 66$)	33 ± 4 ($n = 72$)
Number of ST-A per antenna	47.3 ± 27.2 ($n = 6$)	10.5 ± 6.2 ($n = 6$)	36.2 ± 11.9 ($n = 6$)	17.8 ± 8.8 ($n = 6$)
Number of ST-B per antenna	11.5 ± 4.2 ($n = 6$)	15.3 ± 3.8 ($n = 6$)	10.2 ± 2.4 ($n = 6$)	11.7 ± 2.3 ($n = 6$)
Number of ST-C per antenna	421.3 ± 57.6 ($n = 6$)	410 ± 48.3 ($n = 6$)	448.5 ± 41.8 ($n = 6$)	432.7 ± 70.1 ($n = 6$)
Number of ST-D per antenna	1.2 ± 2.4 ($n = 6$)	8.7 ± 6.7 ($n = 6$)	26.3 ± 17.5 ($n = 6$)	7 ± 8.6 ($n = 6$)
Number of SCo per antenna	2.5 ± 2.3 ($n = 6$)	2.3 ± 2 ($n = 6$)	5.2 ± 2.7 ($n = 6$)	2 ± 2 ($n = 6$)
Number of SCa per antenna	0.8 ± 1 ($n = 6$)	0.5 ± 0.8 ($n = 6$)	0.8 ± 1.2 ($n = 6$)	0.5 ± 0.8 ($n = 6$)
Number of SB per antenna	0.5 ± 0.8	1.2 ± 1.8	4 ± 3.8 ($n = 6$)	1.5 ± 1 ($n = 6$)
Number of SP per antenna	32.5 ± 11.1 ($n = 6$)	38.5 ± 8.1 ($n = 6$)	31.8 ± 5.8 ($n = 6$)	45.5 ± 5.8 ($n = 6$)
ST-A length (μm)	7 ± 1 ($n = 45$)	7 ± 1 ($n = 36$)	8 ± 1 ($n = 43$)	9 ± 1 ($n = 45$)
ST-B length (μm)	16 ± 2 ($n = 48$)	18 ± 3 ($n = 62$)	16 ± 1 ($n = 47$)	17 ± 2 ($n = 53$)
ST-C length (μm)	18 ± 4 ($n = 66$)	17 ± 3 ($n = 72$)	15 ± 2 ($n = 66$)	16 ± 2 ($n = 72$)
ST-D length (μm)	6 ± 2 ($n = 4$)	5 ± 1 ($n = 19$)	5 ± 1 ($n = 22$)	6 ± 1 ($n = 14$)
SCo hole diameter (μm)	4 ± 0 ($n = 15$)	3 ± 0 ($n = 14$)	3 ± 0 ($n = 31$)	3 ± 0 ($n = 12$)
SCa dome diameter (μm)	7 ± 0 ($n = 5$)	7 ± 0 ($n = 3$)	7 ± 0 ($n = 5$)	7 ± 0 ($n = 3$)
SCa knob diameter (μm)	1 ± 0 ($n = 2$)	1 ± 0 ($n = 2$)	1 ± 0 ($n = 2$)	1 ± 0 ($n = 2$)
SB length (μm)	5 ± 1 ($n = 2$)	5 ± 1 ($n = 4$)	4 ± 1 ($n = 16$)	4 ± 1 ($n = 6$)
SP length (μm)	72 ± 6 ($n = 58$)	65 ± 6 ($n = 68$)	78 ± 12 ($n = 56$)	73 ± 9 ($n = 69$)
SP width (μm)	3 ± 1 ($n = 56$)	3 ± 0 ($n = 58$)	4 ± 1 ($n = 52$)	3 ± 1 ($n = 67$)
SP height (μm)	3 ± 1 ($n = 56$)	3 ± 0 ($n = 61$)	3 ± 1 ($n = 52$)	3 ± 0 ($n = 63$)

Values are expressed as means ± standard deviations; in brackets the sample sizes (n) are reported. —, not applicable, since this sensilla type lacks in this species.

Table 2. Results of the analysis of covariance (ANCOVA) carried out to test for the effects of species, sex, and flagellomere on the variance of size and number of the different types of sensilla in the antennae of *Alloxysta* spp.

Dependent variable	Model	Species effect	Sex effect	Flagellomere effect
Flagellum length (μm)	$F_{2,23} = 4.65$, SS = 114104.4, $P = 0.021$	$t = -2.94$, $P = 0.008$	NS	NS
Flagellomere length (μm)	$F_{3,45} = 17.44$, SS = 5104.56, $P < 0.0001$	$t = -4.22$, $P = 0.0001$	NS	$t = -5.68$, $P < 0.0001$
Flagellomere width (μm)	$F_{3,45} = 9.33$, SS = 213.78, $P < 0.0001$	NS	$t = 2.05$, $P = 0.03$	$t = 4.69$, $P < 0.0001$
Number of ST-A	$F_{3,45} = 21.25$, SS = 173.87, $P < 0.0001$	NS	$t = 5.79$, $P < 0.0001$	$t = 5.9$, $P < 0.0001$
Number of ST-B	$F_{1,45} = 11.9$, SS = 16.19, $P < 0.0001$	NS	NS	$t = 5.83$, $P < 0.0001$
Number of ST-C	$F_{2,45} = 24.4$, SS = 4754.26, $P < 0.0001$	NS	NS	$t = 8.3$, $P < 0.0001$
Number of ST-D	$F_{1,45} = 3.12$, SS = 24.66, $P = 0.03$	$t = -2.13$, $P = 0.039$	NS	NS
Number of SB	$F_{2,45} = 4.1$, SS = 0.79, $P = 0.12$	$t = -2.21$, $P = 0.02$	NS	$t = 2.41$, $P = 0.032$
Number of SP	$F_{2,45} = 21.51$, SS = 36.38, $P < 0.0001$	NS	$t = -2$, $P = 0.049$	$t = 7.49$, $P < 0.0001$
Number of SCo	$F_{3,45} = 11.1$, SS = 1.66, $P < 0.0001$	NS	$t = 2.91$, $P = 0.006$	$t = 4.94$, $P < 0.0001$
Number of SCa	$F_{1,45} = 0.88$, SS = 0.01, $P = 0.88$	—	—	—
ST-A length (μm)	$F_{3,36} = 14.22$, SS = 33. 9, $P = 0.001$	$t = -5.91$, $P < 0.0001$	NS	NS
ST-B length (μm)	$F_{3,40} = 6.33$, SS = 40.71, $P = 0.006$	NS	$t = -3.12$, $P = 0.003$	$t = -2.78$, $P = 0.008$
ST-C length (μm)	$F_{3,45} = 24.97$, SS = 142.71, $P < 0.0001$	$t = 4.18$, $P = 0.0001$	NS	$t = -7.57$, $P < 0.0001$
ST-D length (μm)	$F_{3,35} = 0.49$, SS = 2.42, $P = 0.69$	—	—	—
SCo diameter (μm)	$F_{2,24} = 2.85$, SS = 0.4, $P = 0.08$	—	—	—
SP length (μm)	$F_{3,40} = 17.89$, SS = 902.4, $P < 0.0001$	$t = -5.68$, $P < 0.0001$	$t = 4.42$, $P < 0.0001$	NS
SP width (μm)	$F_{3,40} = 34.69$, SS = 2.69, $P < 0.0001$	$t = -8.45$, $P < 0.0001$	$t = 3.46$, $P = 0.01$	$t = -4.55$, $P < 0.0001$
SP height (μm)	$F_{3,40} = 56.46$, SS = 3.32, $P < 0.0001$	$t = 12.05$, $P < 0.0001$	$t = 4.83$, $P < 0.0001$	NS

