



Morphological characterization of the antenna of *Torymus sinensis* (Hymenoptera: Torymidae) and a comparison within the superfamily Chalcidoidea



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ABSTRACT

The parasitoid *Torymus sinensis* (Hymenoptera: Torymidae) has been successfully used in Italy since 2005 for biological control of the invasive cynipid *Dryocosmus kuriphilus* (Hymenoptera: Cynipidae), highly destructive for the economically relevant *Castanea sativa* (Fagales: Fagaceae). In order to investigate the morphological aspects related to sensorial behavior, a fine morphology study of the antennae and their sensilla was conducted by scanning electron microscopy on both sexes of *T. sinensis*. The antennae, composed of a scape, a pedicel and a flagellum with ten flagellomeres, had chaetic sensilla of six subtypes, placoid sensilla of three subtypes, trichoid sensilla, sensilla with a roundish grooved tip, and coeloconic sensilla. The chaetic sensilla of the first three subtypes were found in the scape and in the pedicel, and those of the last three subtypes, together with trichoid, roundish grooved tip and coeloconic sensilla, were found only on flagellomeres. Sexual dimorphism was detected in the morphology of the proper pedicel and the flagellum, and in the presence and distribution of the sensilla and their subtypes. The morphological aspects of the antenna of *T. sinensis* and of its sensilla were compared with those found in the family Torymidae and in other families of the extremely diverse superfamily Chalcidoidea.

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1. Introduction

The Chinese chalcid wasp *Torymus sinensis* Kamijo, 1982 (Hymenoptera: Torymidae), is a natural enemy of the invasive Asian chestnut gall wasp, the cynipid *Dryocosmus kuriphilus* Yasumatsu, 1951 (Hymenoptera: Cynipidae), the worst worldwide pest of the genus *Castanea* Mill., 1754 (Fagales: Fagaceae) (Quacchia et al., 2008). The cynipid, originating from China, invaded Japan in 1941 and progressively colonized other countries in Asia and North America (Quacchia et al., 2008). In Europe, it was found for the first

time in Italy in 2002 on the European chestnut tree, *Castanea sativa* Mill., 1768 (Fagales: Fagaceae) (Brussino et al., 2002; Graziosi and Santi, 2008; Quacchia et al., 2010; Bernardo et al., 2013) and later in France and other European countries (Aebi et al., 2007; EFSA Panel on Plant Health, 2010; Amorim et al., 2022).

Torymus sinensis has been used widely and successfully as parasitoid in Italy since 2005 for biological control of larvae of *D. kuriphilus*, significantly contributing to the reduction of damages to the chestnut tree economy (Ferracini et al., 2019, 2022a). Similar results were also reported in several European countries (Borowiec et al., 2014; Matošević et al., 2017; Amorim et al., 2022; Avtzis et al., 2023).

Although phylogenetic data (Pogolotti et al., 2019; Gil-Tapetado et al., 2022; Viciruc et al., 2023), transcriptomic and proteomic

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information (Scieuzo et al., 2021), and morphological aspects by stereomicroscopy (Viciriuc et al., 2023) have been recently obtained for *T. sinensis*, some morphological aspects related to behavior of this species have not yet been investigated. Previous and very limited ultrastructural morphological data on antennae of *T. sinensis* only concerned the terminal segment of the flagellum (flagellomere 10) which was compared with the same structure of *Torymus beneficus* Yasumatsu and Kamijo 1979 (Hymenoptera: Torymidae) (Otake, 1987).

A detailed study on antennal and sensillar morphology may provide relevant information about the biology of *T. sinensis*, which could improve chestnut pest management. For this reason, an investigation by scanning electron microscopy (SEM) was carried out on the antennae of adults of both sexes of *T. sinensis*, focusing on morphology and distribution of the various types of sensilla.

2. Materials and methods

2.1. Insects

Torymus sinensis adults were reared from 200 galls of *D. kuriphilus* collected in the municipality of Villar Focchiardo (Turin, Italy), in a managed chestnut orchard. Galls were randomly collected by hand from low branches and with the help of lopping shears from the medium–high canopy (Ferracini et al., 2015, 2022a, 2022b). The collected galls were isolated inside cardboard boxes and kept outdoors until the emergence of *T. sinensis* adults. A total of 15 females and 15 males were anaesthetized with CO₂, individually placed in 1.5 ml plastic tubes and immediately frozen at –20 °C, where they were stored.

2.2. Scanning electron microscopy

For scanning electron microscopy (SEM), debris residues were removed by soaking the samples in a 0.05 % trypsin solution (BD Difco, Becton Dickinson, Sparks, MD) and 0.02 % sodium ethylenediaminetetraacetic acid (Merck, Darmstadt, Germany), and rinsing them three times for 10 min in phosphate buffer (0.1M; pH 7.2–7.4). Afterwards, the heads of seven females and seven males were detached and fixed in 3 % glutaraldehyde for 24 h, briefly rinsed in 0.1M phosphate buffer, postfixed in 1 % osmium tetroxide for 12 h and rinsed again in 0.1M phosphate buffer. The samples were then dehydrated in a graded ethanol series from 50 % to 100 % and then in increasing concentrations of hexamethyldisilazane (HMDS) and decreasing concentrations of ethanol, to 100 % HMDS. The samples in HMDS were then dry evaporated, fixed on stubs and coated with gold-palladium in an S150 Edwards sputter coater (HHV Ltd, Crawley, UK). For the other 16 samples, the antennae were removed and prepared for SEM as previously indicated. All samples were examined with a Zeiss EVO 40 SEM (Zeiss, Milan, Italy) at the Electron Microscopy Centre of the University of Ferrara. Observations by SEM allowed morphometric measurements through the software ZEISS SmartSEM v.5.09.SP10 (Carl Zeiss Ltd, Oberkochen, Germany), expressing data as averages with standard deviation. The classification of sensilla followed those of previous studies (Altner and Prillinger, 1980; Zacharuk, 1985; Amornsak et al., 1998; Huang et al., 2017). For the sensilla with dubious denomination, the name was chosen based on sensillar morphology.

3. Results

3.1. General morphology

The general morphology of antennae of the adult female and

male of *T. sinensis* is shown in Fig. 1a and b. The antennae, geniculate, are located in the frontal region of the head, between the compound eyes (Fig. 1a, inset). Each antenna is composed of three regions, the scape, the pedicel and the flagellum (Fig. 1a–c). The scape is divided into a proximal region, the radicula (Fig. 1d and e), and a distal cylindrical one, the proper scape (Fig. 1c and f). The pedicel is divided in a proximal region, the proper pedicel, and a distal conical one, the annellus (Fig. 1a, b and g–i). The flagellum is divided into 10 flagellomeres (Fig. 1a and b). Unlike in males (Fig. 1b), in females the diameter of the flagellum slightly increases from flagellomere 6 to flagellomere 8 (Fig. 1a). The radicula, the proper scape, the proper pedicel and the annellus are characterized by the presence of chaetic sensilla, and the flagellomeres by the presence of chaetic, placoid, trichoid, roundish grooved tip and coeloconic sensilla (Table 1).

3.2. Scape

The scape, the region connecting the antenna with the head, is composed of the conical radicula (49.4 ± 1.7 µm long in females and 49.2 ± 2.0 µm in males), and the cylindrical proper scape (270.5 ± 6.2 µm long in females and 213.1 ± 4.6 µm in males) (Fig. 1a–c). In both sexes, the radicula is characterized by a smooth surface and by two clusters of chaetic sensilla of subtypes I (CS-1, longer, Fig. 1j) and II (CS-2, shorter, Fig. 1k). The first cluster, composed of nine chaetic sensilla, is located in the anterior part of the radicula (Fig. 1d), and the second cluster, composed of 6–7 chaetic sensilla, is located in the posterior part (Fig. 1e). All sensilla of both clusters of the radicula have a conical shape, a rounded tip and a smooth surface (Fig. 1j and k). In the anterior part of the radicula, other CS-1 are visible near the boundary between the radicula and the head (Fig. 1d, inset). The distal part of the scape is characterized by an imbricated surface (Fig. 1f). The only sensilla found on the distal part of the scape are chaetic sensilla of subtype III (CS-3), characterized by a long, articulated and conical bristle, with shallow longitudinal grooves and a pointed tip (Fig. 1f and l).

3.3. Pedicel

The pedicel is composed of the proximal proper pedicel (90.1 ± 6.1 µm long in females and 85.0 ± 3.7 µm in males), articulated with the scape, and the distal annellus (23.2 ± 2.7 µm long in females and 18.2 ± 2.6 µm in males), articulated with the flagellum (Fig. 1g–i). In males, the proper pedicel has a stockier shape than in females (Fig. 1h, inset, and i). The aspect of the surface of both regions of the pedicel is similar to that of the scape and the only type of sensilla found is the CS-3.

3.4. Flagellum

The most distal region of the antenna, the flagellum (793.1 ± 34.1 µm long in females and 1011.5 ± 23.8 µm in males), is composed of ten segments (flagellomeres 1–10) articulated among themselves. The first flagellomere (flagellomere 1) has the proximal region articulated with the annellus of the pedicel (Fig. 1a–c). All flagellomeres are cylindrical except the last one, which is conical, but in females, the diameter of the flagellum slightly increases from flagellomere 6 to flagellomere 8.

In all flagellomeres of both sexes, there are chaetic sensilla of subtype IV (CS-4), placoid sensilla and sensilla with roundish grooved tip (Table 1). Other types and subtypes of sensilla are present either only in one sex and/or in some flagellomeres (Table 1).

The CS-4 are characterized by a long, articulated and conical bristle, with deep longitudinal grooves and a pointed tip

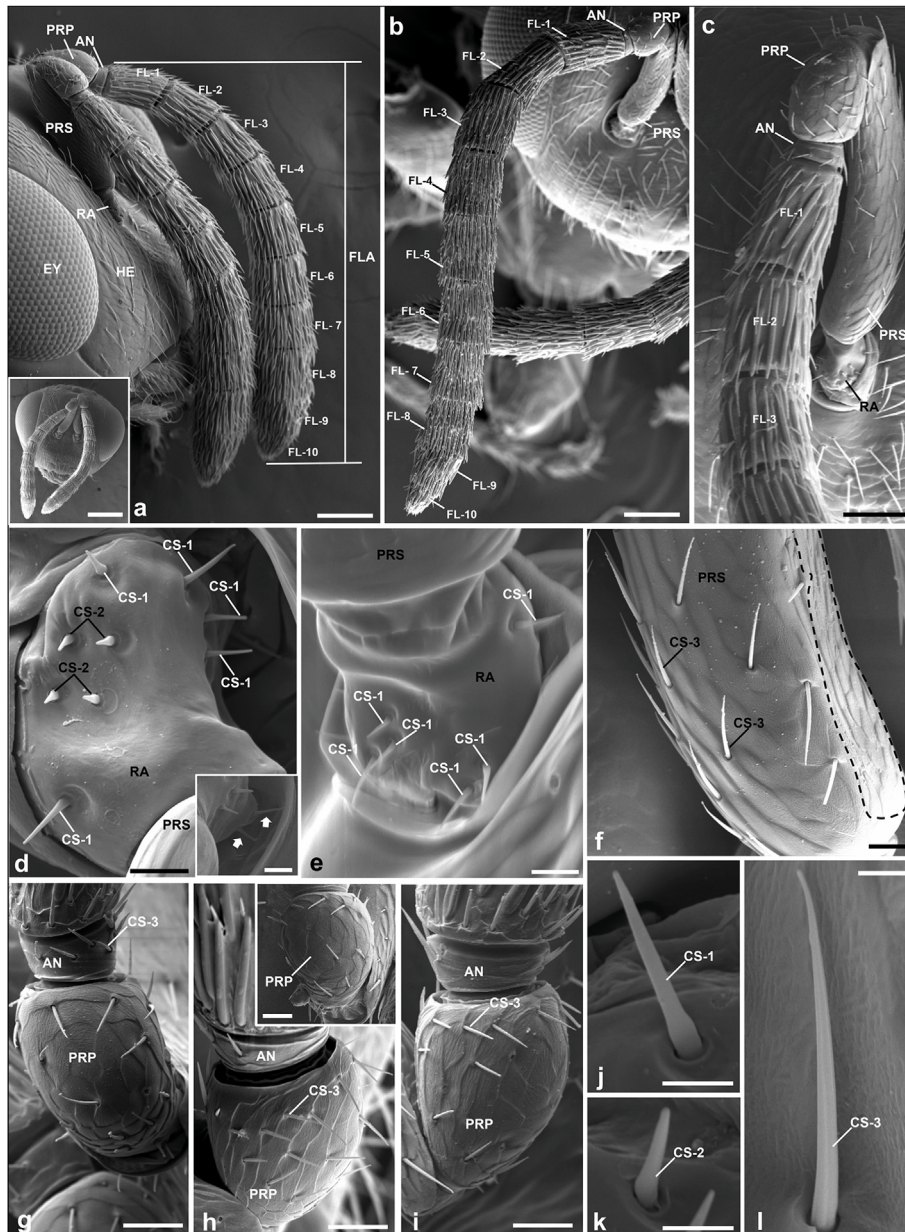


Fig. 1. Scanning electron micrographs of the antenna of *Torymus sinensis*. (a) Lateral view of the head of the female, showing the antennae *in toto*. Scale bar = 100 μm . Inlay: Frontal view of head of the female *in toto*. Scale bar = 250 μm . (b) Dorsal view of the right antenna of the male *in toto*. Scale bar = 100 μm . (c) Frontal view of the head of the female, showing the scape, the pedicel and the first three flagellomeres of the flagellum. Scale bar = 50 μm . (d) Anterior area of the radícula of the female, showing chaetic sensilla of subtypes I (CS-1, longer), and II (CS-2, shorter). Scale bar = 10 μm . Inlay: CS-1 (arrows) near the boundary between the radícula and the head of a male. Scale bar = 10 μm . (e) Posterior area of the radícula of the female. Scale bar = 10 μm . (f) Detail of the anterior part of the proper scape of the female, showing the imbricated surface, with one side with chaetic sensilla of subtype III (CS-3) and the other side (dashed line) hairless. Scale bar = 10 μm . (g) Dorsal view of the proper pedicel of the female surmounted by the annellus. Scale bar = 25 μm . (h) Lateral view of the proper pedicel of the male surmounted by the annellus. Scale bar = 25 μm . Inlay: Dorsal view of the proper pedicel of the male. Scale bar = 25 μm . (i) Lateral view of the proper pedicel of the female surmounted by the annellus. Scale bar = 25 μm . (j) Detail of the anterior part of the radícula, showing a CS-1. Scale bar = 5 μm . (k) Detail of the anterior part of the radícula, showing a CS-2. Scale bar = 5 μm . (l) Detail of the proper scape showing a CS-3 with longitudinal grooves. Scale bar = 2.5 μm . Abbreviations: AN, annellus; CS-1, chaetic sensillum of subtype I; CS-2, chaetic sensillum of subtype II; CS-3, chaetic sensillum of subtype III; EY, eye; FL-1 to FL-10, flagellomeres 1–10; FLA, flagellum; HE, head; PRP, proper pedicel; RA, radícula; PRS, proper scape.

(Fig. 2a–c). The articulated base of the bristle is surrounded by a raising of the cuticle (Fig. 2c and d). The CS-4 are homogeneously distributed in all flagellomeres, except in flagellomere 10 where they are absent in the apical region.