NS: the independent variable did not account for the variability of the dependent morphological variable. —, the test for the effect of the three independent variables did not apply since the overall model was not significant (no difference across species, sexes and flagellomeres was detected).

(Fig. 1), fall in two distant clusters. In addition, basal families of Cynipoidea (Ibaliidae and Liopteridae) were intermixed within a cluster including also Aspicerinae and Anacharitinae. Charipinae presents a set of sensillar types more similar to that of Figitinae than to that of Eucoilinae (Fig. 4), maybe giving new preliminary elements in support of the phylogenetic relationships depicted by one recent analysis (Fig. 1B) and against the other available ones (Fig. 1A and C). More in general, species of the same subfamily clustered closely together only for Plectocynipinae, so that overall for Figitidae the variability in absence/presence of sensilla type does not seem a valid character in phylogenetic studies (Fig. 4).

The morphology and distribution of the eight types of sensilla found in *Alloxysta* spp. are described in detail below.

Sensilla Trichoidea (ST-A, ST-B, ST-C, and ST-D)

The sensilla trichoidea are characterized being setiform and pointed at the tip; they are very variable in length (HAO reference: http://

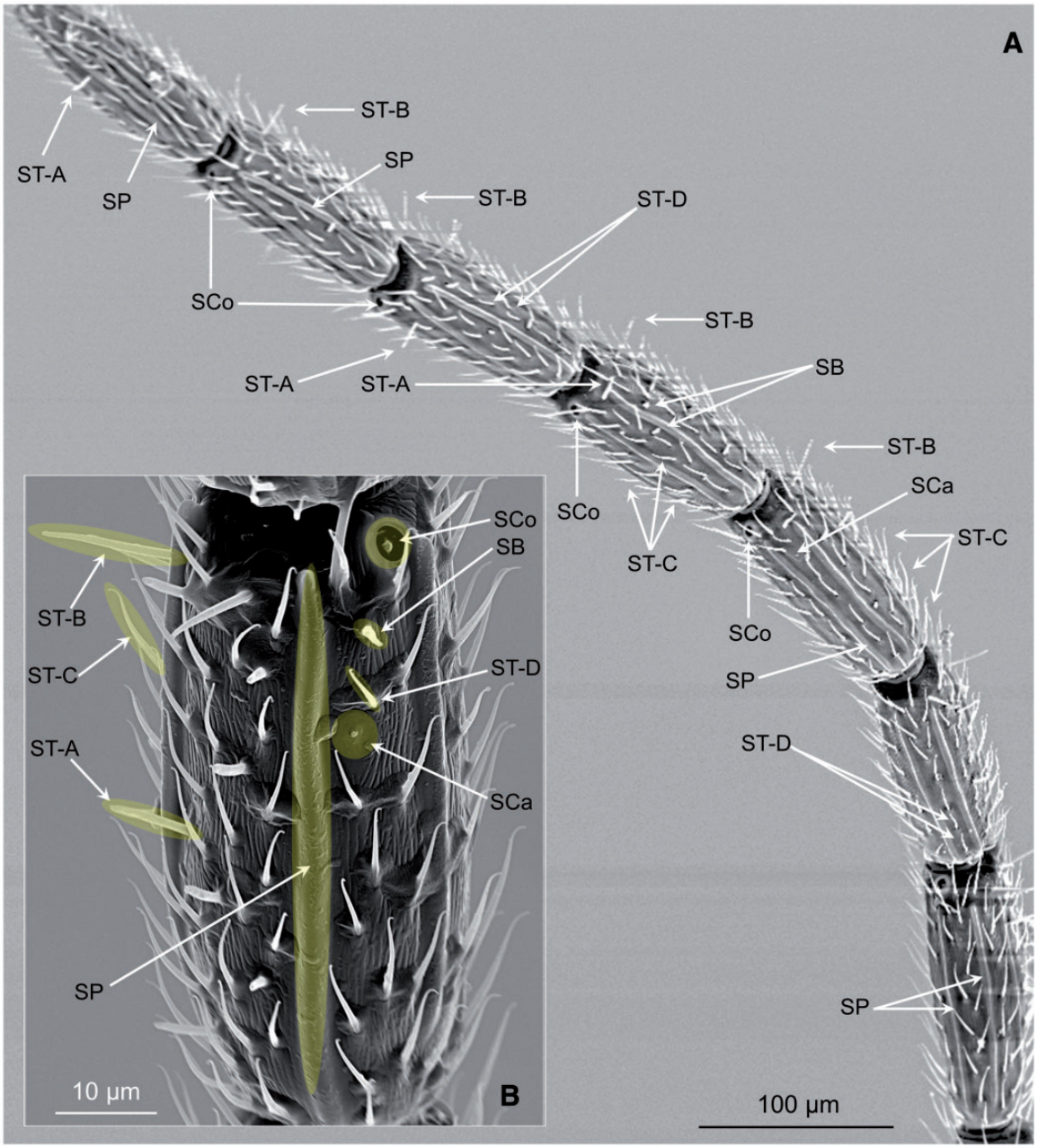

Fig. 3. Representative distribution of the eight types of sensilla observed in an antenna (A, *A. victrix* female in ventral-lateral view, from F_5 to F_{11}) and an antennal flagellomere (B, ventral view of F_8 of *A. victrix* female). (A) Several sensilla of each type are arrowed. (B) One sensillum of each type is arrowed and highlighted in yellow.

purl.obolibrary.org/obo/HAO_0002299). All the four types here detected are presented in both species and sexes.

The sensilla trichoidea type A (ST-A) (Fig. 5B, C, and H) have a grooved surface and were relatively abundant sensilla on the antennae (about 10–50 on average across species and sexes) and more abundantly in the distal flagellomeres (Tables 1 and 2). ST-A were observed almost exclusively dorsally or dorsal-laterally on the antennae (Supp Fig. 1A–C [online only]). Their shape differs from the other types of sensilla trichoidea in particular because of the tip of the organ, which is almost thick as the base. They were more numerous in females, not differing between species (Tables 1 and 2). Their length ranged from 7 to 9 µm on average and it was greater in *A. victrix* (Tables 1 and 2).