The placoid sensilla are of two subtypes in females (PS-1 and PS-2) (Fig. 2a, e and 3a–c) and one subtype in males (PS-3) (Figs. 2b, 3d and 3e). In females, PS-1 and PS-2 apparently alternate with each other (Figs. 2a and 3a). These sensilla are all long and subcylindrical, connected with the entire flagellomere surface except at the blunt

tip, which is free (Fig. 2a and b). In females, their different thickness and number of microperforations characterizes the PS-1 (larger and with more microperforations) and the PS-2 (thinner and with less microperforations) (Fig. 3a–c). In males, the number of microperforations of PS-3 is similar to that of PS-1 of females (Fig. 3d and e).

In females, there are three patterns of distribution of these sensilla in flagellomere 1. The first pattern is characterized by PS-1 and PS-2 distributed in two groups, one with the base near the

Table 1

Distribution of the different types of sensilla in regions and segments of both sexes of *Torymus sinensis*. Abbreviations: AN, annellus; COS, coeloconic sensilla; CS-1 to CS-6, chaetic sensilla of subtypes I-VI; FL-1 to FL-10, flagellomeres 1–10; PRP, proper pedicel; PRS, proper scape; PS-1 to PS-3, placoid sensilla of subtypes I-III; RS, sensilla with roundish grooved tip; RA, radícula; TS, trichoid sensilla; X, presence of sensilla.

Regions	Segments	COS	CS-1	CS-2	CS-3	CS-4	CS-5	CS-6	PS-1	PS-2	PS-3	RS	TS
Scape	RA ♂		X	X									
	RA ♀		X	X									
	PRS ♂				X								
	PRS ♀				X								
Pedicel	PRP ♂				X								
	PRP ♀				X								
	AN ♂				X								
	AN ♀				X								
Flagellum	FL-1 ♂					X					X	X	
	FL-1 ♀					X			X	X	X	X	
	FL-2 ♂					X					X	X	X
	FL-2 ♀					X			X	X	X	X	X
	FL-3 ♂					X					X	X	X
	FL-3 ♀					X			X	X	X	X	X
	FL-4 ♂					X					X	X	X
	FL-4 ♀					X			X	X	X	X	X
	FL-5 ♂					X					X	X	X
	FL-5 ♀					X			X	X	X	X	X
	FL-6 ♂					X					X	X	X
	FL-6 ♀					X			X	X	X	X	X
	FL-7 ♂					X					X	X	X
	FL-7 ♀					X			X	X	X	X	X
	FL-8 ♂					X					X	X	X
	FL-8 ♀	X				X			X	X	X	X	X
	FL-9 ♂					X					X	X	X
	FL-9 ♀	X				X	X	X	X	X	X	X	X
	FL-10 ♂					X	X	X	X	X	X	X	X
	FL-10 ♀	X				X	X	X	X	X	X	X	X

proximal boundary of the flagellomere and the sensillar tips extending to its middle part, and the other with the base in the middle part of the flagellomere and the sensillar tips near its distal limit (Fig. 4a). In some cases, the sensillar tips exceed the edge, invading the next flagellomere. As in the first pattern, the second one is characterized by PS-1 and PS-2 distributed in two groups, but the sensilla in the first group, based near the proximal boundary of the flagellomere, have their tips near its distal border, sometimes overlapping the edge and invading the next flagellomere (Fig. 4b). The third pattern is characterized by a single group of PS-1 and PS-2, based near the proximal border of the flagellomere, and with the tips in a position similar to that of the second group in the first pattern (Fig. 4c). The distribution of PS-1 and PS-2 in flagellomeres 2–8 of females follows that of the second pattern of flagellomere 1 (Fig. 4d). In flagellomeres 9 and 10, the distribution of PS-1 and PS-2 is different in comparison to that of the other flagellomeres, because these sensilla are organized in a single group (a cluster) with the base near the proximal border of the flagellomere and the tips near its distal border (Fig. 4e).

In males, the PS-3 are distributed in three rows in flagellomere 1 (Fig. 4f). In flagellomeres 2–6 the middle row of PS-3 tends to decrease (Fig. 4g), until in flagellomeres 7 and 8 only two rows of PS-3, and in flagellomere 9 two rows or only one row are found (Fig. 4h and i). As in females, in flagellomere 10 there is only one row of PS-3 (Fig. 4i).

The sensilla with roundish grooved tips (RS) are characterized by a conical base, a smooth cylindrical stalk, and a roundish tip with longitudinal grooves. Each RS is located in a shallow depression (Fig. 2d and inlay). In both sexes, these sensilla are detected near and on the distal boundary of all flagellomeres (Fig. 2a, b, d, e and 5a). In flagellomere 10, the RS are located near the tip (Fig. 5a and b).

The trichoid sensilla (TS) are characterized by a long, non-

articulated conical structure covered by microperforations and with a sharp tip (Fig. 2a–c). In both sexes, the base of TS is located in a shallow depression and has a small, crown-shaped cuticular structure on the external side (Fig. 2c). The TS are uniformly distributed on flagellomeres 2–10 and their tips protrude on the next flagellomere (Fig. 2a and b).

In females, very rare coeloconic sensilla (COS) characterized by a ring with a raised border, surrounding small radial bulges, are found on flagellomeres 8–10, one or two for each flagellomere (Fig. 5a, c and d). The COS are not found in males.

In females, chaetic sensilla of other two subtypes, CS-5 and CS-6, are found on flagellomeres 9 and 10. These sensilla are organized in groups, localized on the ventral side in flagellomeres 9 and 10, and also on the apex of flagellomere 10 (Fig. 5a and e). In males, the groups of CS-5 and CS-6 are not found on flagellomere 9, but they are present in the ventral side and in the apex of flagellomere 10, although more sparse (Fig. 5b and f). In both sexes, in the apex of flagellomere 10 the CS-5 and CS-6 are grouped in a circular cluster, one side of which has the surface in continuity with the surface of the flagellomere, and the other side is outlined by a furrow (Fig. 5b, g and h).

In both sexes, the CS-5 and CS-6 have a long articulated bristle with longitudinal grooves (Fig. 5a, b and g–i). The CS-5 are characterized by a tip with a single pore (Fig. 5j), and the CS-6 by a thin pointed tip (Fig. 5a).

4. Discussion

The extremely diverse superfamily Chalcidoidea is composed of 23 families, 22 extant and one extinct (Aguilar et al., 2013). The classification of the family Torymidae is currently under revision: molecular data suggest that the two subfamilies Toryminae and Megastigminae should be elevated to two families, Torymidae

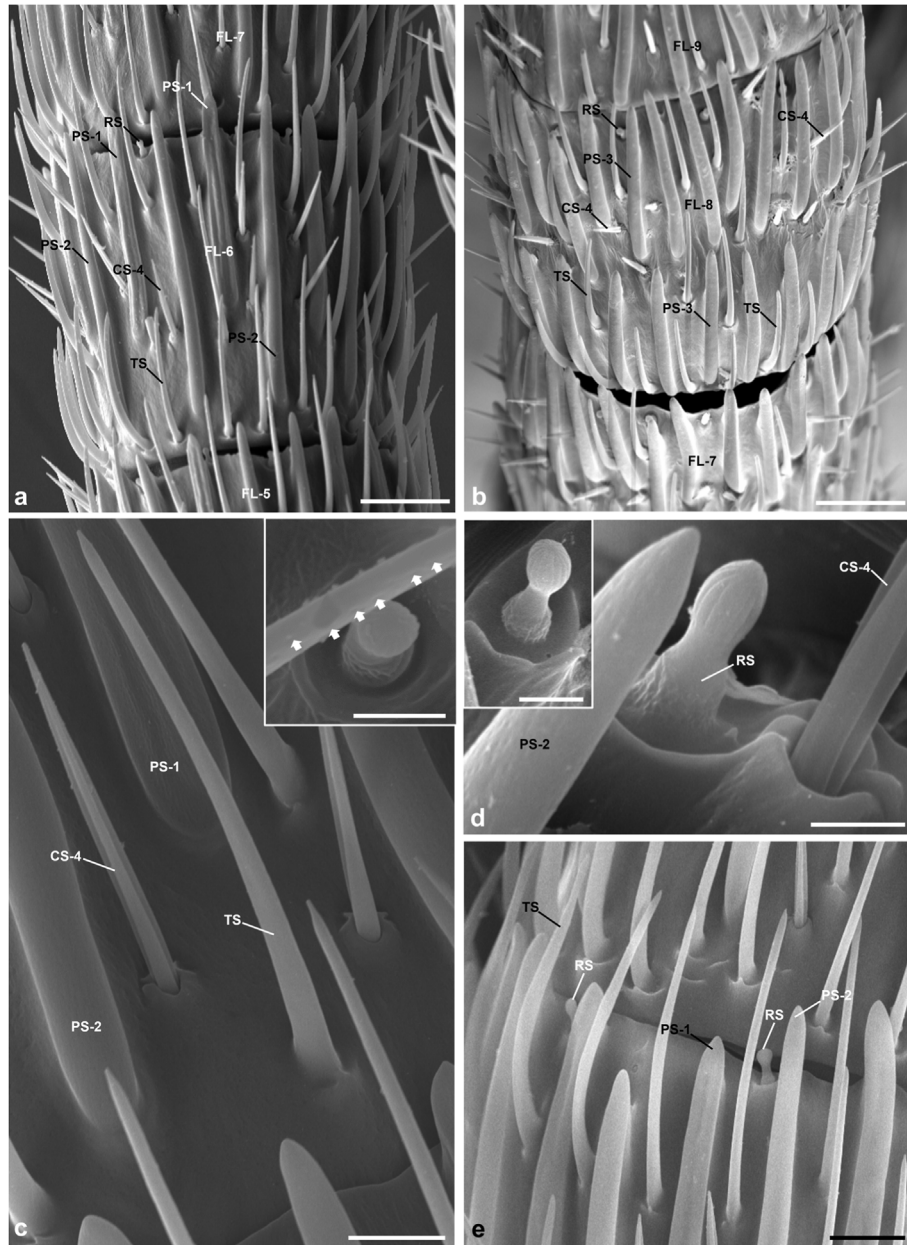


Fig. 2. Scanning electron micrographs of the flagellomeres of *T. sinensis*. (a) Overview of flagellomeres 5–7 in the female, including the distal part of flagellomere 5 and the proximal part of flagellomere 7. Chaetic sensilla of subtype IV (CS-4), placoid sensilla of subtypes I (PS-1) and II (PS-2), trichoid sensilla (TS) and grooved tip sensilla (RS) are visible. Scale bar = 25 μm . (b) Overview of flagellomeres 7–9 in the male, including the distal part of flagellomere 7 and the proximal part of flagellomere 9. Chaetic sensilla of subtype IV (CS-4), placoid sensilla of subtype III (PS-3), trichoid sensilla (TS) and grooved tip sensilla (RS) are visible. Scale bar = 25 μm . (c) Surface of a flagellomere of the female showing CS-4, PS-1, PS-2 and TS. Scale bar = 5 μm . Inlay: detail of TS showing microperforations (arrows). Scale bar = 2.5 μm . (d) Detail of the distal border of a flagellomere of the female, showing a RS, part of a PS-2 and the base of a CS-4 with longitudinal grooves. Scale bar = 2.5 μm . Inlay: Detail of a RS of a male, showing the shallow depression in which the sensillum is located. Scale bar = 2.5 μm . (e) Overview of the boundary between two flagellomeres in the female, showing two RS near the boundary. Scale bar = 10 μm . Abbreviations: CS-4, chaetic sensillum of subtype IV; PS-1, placoid sensillum of subtype I; PS-2, placoid sensillum of subtype II; PS-3, placoid sensillum of subtype III, RS, sensillum with roundish grooved tip; TS, trichoid sensillum. Other abbreviations as in Fig. 1.

sensu stricto and Megastigmidae (Janšta et al., 2018), increasing the number of extant families of Chalcidoidea to 23.

Among the species listed in the extant Torymidae, a detailed description of antennal sensilla by SEM was provided for *Megastigmus sichuanensis* Doğanlar and Zheng, 2017 (Huang et al., 2017). Recently, SEM images of the antenna were shown for *Pseudotorymus jaapiellae* Yang et Chen (Zhu et al., 2021), presumably belonging to the family Torymidae. However, neither the status of species nor the classification of *P. jaapiellae* within the family Torymidae have been confirmed to date.

Within the genus *Torymus* Dalman, 1820, an early SEM study on *Torymus varians* (Walker, 1833) showed the ventral side of the last two flagellomeres and details of the sensilla (Domenichini, 1977–1978). The distal part of the antenna of females and an unidentified flagellomere were also shown in *Torymus warreni* (Cockerell, 1911) (Barlin and Vinson, 1981). Some SEM images of the apical part of the last flagellomere and of the ventral side of the last two flagellomeres were shown for adults of both sexes of *T. beneficus*, together with one SEM image of the apex of the last flagellomere of a *T. sinensis* female (Ōtake, 1987). No detailed description or further

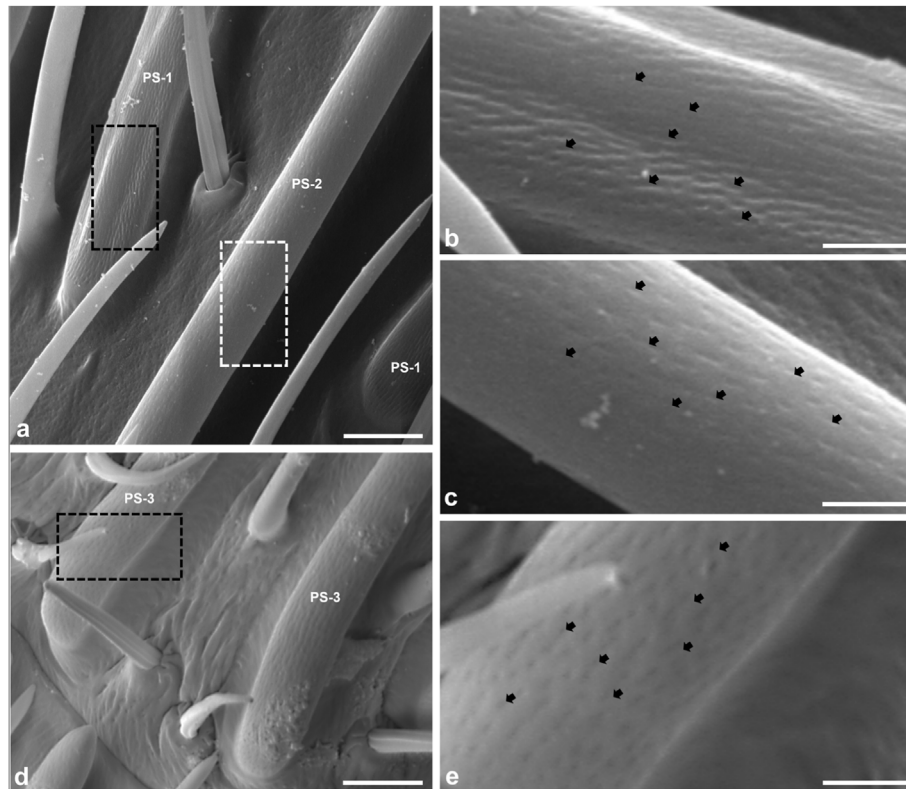


Fig. 3. Scanning electron micrographs of the placoid sensilla of *T. sinensis*. (a) Surface of a flagellomere in the female, showing details of a PS-1 and a PS-2. Scale bar = 5 μm . (b) Detail of the surface of the PS-1 in the black rectangle of Fig. 3a. Arrows indicate microperforations. Scale bar = 1.5 μm . (c) Detail of the surface of the PS-2 in the white rectangle of Fig. 3a. Arrows indicate microperforations. Scale bar = 1.5 μm . (d) Surface of a flagellomere in the male, showing parts of two PS-3. Scale bar = 5 μm . (e) Detail of the surface of the PS-3 in the black rectangle of Fig. 3d. Arrows indicate microperforations. Scale bar = 1.5 μm . Abbreviations as in Fig. 2.