The sensilla trichoidea type B (ST-B) (Fig. 5A, D, F, G, and I) are not very abundant (about 10–15 per antenna across species and sexes) but very conspicuous organs and clearly differing from the other sensilla trichoidea in length and location. The tip is clearly thinner than the base. The average length is about 16–18 µm, thus being long sensilla (Tables 1). They have longitudinal grooves. Opposing pairs are placed laterally at the distal end of many flagellomeres and are directed outward the flagellomere, so that they are best visible in dorsal or lateral view than in ventral view (Fig. 5A). However, their number is generally greater in the last flagellomere ($F_{11/12}$) (Fig. 5G), where they can be up to 8, so that an effect of flagellomere on ST-B number was significant (Table 2). In parallel with this increase in number distally, their length decreased (Table 2).

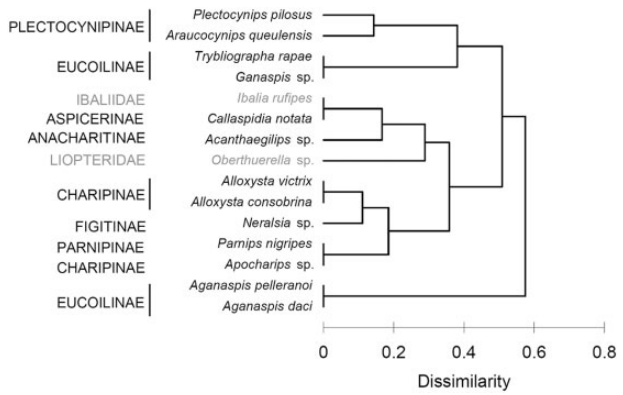

Fig. 4. Dendrogram depicted by the cluster analysis (Jaccard index) based on the matrix of presence/absence of the nine different types of sensilla described overall for females Figitidae. Ancestral taxa (Ibaliidae and Liopteridae) are in grey.

The two species and sexes have similar number of ST-B (Tables 1 and 2). On the other hand, ST-B was longer in males than in females (Tables 1 and 2).

The sensilla trichoidea type C (Fig. 5D, F, and G) were very widespread in both species and they are those with highest number on the antennae (about 400 across species and sexes) and they can be found in all flagellomeres (Table 1), dorsally, laterally and ventrally. They are on average a little smaller than ST-B (15–18 µm) but they can be easily recognized from that type because of their strong inclination, almost laying on the antennal surface, and by their somehow reduced thickness compared with ST-A and ST-B. The distal part is thinner than the base and often ends with a "down and up" curve (Fig. 5G). The two species and sexes have similar number of ST-C, and distal flagellomeres have more sensilla than proximal ones (Tables 1 and 2). As occurs with ST-B, the length of ST-C decreased distally (Table 2). *A. consobrina* have longer ST-C than *A. victrix* (Table 2).