SEM data are currently available for the antennae of *T. sinensis*.

In order to compare the sensilla found in this study on the antennae of both sexes of *T. sinensis* with similar sensilla found in other genera and species of the family Torymidae, and of other families of Chalcidoidea, the original names of the sensilla reported in the literature are indicated in detail, together with the corresponding reference or references.

4.1. Chaetic sensilla

In the antenna of both sexes of *T. sinensis*, six subtypes of chaetic sensilla are found. In the radicula, the chaetic sensilla are of subtypes I and II (CS-1 and CS-2) in both sexes. Within the family Torymidae, similar sensilla, but only of one type (“sensillum chaetica I”), were described in *M. sichuanensis* (Huang et al., 2017) and in *P. jaapiellae* (“type I sensilla basiconic”) (Zhu et al., 2021).

In Encyrtidae, similar chaetic sensilla of two types were identified in the radicula of both sexes of *Metaphycus parasaissetiae* Zhang and Huang 2007 (Zhou et al., 2013). These sensilla were distinguished based on length and tip shape as “sensilla chaetica-1 and -2” (Zhou et al., 2013). In *Ooencyrtus phongi* Trjapitzin, Myartseva & Kostjukov, 1977 similar chaetic sensilla of only one type were found and identified as “sensilla chaetica type Ra” (Xi et al., 2011). In Agaonidae, similar chaetic sensilla of only one type but smooth and of variable length (“sensilla chaetica type 1”) were also detected in the radicula of *Ceratosolen solmsi marchali* Mayr, 1906 (Li et al., 2009). In Trichogrammatidae, similar chaetic sensilla, smooth, without pores and not articulated, were detected in the radicula and the pedicel of *Pseudoligosita yasumatsui* (Vigiani and Subba Rao, 1978), and described as “trichoid sensilla type

5” (Wong et al., 2019).

In Eulophidae, similar chaetic sensilla were described in the radicula of both sexes of *Trichospilus pupivorus* Ferrière, 1930, and identified as “sensilla chaetica”. They were very few, short, smooth and located in cuticular depressions (da Silva et al., 2016). In both sexes of *Tamarixia radiata* (Waterston, 1922), chaetic sensilla similar to those of *T. sinensis* were found in the radicula and pedicel. They were smooth and located in cuticular depressions, and were identified as “type II aporous sensilla trichoidea” (Onagbola et al., 2009).

In Pteromalidae, “non-porous sensilla chaetica” of only one type similar to the chaetic sensilla of *T. sinensis* were described in the radicula of both sexes of *Pteromalus puparum* (Linnaeus, 1758) (Dweck, 2009). In both sexes of *Pteromalus cerealellae* (Ashmead, 1902), “aporous sensilla trichodea type IV” similar to the chaetic sensilla of *T. sinensis* were also described in the radicula (arranged in groups) and in the pedicel (Onagbola and Fadamiro, 2008). Sensilla morphologically similar to the chaetic ones of *T. sinensis* were found in the radicula and the pedicel of *Jaliscoa hunteri* (Crawford, 1908), and identified as “poreless type 4 sensilla trichoid” in “deep wells” (Gómez-Dominguez et al., 2018).

In *T. sinensis*, chaetic sensilla of subtype III (CS-3), articulated and grooved, are found in the proper scape, in the proper pedicel and in the annellus of both sexes, and chaetic sensilla of subtype IV (CS-4) in all flagellomeres. Within the family Torymidae, chaetic sensilla similar to CS-3 and CS-4 were found in all antennal segments (except the radicula) of both sexes of *M. sichuanensis*, and identified as “sensilla chaetica II” (Huang et al., 2017). In both sexes of *P. jaapiellae*, similar sensilla, grooved and tapered, were found in all antennal segments (except the radicula) and identified as “type II

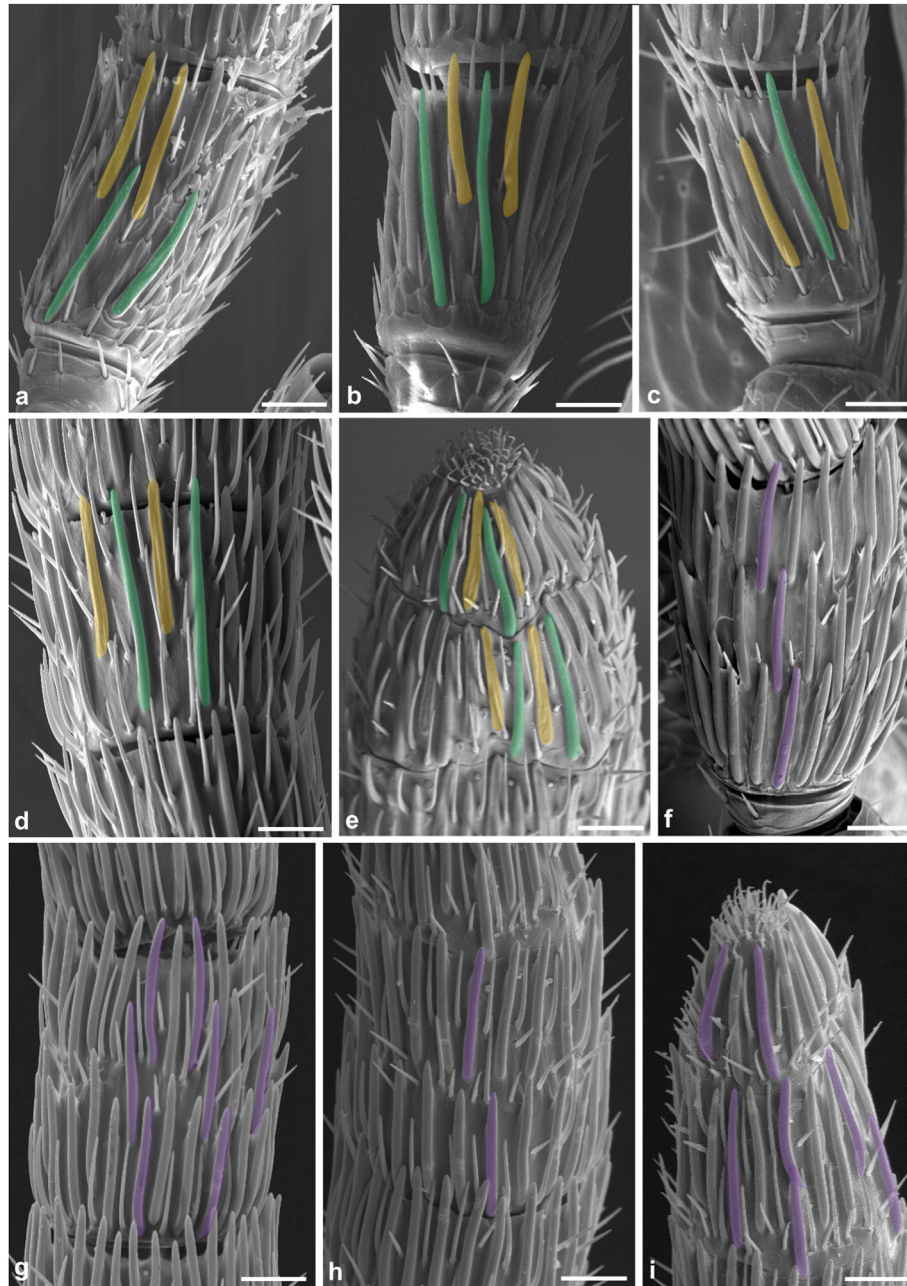


Fig. 4. Distribution of the placoid sensilla on the flagellomeres of *T. sinensis*. (a), (b), (c) Placoid sensilla in flagellomere 1 of the female. Some PS-1 are indicated in yellow and some PS-2 in green. Scale bar = 25 μm . (d) Placoid sensilla in flagellomere 6 of the female. Some PS-1 are indicated in yellow and some PS-2 in green. Scale bar = 25 μm . (e) Placoid sensilla in flagellomeres 9 and 10 of the female, in dorsal view. Some PS-1 are indicated in yellow and some PS-2 in green. Scale bar = 25 μm . (f) Placoid sensilla (PS-3) in flagellomere 1 of the male. Some PS-3 are indicated in purple. Scale bar = 25 μm . (g) Placoid sensilla in flagellomere 5 of the male. Some PS-3 are indicated in purple. Scale bar = 25 μm . (h) Placoid sensilla in flagellomere 8 of the male. Some PS-3 are indicated in purple. Scale bar = 25 μm . (i) Placoid sensilla in flagellomeres 9 and 10 of the male, in dorsal view. Some PS-3 are indicated in purple. Scale bar = 25 μm .

sensilla trichodea” (Zhu et al., 2021).

In Pteromalidae, sensilla morphologically similar to CS-3 and CS-4 of *T. sinensis* were found in all antennal segments (except the radicula) of both sexes of *Pt. cerealellae* (Onagbola and Fadamiro, 2008) and *J. hunteri* (Gómez-Dominguez et al., 2018). They were respectively identified as “aporous type 2 sensilla trichodea” and “poreless type 2 sensilla trichoid”. In females of *Pt. puparum*, sensilla similar to CS-3 and CS-4 were described in the scape, the pedicel, the annellus and the first flagellomere, and identified as “nonporous sensilla trichodea type 1” (Dweck, 2009).

In *T. sinensis*, the distribution of chaetic sensilla of subtypes V

and VI (CS-5 and CS-6) show sexual dimorphism. In females, CS-5 and CS-6, organized in groups, were found on the ventral side in flagellomeres 9 and 10, and on the apex of flagellomere 10. In males, CS-5 and CS-6, absent in flagellomere 9, are present on the ventral side and apex of flagellomere 10.

Similar sensilla were previously observed in females of *T. sinensis* (Ōtake, 1987) and *T. varians* (Domenichini, 1977-1978), but in both cases they were not clearly identified. When flagellomeres 9 and 10 were examined in males of *T. beneficus*, the distribution of sensilla similar to CS-5 and CS-6 paralleled that of *T. sinensis* males (Ōtake, 1987).

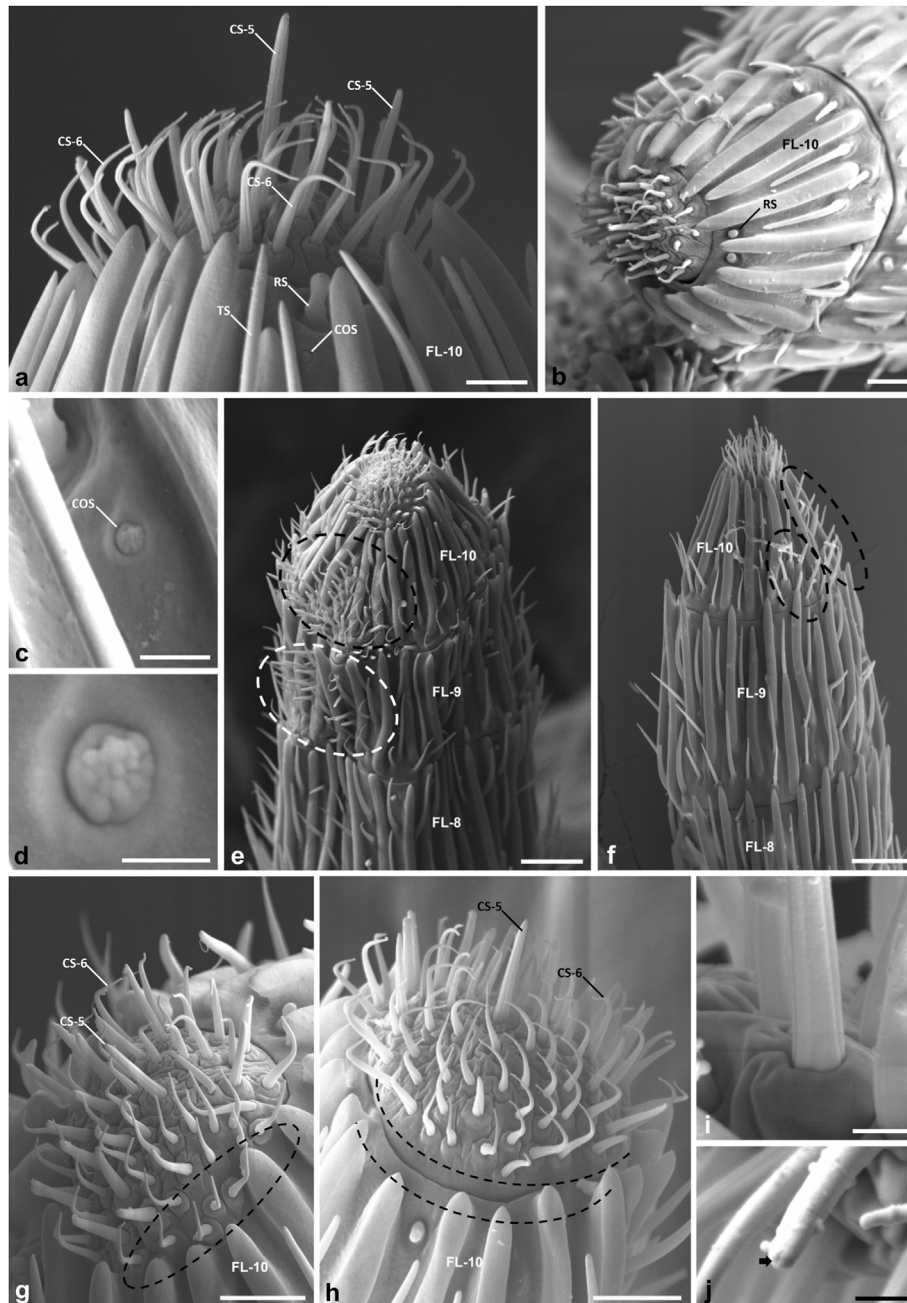


Fig. 5. Scanning electron micrographs of the flagellomeres of *T. sinensis*. **(a)** Terminal part of flagellomere 10 of the female, showing the circular cluster of chaetic sensilla of subtypes V (CS-5) and VI (CS-6). Scale bar = 5 μm . **(b)** Overview of the apex of flagellomere 10 of the male. Scale bar = 10 μm . **(c)** Detail of the surface of flagellomere 10 of the female showing a coeloconic sensillum (COS). Scale bar = 2.5 μm . **(d)** Close view of the COS in Fig. 4c. Scale bar = 1 μm . **(e)** Overview of the dorsal side of flagellomeres 8–10 of the female. The white and black ovals respectively indicate the clusters of CS-5 and CS-6 in flagellomeres 9 and 10. Scale bar = 25 μm . **(f)** Overview of the lateral-ventral side of flagellomeres 8–10 of the male. The black ovals indicate the cluster of CS-5 and CS-6 in flagellomere 10. Scale bar = 25 μm . **(g)** Side view of the apical part of flagellomere 10 of the female, showing the surface without furrows. Scale bar = 10 μm . **(h)** Opposite side of the apical part of flagellomere 10 of the female, showing a furrow between the apical part and the rest of flagellomere 10. Scale bar = 10 μm . **(i)** Base of a CS-5 in the female, with grooved surface. Scale bar = 1.5 μm . **(j)** Apical part of a CS-5 in the female, showing a terminal pore (arrow). Scale bar = 1.5 μm . Abbreviations: COS, coeloconic sensillum; CS-5, chaetic sensillum of subtype V; CS-6, chaetic sensillum of subtype VI. Other abbreviations as in Figs. 1 and 2.