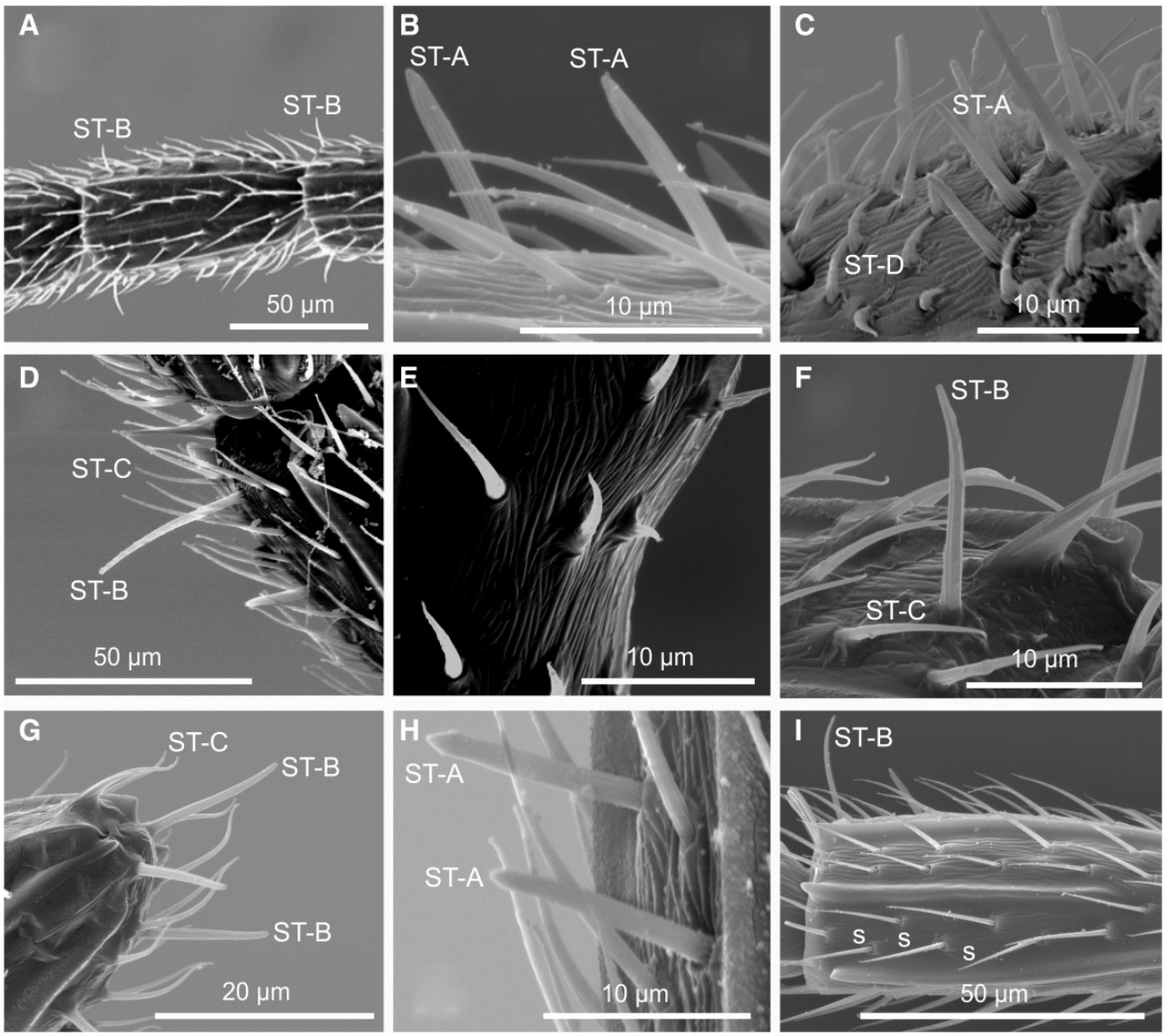

Fig. 5. Sensilla trichoidea in the antennae of *Alloxysta* spp. (A) ST-B in female *A. consobrina*. (B) ST-A in female *A. consobrina*. (C) ST-A and ST-D in female *A. consobrina*. (D) ST-B and ST-C in male *A. consobrina*. (E) ST-D in male *A. consobrina*. (F) ST-B and ST-C in female *A. victrix*. (G) ST-B and ST-C in female *A. victrix*. (H) ST-A in male *A. victrix*. (I) ST-B and setae (s) in female *A. consobrina*.

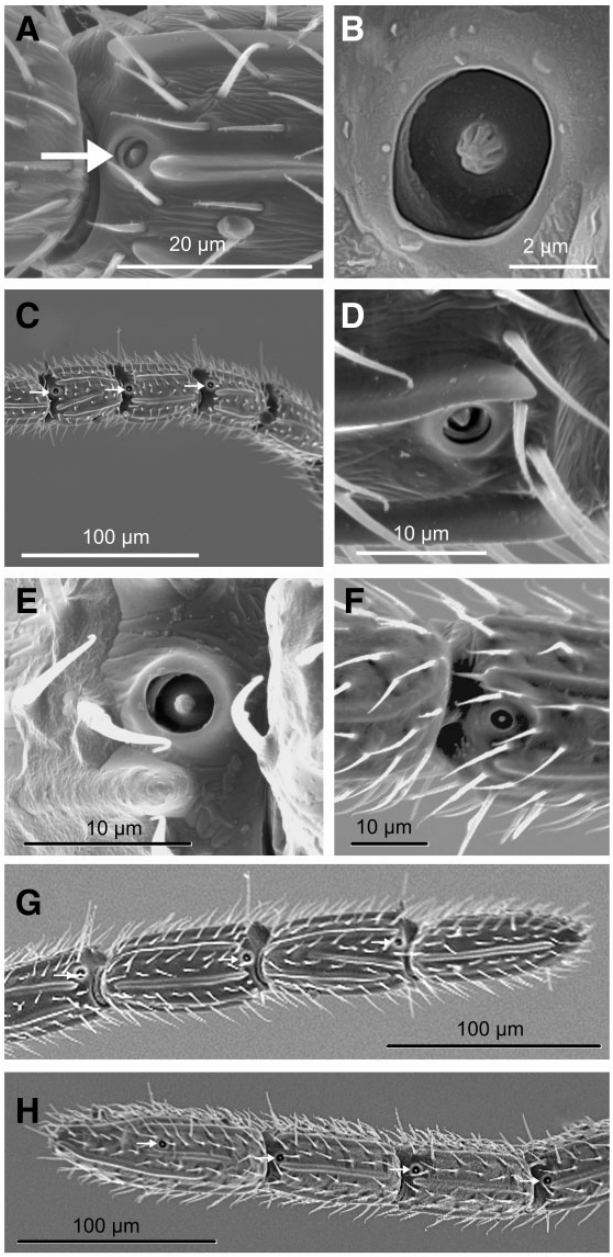

Fig. 6. SCo in the antennae of *Alloxysta* spp. (A–B) Female *A. consobrina* (note arrow in A). (C–D) Male *A. consobrina* (note arrows in C). (E) Female *A. victrix*. (F) Male *A. victrix*. (G) Apical flagellomeres of *A. consobrina* male antennae (F_9–F_{12}) (arrows point to SCo). (H) Apical flagellomeres of *A. victrix* female antennae (F_8–F_{11}) (arrows point to SCo).

The sensilla trichoidea type D (Fig. 5C and E) are very variable in number across species and sexes (from about 1 to 26 per antenna) and can be found from F_1 to the apical flagellomere, though generally not in all segments. They seemed to be almost confined to the external lateral sides of the antennae (Supp Fig. 1D [online only]). ST-D number was greater in *A. victrix* was similar in the two sexes and it did not change with flagellomere (Table 2). ST-D differ from the other sensilla trichoidea by their smaller size (about 5 µm in all species and sexes), their bulb-like base, and their little tilt angle with the antennal surface (Fig. 5 and Table 1). Their surface is smooth.

In addition to the hair-like sensilla described above, the antennae of both species present setae (Fig. 5I and Supp Fig. 1A [online only]), i.e., non-innervated hair-like structures, which being not involved in sensing (Ågren 1977) were not further analyzed here.

Sensilla Coeloconica

The SCo (Fig. 6) are "basiconic pegs," located in cuticular depressions and having circular outline and appearance of rosette (Fig. 6B, D, and E) (HAO reference: http://purl.obolibrary.org/obo/HAO_0002001). Two types were described for Cynipoidea, being the largest the type A (Polidori and Nieves-Aldrey 2014). In *Alloxysta*, only the SCo of type A was found (Fig. 6), so in the following text SCo refers to SCo-A only. A donut-shaped ring of about 3–4 µm diameter surrounds the peg (Table 2). These sensilla are rare and located on the distal end of the flagellomeres from F_4 to F_{11} on the antennae of both sexes and species, generally no more than one in a flagellomere (Fig. 6C, G, and H). Such distribution translates into a significant effect of the flagellomere on their number (Table 2). SCo are typically located laterally on the antennae. In females, the diameter of the pit is small compared to F_{10} width (ratio between 0.03 and 0.05). Males always lack SCo in their apical flagellomere (F_{12}) (Fig. 6G). SCo are typically located on or close the distal margin of a flagellomere (Fig. 6A and F), except in F_{11} of females, where they are often located in the middle of F_{11} (Fig. 6H); sometimes an additional sensillum of this type is also presented in the distal part of female F_{11}, thus causing an effect of sex on their number (greater in females) (Table 2). There was not significant variation in either SCo number or SCo pit diameter across species (Table 2).

Sensilla Campaniformia

SCa (Fig. 7A–C) are characterized by a button-like knob about 1 µm in diameter with a small irregular surface emerging from an opening in the center of a domed, smooth, circular cuticular disk of about 7 µm in all species and sexes (HAO reference: http://purl.obolibrary.org/obo/HAO_0001973) (Table 1). SCa were presented in both sexes and species but are rare along the antenna, typically with a maximum of one sensillum in a flagellomere, and often, though not always, close to the SCo-A (Fig. 7B). They are found from F_4 to F_{11}, but never in all of these flagellomeres. SCa number did not vary significantly across species and sexes (Table 2).

Sensilla Basiconica

SB (Fig. 7F–G) have a cone-like peg, a grooved surface, and a pored apex, and project almost perpendicularly with respect to the axis of the antenna (HAO reference: http://purl.obolibrary.org/obo/HAO_0002300). The pegs of SB arise from a shallow socket and they are generally not or weakly curved. SB were observed dorsally, laterally, and ventrally on the antennae. SB can be differentiated from sensilla trichoidea because they are the shortest hair-like sensilla (about 4–5 µm in all species and sexes) on the antennae (Table 1) and because of their thick shape. SB was very rare on the antennae; however, they seem to be a bit more numerous in *A. victrix* and in the distal flagellomeres (Tables 1 and 2).

Sensilla Placoidea

The SP (Fig. 7B and D–E) are the largest sensilla on the antennae. In Cynipoidea, they are multiporous, elongate, plate-like sensilla with a large surface area (HAO reference: http://purl.obolibrary.org/obo/HAO_0000640). In *Alloxysta*, they are abundant (about 30–40 per antenna) (Table 1) and they can be found from F_1 to the apical flagellomere, dorsally, laterally, and ventrally. Their number was similar in the two species but greater in males (Table 2). The number of SP visible in each row ranged from 1 to 7, and only one row of SP is