In Pteromalidae, clusters of sensilla similar to CS-5 and CS-6, respectively identified as “unipore chaetic sensilla” and “poreless type 1 sensilla trichoid”, were found in the apical flagellomere of both sexes of *J. hunteri*, but they were much fewer in males (Gómez-Domínguez et al., 2018), as found in the present study in *T. sinensis*.

Clusters of sensilla similar to CS-5 and CS-6 were also found in the apex of the flagellum of both sexes of *Nasonia vitripennis* (Walker, 1836), and respectively described as “thick-walled

chemoreceptors” and “short, curved, tactile hair” (Miller, 1972). Similar sensilla were found in both sexes of *Peridesmia discus* (Walker, 1835), but in this species they were found also on the ventral side of the apex (Miller, 1972).

In *Pt. cerealellae*, sensilla similar to CS-5 and CS-6 were found in the last two flagellomeres of females and in the terminal flagellomere of males. These sensilla were respectively identified as “uniporous chaetica sensilla” and “aporous type 1 sensilla

trichodea" (Onagbola and Fadamiro, 2008). Sensilla similar to CS-5 and CS-6 were also found in the last two flagellomeres of females of *Pt. puparum*, and respectively identified as "uniporous sensilla trichodea type 4" and "nonporous sensilla trichodea type 2" (Dweck, 2009).

Concerning the possible functions of the chaetic sensilla without the apical pore, generally they have been considered as mechanoreceptors (Zacharuk, 1985). In *Rhopalicus tutela* (Walker, 1836) (Pteromalidae) a tactile mechanosensory function has been proposed (Pettersson et al., 2001). A proprioceptive role in the movement of the antenna was suggested for the chaetic sensilla of *Pt. puparum* (Dweck, 2009). For chaetic sensilla with an apical pore, a general mechanosensitive and chemosensitive function was suggested in insects (Altner and Prillinger, 1980; Zacharuk, 1985). In *Cotesia plutellae*, presently *Cotesia vestalis* (Haliday, 1834) (Braconidae), belonging to the superfamily Ichneumonoidea, these sensilla were classified as "gustatory" (van Baaren et al., 2007).

4.2. Placoid sensilla

In *T. sinensis* females, the placoid sensilla are of two subtypes (PS-1 and PS-2), the first one larger and with abundant microperforations and the second one thinner and with less microperforations. In males, the placoid sensilla are of a third subtype (PS-3), with microperforations similar to those of PS-1.

"Plate sensilla" very similar to the placoid ones found in the present study were previously found in the flagellum of females of *T. sinensis* and in both sexes of *T. beneficus* (Ōtake, 1987). An image of the last two flagellomeres suggests the presence of these sensilla also in *T. varians* females (Domenichini, 1977–1978). Placoid sensilla comparable to those of *T. sinensis* and of two subtypes, one with microperforations ("multiporous plate sensilla type 1") and the other apparently without microperforations ("multiporous sensilla type 2"), were found in females of *T. warreni* (Barlin and Vinson, 1981). "Longitudinal sensilla", probably identifiable as "placoid sensilla" were recently described in 14 Japanese species of the genus *Torymus* (Matsuo, 2020). In other Torymidae, "sensilla placodea" without information about microperforations were found in the flagellum of both sexes of *M. sichuanensis* (Huang et al., 2017) and "sensilla placodea" with numerous pores in both sexes of *P. jaapiellae* (Zhu et al., 2021).

In Agaonidae, "multiporous placoid sensilla" of two types were found in females of *C. solmsi marchali*, the first type in flagellomeres 3–8 and the second one only in flagellomere 2 (Li et al., 2009). In Aphidiidae, only one type of placoid sensilla ("elongate placoid sensilla") was found in both sexes of *Aphidius smithi* Sharma and Subba Rao, 1959, in all flagellomeres of males and in flagellomeres 1–17 of females (Borden et al., 1978).

In Encyrtidae, only one type of placoid sensilla was found in flagellomeres 2–10 of females of *Leptomastix dactylopii* Howard, 1885, and in all flagellomeres of females of *Epidinocarsis lopezi*, presently *Anagyrus lopezi* (De Santis, 1964) (van Baaren et al., 1996). Placoid sensilla were also identified in *O. phongi* in all flagellomeres of females and in all flagellomeres except the "claval segments 2 and 3" of males (Xi et al., 2011), and from flagellomere 5 onwards in both sexes of *Me. parasaissetiae* (Zhou et al., 2013). "Multiporous plate sensilla" of two types in females and one type in males were also reported in *Anagyrus pseudococci* (Girault, 1915), and of two types in females of *Comperiella bifasciata* Howard, 1906, but for these two species there were no images supporting the data (Barlin and Vinson, 1981). Recently, "multiporous plate sensilla" of only one type were described in all flagellomeres of females of *Anagyrus vladimiri* Triapitsyn, 2019 (Sevarika et al., 2022).

In Eulophidae, "multiporous plate sensilla" of two types ("type 1" and "type 2") were found in the flagellum of females, and of only

one type ("type 1") in the flagellum of males of *Tetrastichus hagenowii*, presently *Aprostocetus hagenowii* (Ratzeburg, 1852) (Barlin and Vinson, 1981; Barlin et al., 1981), and of *Elasmus polistis* Burks, 1971, but in *E. polistis* no SEM images were provided to support the data (Barlin and Vinson, 1981). The "multiporous plate sensilla" were also found in the flagellum of both sexes of *Sympiesis sericeicornis* (Nees, 1834) (Meyhöfer et al., 1997), and of *Quadrastichus erythrinae* Kim, 2004, but in *Q. erythrinae* two types were identified, "MPS I" and "MPS II" (Li et al., 2013). "Multiporous placoid sensilla" of only one type were found in the flagellum of both sexes of *Ta. radiata* (Onagbola et al., 2009). In *Tetrastichus brontispae* (Ferrière, 1933) (Xin et al., 2010), the "sensilla multiporous plate" found in the flagellum of both sexes and the "sensilla auricillica" in the flagellum of females were comparable to the placoid sensilla identified in *T. sinensis*.

In Aphelinidae, "multiporous plate sensilla" of two types were reported in the flagellum of females of *Coccophagus pulvinariae* Compere, 1931 and of only one type in both sexes of *Aphytis melinus* DeBach, 1959, but in *Ap. melinus* no images were provided to support the data (Barlin and Vinson, 1981).

Concerning Pteromalidae, only one type of "multiporous sensilla placodea" were identified in all flagellomeres (except the last one) in females of *Pt. puparum* (Dweck, 2009) and only one type of "placoid sensilla" in the flagellum of *R. tutela*, presumably of both sexes (Pettersson et al., 2001). "Multiporous plate sensilla" of only one type were identified in all flagellomeres of both sexes of *J. hunteri* (Gómez-Domínguez et al., 2018). "Multiporous placoid sensilla" of only one type were also found in all flagellomeres (except the first two and the last one) of both sexes of *Pt. cerealellae* (Onagbola and Fadamiro, 2008).