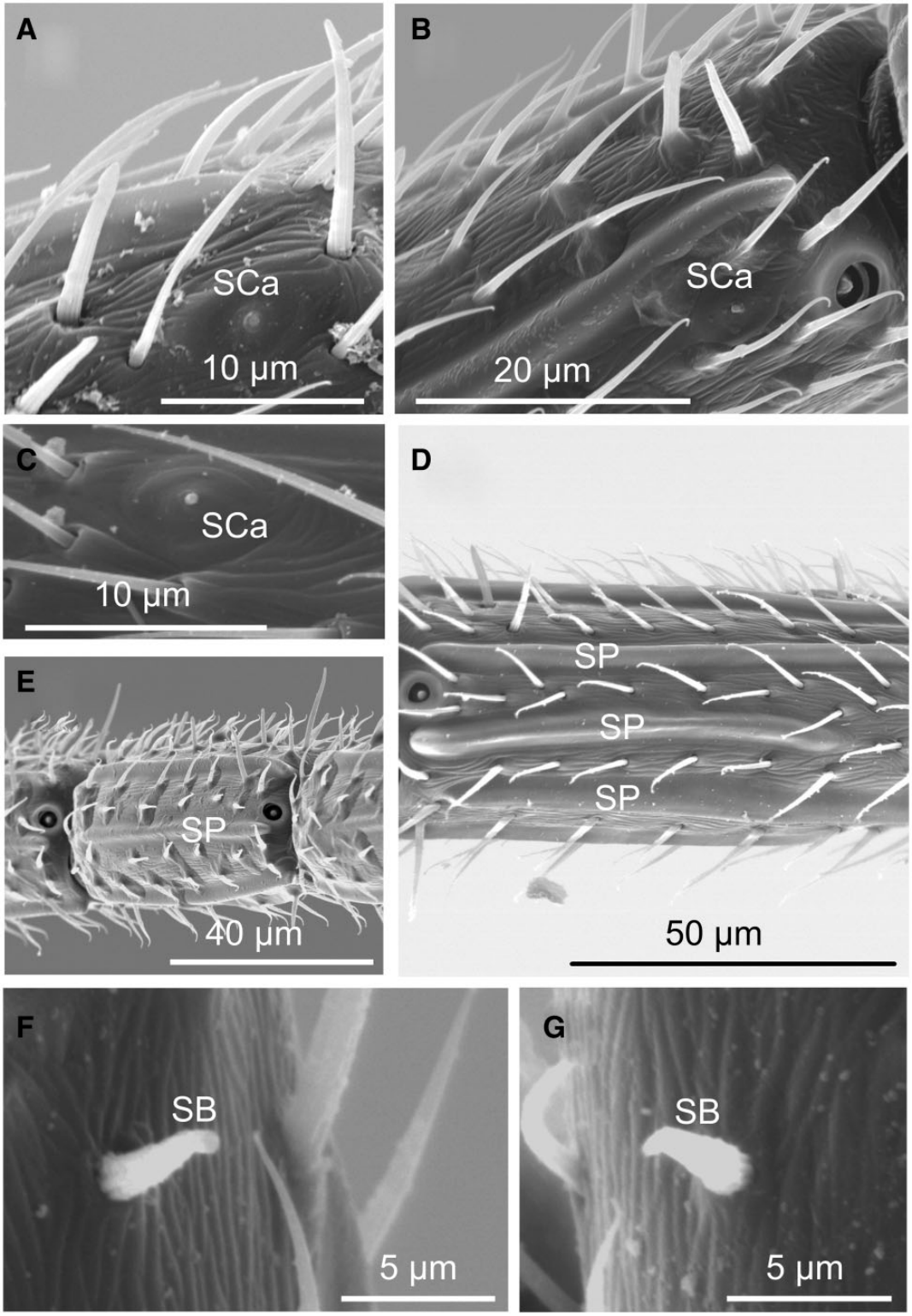

Fig. 7. SCa, SB, and SP in the antennae of *Alloxysta* spp. (A) SCa in female *A. victrix*. (B) SCa and SP in female *A. consobrina*. (C) SCa in female *A. consobrina*. (D) SP in female *A. victrix*. (E) SP in male *A. victrix*. (F) SB in female *A. victrix*. (G) SB in male *A. victrix*.

presented in a single flagellomere (Fig. 7D–E). On F_{10} in females, 3–5 SP per row were presented (Fig. D). SP number increased distally (Table 2). SP appeared to be widely separated (>as width of a sensillum) (Fig. 7D–E). Their average length in *Alloxysta* spp. was about 70 µm, widely covering longitudinally the flagellomeres (Table 1). SP was longer and wider in *A. victrix* and in females, but SP height was greater in *A. consobrina* (Tables 1 and 2). The proximal flagellomeres owned narrower SP (Table 2). Almost flat SP, only slightly or not rising on the segment, were detected in females of both species, and in both species SP have a surface always

constantly smooth, not with longitudinal furrows or depressions. SP more or less overlaps the distal margin of the flagellomere (Fig. 7D–E). Along the flagellomere, SP develops roughly linear (Fig. 7D–E).

Discussion

Sensillar Equipment in *Alloxysta* within Figitidae

This study is the first which characterizes the antennal sensilla of any species of *Alloxysta* and the first study of both sexes in any Charipinae, thus it improves our knowledge on the sensillar equipment of Figitidae as a whole. On the whole, our results show that the sensillar equipment on the antennae of *Alloxysta* shows some similarities with that previously described for the 11 Figitidae previously studied (Butterfield and Anderson 1994, Tormos et al. 2013, Polidori and Nieves-Aldrey 2014), as well as some similarities with that described for other species of parasitoids from different families of Hymenoptera (e.g., van Baaren et al. 1996, 1999; Amornsak et al. 1998; Bleeker et al. 2004; Bourdais et al. 2006; Dweck and Gandallah 2008; Wang et al. 2010).

However, we found some differences between the two *Alloxysta* species and between sexes in these two species, and our analysis of literature data shows that there are also some differences among species of Figitidae.

The total number of sensilla types observed in *Alloxysta* spp. was eight, thus falling in the range known for Cynipoidea (4–9 in females, depending on species) (Polidori and Nieves-Aldrey 2014). *Alloxysta* spp. had the same number of sensillar types of *Apocharips* sp. (also in the Charipinae) and *Parnips niger* (Barbotin) (Parnipinae) (Polidori and Nieves-Aldrey 2014). One particular type of sensilla lacked in both *Alloxysta* species, the "large disc sensilla," which is exclusively owned by members of the subfamily Plectocynipinae (Polidori and Nieves-Aldrey 2014). *Alloxysta* species also lacked a small sub-type of SCo (type B in Polidori and Nieves-Aldrey [2014]) which was detected to date in five figitids belonging to four subfamilies (including *Apocharips* [Charipinae]) (Polidori and Nieves-Aldrey 2014).

Overall, it seems that Eucoilinae and Anacharitinae have the lowest number of sensillar types among Figitidae (4–5), while the highest number occurs in Charipinae, Parnipinae, and Figitinae (8–9) (Polidori and Nieves-Aldrey 2014). Basal cynipoids have few to moderately numerous types of sensilla (4–6). Thus, the total number of sensillar types apparently seems to have repeatedly increased and decrease during cynipoid evolution in a complex way (Fig. 1). On the other hand, the presence/absence of each of the different sensillar types did not agree with recent phylogenetic scenarios depicting relationships between subfamilies (Figs. 1–3). However, the cluster analysis based on presence/absence of sensilla types may preliminary agree with one of the two phylogenetic hypotheses available to date (Fig. 1B).

The host type seems also unlikely to be related with the sensillar equipment. For example, while Charipinae (attacking aphids via Hymenoptera) and Parnipinae (attacking plant-galling Hymenoptera) present similar sensillar bouquet, members of subfamilies attacking Diptera distributed in unpredictable position in the cluster analysis (e.g., Eucoilinae: distant from all the other parasitoids of Diptera; Figitinae: close to parasitoids of Hymenoptera).

The sensilla trichoidea are the most abundant throughout the antennomeres of the two species in both males and females, as it occurs in many parasitoid Hymenoptera (e.g., van Baaren et al. 1996, 2007). Sensilla trichoidea have been associated with different functions: olfactive (Dietz and Humphreys 1971), gustative (Esslen and Kaissling 1976), mechanoreceptive (Daly and Ryan 1979), and thermosensitive (Zachuruk 1985). It is not clear from the literature if longer and shorter sensilla trichoidea are associated to different functions. In Cynipoidea, some sensilla trichoidea may be chemoreceptors by contact (gustatory) and some others may be mechanoreceptors (Butterfield and Anderson 1994, Romani et al. 2010), though only a histological study would provide evidence for these functions in all the studied species. In *A. victrix*, contact kairomones mediate the foraging behavior and could be processed through some of the types of sensilla trichoidea described here (Grasswitz 1998).

The ST-A described here were found in all the previously studied Cynipoidea (Polidori and Nieves-Aldrey 2014) with the notable exception of *Ganaspis* and *Aganaspis* (Eucoilinae), possibly the group of Figitidae with overall simpler sensillar equipment among Cynipoidea (Butterfield and Anderson 1994, Tormos et al. 2013, Polidori and Nieves-Aldrey 2014). ST-B is very long and has the typical arrangement of being located in pairs toward the distal end of the flagellomeres. However, in some *Alloxysta* individuals, ST-B can be as many as eight in the apical flagellomere (more commonly 3–5).

The most abundant sensilla trichoidea were the ST-C, which cover most flagellar segments. In Cynipoidea, this type of sensilla was detected in all studied species so far (Polidori and Nieves-Aldrey 2014), though with varying density. For example, Eucoilinae possess very low ST-C density along flagellomeres, while *Acanthaegilips* sp. (Anacharitinae) and Charipinae have a very high ST-C density (Polidori and Nieves-Aldrey 2014). Because basal Cynipoidea tend to have higher ST-C density and the more derived groups (Eucoilinae and Charipinae) either low or high density, it could be suggested that within Figitidae ST-C density increased and decreased several times during evolution. Interestingly, it seems that species attacking Hymenoptera (or other groups via Hymenoptera) and Neuroptera tend to have higher ST-C density (the number of ST-C on female F_{10}, measured in a row along its length was generally >10) than those attacking Diptera (this number was generally 1–2) (Polidori and Nieves-Aldrey 2014). ST-D was rare, and they were previously observed in only two species of Figitidae (one gall-inquiline and one parasitoid of unconcealed host). We thus provide here the first evidence of their presence in Charipinae.

The SCo present in *A. victrix* and *A. consobrina*, which belong to the previously described type A (Polidori and Nieves-Aldrey 2014), have been also found in other parasitoid Hymenoptera (e.g., Bourdais et al. 2006) and closely resemble those observed in other Cynipoidea (Polidori and Nieves-Aldrey 2014). Among Figitidae, there is some variability in their number and distribution. For example, although they typically start in the middle part of female flagellum (F_5–F_8 to F_{11}), in five figitid species they start in the proximal part of flagellum (F_2–F_4 to F_{10}). In addition, despite in most cases one SCo is present in a flagellomere, *Aganaspis pellerenoi* and *Plectocynips pilosus* have 2–3 SCo in few flagellomeres (Tormos et al. 2013, Polidori and Nieves-Aldrey 2014). Males and females of the *A. victrix* and *A. consobrina* species here studied have coeloconic sensilla from F_4 to F_{11}; F_{12} of males lacks this type of sensilla in both species. The unique location of the SCo in the F_{11} of females (often in the middle of the flagellomere), together with the rare presence of two SCo, make suspect that originally female antennae had the same number of flagellomeres than males, and that F_{11} of females corresponds to two fused flagellomeres ($F_{11} + F_{12}$).

Most Figitidae have SCo far from the flagellomere's distal margin. However, Charipinae, Figitinae, and Parnipinae have SCo on or close to the distal margin. The relative width of the SCo pit (pit diameter/width of F_{10}) in *Alloxysta* is short, as in all the other figitids

studied to date, and similar to herb-gallers within Cynipidae (Aylacinii *sensu* lato) and differently from the larger pits of most of wood-gallers within Cynipidae (e.g., some Cynpini, Escathocerini) (Polidori and Nieves-Aldrey 2014). According to the literature, their function is probably thermo- and hygro-receptive (Altner et al. 1977, 1983; Yokohari 1978).

The SCa found in *Alloxysta* closely resemble those found in other parasitoid wasps (including other Cynipoidea) (Amornsak et al. 1998, Ahmed et al. 2013, Polidori and Nieves-Aldrey 2014). In Figitidae, SCa are now reported in 8 out of 13 studied species (Polidori and Nieves-Aldrey 2014, this study). They also occur in many other Cynipoidea (Polidori and Nieves-Aldrey 2014). On the other side, this sensillar type seems rarer in other parasitoid lineages, such as in Ichneumonoidea (Ochieng et al. 2000, Roux et al. 2005, Ahmed et al. 2013) and in Chalcidoidea (van Baaren et al. 1999, Onagbola et al. 2009, Li et al. 2013). Electrophysiological studies suggest that SCa are thermo-hygroreceptors (Merivee et al. 2003).

SB in Figitidae was detected in about half of the studied species to date (Polidori and Nieves-Aldrey 2014, this study). Possibly Platygastroidea possess the SB more similar to those found in Cynipoidea (Isidoro et al. 2001). It is possible that SB involve a bimodal function as chemo- and thermoreceptors (Isidoro et al. 1996).

The SP are very common in Hymenoptera, being typically large and elongated in the Terebrantia (including Ichneumonoidea, Chalcidoidea, Cynipoidea) (e.g., Basibuyuk and Quicke 1999) and small and roughly oval/circular in the Aculeata, particularly in the Apoidea (bees and apoid wasps) (e.g., Polidori et al. 2012). The SP found in this study are morphologically very similar to those described in the previously studied Cynipoidea (Butterfield and Anderson 1994, Tormos et al. 2013, Polidori and Nieves-Aldrey 2014). Almost all figitids seem to lack SP on F_1 (*Alloxysta* is one of these exceptions) and most of the species have SP on F_{10} arranged in one row (Polidori and Nieves-Aldrey 2014, this study).

The number of SP visible in each row on F_{10} seems to be also weakly variable: Plectocynipinae, Parnipinae, Figitinae, Aspicerinae and Anacharitinae have 6–8 SP per row, while Charipinae and almost all Eucoilinae have 3–5 SP per row (Tormos et al. 2013, Polidori and Nieves-Aldrey 2014, this study). The fact that Charipinae and Eucoilinae share this character would be in accordance with their close phylogenetic positions depicted by the most recent Parsimony analysis (Fig. 1A). On the other hand, SP extends at most only reaching the distal margin of segment in most figitids but they more or less overlap the distal margin of segment in Charipinae and Aspicerinae (Polidori and Nieves-Aldrey 2014, this study), supporting the relationships depicted by the most recent Bayesan analysis (Fig. 1B). Thus, at the moment it is unlikely that SP abundance, morphology, and distribution are useful to support subfamily-level phylogenetic reconstructions of Figitidae. According to the literature, these sensilla are associated with olfactory functions (Kaissling and Renner 1968, Ochieng et al. 2000).

Interspecific and Intersexual Differences in *Alloxysta*

Sexual dimorphism in antennal and sensillar morphology and sensilla abundance was detected in *Alloxysta*. In particular, male antennae harbor more SP and have longer ST-B, while females have wider flagella, possess more ST-A and more SCo, and have larger SP. Differences between sexes were reported for a several species of parasitoid wasps. For example, in *Trichogramma australicum* (Girault) (Chalcidoidea: Trichogrammatidae), male antennae possess two types of trichoid sensilla which are absent in females, possibly because they are associated with courtship behavior (Amornsak et al. 1998). In two species of *Aganaspis*, two species of *Cotesia* (Braconidae), and *Microplitis croceipes* Cresson (Ichneumonoidea: Braconidae), males have a greater abundance of SP compared with females (Ochieng et al. 2000, Bleeker et al. 2004, Tormos et al. 2013), similar to what found here for *Alloxysta*. In *Pteromalus cerealellae* (Ashmead) (Chalcidoidea: Pteromalidae) and *Tamarixia radiata* (Waterston) (Chalcidoidea: Eulophidae) are females those having more SP, while males have a greater number of certain types of sensilla trichoidea (Onagbola and Fadamiro 2008, Onagbola et al. 2009). On the other hand, in *Alloxysta*, females have a greater number of a type of sensilla trichoidea (ST-A) than males. In most Figitidae, males are attracted to the female sex pheromones (Chapman 1982), and males of *A. consobrina* and *A. victrix* could use SP to detect such pheromones, possibly in conjunction with the host's odor (Bleeker et al. 2004). On the other hand, females can rely on the more abundant ST-A during foraging, that it was shown for *Alloxysta* to be mediated by contact kairomones (Grasswitz 1998). Independently from the role that each type of sensilla plays in the wasp's life, the trend to have sexes developing contrast density of some types of sensilla may suggest their different function (e.g., perception of mate-related volatile cues vs. perception of host-related volatile cues) (Onagbola et al. 2009). However, cases of no sexual dimorphism in sensillar equipment are also known (van Baaren et al. 1999). In addition to differences in sensilla, males of both *Alloxysta* species possess an excavated area on F_1 to F_3. This modified area, named "release and spread structure" (RSS) by Isidoro et al. (1999) and typical in Cynipoidea, consists of a ridge and an excavation, both pored and houses a gland which emits a pheromone.

Differences in morphology and distribution of the sensilla may reflect host use in the two studied *Alloxysta* species. In fact, in *A. consobrina*, the greater abundance of SB and ST-D, the smaller size of the SP, the shorter ST-A, and even the overall smaller size of the flagellum, compared with *A. victrix*, may be related with the fact that the latter species is known to attack about 30% more aphid species than *A. consobrina* and about twice the number of aphid parasitoid species attacked by *A. consobrina* (Ferrer-Suay et al. 2014). This would agree with previous studies in other parasitoid lineages showing that that host range affects occurrence and abundance of sensilla (e.g., van Baaren et al. 2007, Das et al. 2011).

A. consobrina and *A. victrix* possess a complex and rich sensillar equipment on the antennae, with a diversity of sensillar types greater than most of previously studied Figitidae. In females in particular, such complexity may be linked to their observed ability to discriminate parasitized from non-parasitized aphids, as well as the age of aphids, through antennal contact alone (i.e., without previous probing with ovipositor) (Grasswitz and Reese 1998), suggesting that they have to process with antennae a complex bouquet of information from aphid environment, aphid species, primary parasitoid species, and aphid age/parasitism stage. The two species seem to have several differences in their sensillar equipment, perhaps in accordance with the different degree of host range; however, because both species can be classified as generalists attacking many aphid host species and many primary parasitoid host species, it would be very interesting to analyze in the future species of this genus with a much stronger host specialization (Ferrer-Suay et al. 2014). Further analyses including histological and ethological data aimed to assess the function of the different sensillar types are required to formally test for this hypothesis. The comparison with the other species of Figitidae studied by far suggests a complex series of morphological changes during evolution of this group, and the taxonomic sample should be thus substantially enlarged to disclose possible trends in sensillar equipment evolution in the family.

Supplementary Data

Supplementary data are available at *Journal of Insect Science* online.

Acknowledgments

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