"Plate organs" apparently similar to the placoid sensilla of *T. sinensis* were identified in the flagellum of both sexes of *N. vitripennis* and *Pe. discus* (Miller, 1972). In *N. vitripennis*, a later study identified two types of "multiporous plate sensilla" in females and one type in males, distinguishing the types by the number of microperforations ("pore concentration") (Wibel et al., 1984). The same situation was observed in *Muscidifurax zaraptor* Kogan and Legner, 1970, and *Cecidostiba dendroctoni*, presently *Dinotiscus dendroctoni* (Ashmead, 1894), but in *D. dendroctoni* no images were provided to support the results (Barlin and Vinson, 1981). Two types of "multiporous plate sensilla" were also reported in females of *Spalangia drosophilae* Ashmead, 1887, but, again, no images were provided to support the data (Barlin and Vinson, 1981).

In Trichogrammatidae, two types of placoid sensilla ("PS1" and "PS2") distinguished by the number of microperforations were found in the apical flagellomere of females of *Trichogramma australicum* Girault, 1912, and only one type in males ("PS1") (Amornsak et al., 1998). Two types of placoid sensilla in the last two flagellomeres of females ("type 1" and "type 2") and one type ("type 1") in males, again based on different distribution of microperforations, were described in *Ps. yasumatsui* (Wong et al., 2019).

In the last flagellomere of females of *Trichogramma galloi* Zucchi, 1988, and *Trichogramma pretiosum* Riley, 1879, only one type of "multiporous sensilla placodea" was found (Cönsoli et al., 1999), and in *Trichogramma nubilale* Ertle and Davis, 1975, only one type of "multiporous pitted sensilla placodea" (Olson and Andow, 1993). "Sensilla placodea" were found in the last flagellomere of females of *Trichogramma confusum* Viggiani, 1976, and *Trichogramma ostrinae* Pang and Chen, 1974 (Gong et al., 2004). Only one type of "sensilla placodea" was found in the last flagellomere of females of *Trichogramma brasiliensis* (Ashmead, 1904) and *Trichogramma evanescens* Westwood, 1833 (Voegelé et al., 1975). In *Tr. evanescens*, only one type of "multiporous sensilla placodea" was reported in both sexes, but with no images to support the data (Barlin and Vinson, 1981).

Summarizing the results about placoid sensilla, in this study they are described for the first time in both sexes of a species belonging to the family Torymidae. These results support what previously found in females of *T. warreni*: no data were available on males of this species (Barlin and Vinson, 1981). In addition, the pattern of distribution of these sensilla, with sexual dimorphism, is described in detail for the first time in *T. sinensis*.

Generally, among the species presently investigated for antennal sensilla, some belonging to different families of Chalcidoidea share the presence of two types of placoid sensilla in females and one type in males, as in *T. sinensis*. Among these families, there are Encyrtidae (Barlin and Vinson, 1981), Eulophidae (Barlin and Vinson, 1981), Pteromalidae (Wibel et al., 1984) and Trichogrammatidae (Wong et al., 2019). Further studies are required to verify whether one or two types of these sensilla are present in the different families, genera and species of Chalcidoidea, and whether these types are present in one or both sexes.

About the function of placoid sensilla, a general olfactory role connected to oviposition was suggested in several species of Chalcidoidea (Barlin and Vinson, 1981). In *Tr. galloi* and *Tr. pretiosum*, it was suggested that they could play a role in perception of pheromones released by the host female, to orient the parasitoid female towards host eggs (Cònsoli et al., 1999). An olfactory function was hypothesized also in *Pt. puparum*, based on the presence of a multiple pore system (Dweck, 2009). In *Ta. radiata*, where placoid sensilla were found in both sexes, the hypothesis advanced was that in females they could play a role in host finding, and in males they could be a mating cue (Onagbola et al., 2009). A general chemoreceptor function of placoid sensilla was suggested in both sexes of *R. tutela* (Pettersson et al., 2001) and in females of *C. solmsi marchali* (Li et al., 2009).

4.3. Grooved tip sensilla

In both sexes of *T. sinensis*, sensilla with roundish grooved tip are found in the flagellum. These roundish grooved tip sensilla (RS) have a conical corrugated base, a smooth cylindrical stalk and a roundish tip with longitudinal grooves. These sensilla have never been previously described in the genus *Torymus*. Sensilla similar to RS were described in *P. jaapiellae* in flagellomeres 1–10 of females and flagellomeres 2–10 in males, and identified as “sensilla coelocónica” (Zhu et al., 2021).

In Encyrtidae, sensilla similar to RS were described in flagellomeres 2–10 of females of *L. dactylopii*, in all flagellomeres of females of *A. lopezi* (van Baaren et al., 1996), in the flagellum of both sexes of *O. phongi* (Xi et al., 2011) and of females of *Cheiloneurus noxius* Compere, 1925 (Weseloh, 1972). In all four species, these sensilla were identified as “basiconic sensilla”. “Sensilla grooved peg” similar to RS were recently described in all flagellomeres except the last one of females of *A. vladimiri* (Sevarika et al., 2022).

In Eulophidae, sensilla similar to RS were detected in the flagellum of both sexes of *Ta. radiata* and described as “basiconic capitate peg sensilla” (Onagbola et al., 2009), and of *Melittobia australica* Girault, 1912, described as “short basiconic capitate pegs” (Dahms, 1984). Comparable sensilla were also found in the flagellum of both sexes of *Ap. hagenowii*, and described as “multiporous pegs” (Barlin et al., 1981). Sensilla similar to RS were found in the flagellum of both sexes of *Tr. pupivorus*, described as “basiconic sensilla-1” (da Silva et al., 2016), of *Te. brontispae*, described as “sensilla mammiliformia” (Xin et al., 2010) and of *Q. erythrinae*, described as “basiconic capitate peg sensilla” (Li et al., 2013).

Within the family Pteromalidae, sensilla similar to RS were described in the flagellum of females of *Pt. puparum* (Dweck, 2009), in both sexes of *J. hunteri* (Gómez-Domínguez et al., 2018) and in both sexes of *Pt. cerealellae* (Onagbola and Fadamiro, 2008). In all

three species, these sensilla were identified as “basiconic capitate peg sensilla”. In the flagellum of both sexes of *N. vitripennis* and of *Pe. discus*, sensilla very similar to RS were found and identified as “basiconic capitate peg” (Miller, 1972; Wibel et al., 1984). Similar sensilla were found also in the flagellum of *R. tutela*, presumably of both sexes, but in this case they were identified as “coelocónica sensilla” (Pettersson et al., 2001).

In Trichogrammatidae, sensilla comparable to RS were found in the flagellum of females of *Tr. galloi* and *Tr. pretiosum* (Cònsoli et al., 1999) and in both sexes of *Ps. yasumatsui* (Wong et al., 2019): in these three species, they were described as “basiconic sensilla”. Sensilla similar to RS were also found in the flagellum of females of *Tr. evanescens* and identified as “sensilla ampullacea” (Voegelé et al., 1975), and of *Tr. nubilale*, described as “multiporous grooved sensilla basiconica” (Olson and Andow, 1993). In *Tr. confusum* and *Tr. ostriniae*, these sensilla were observed in females and identified as “sensilla ampullacea” (Gong et al., 2004).

In Chalcidoidea, the only hypothesis about the function of sensilla with a shape similar to RS was the thermal and/or hygro-sensitive one advanced in *Tr. nubilale* (Olson and Andow, 1993), and in *L. dactylopii* (van Baaren et al., 2007).

4.4. Trichoid sensilla

In both sexes of *T. sinensis*, trichoid sensilla (TS) are found only on the flagellum, from flagellomeres 2 to 10. They have a conical, non-articulated structure covered by microperforations and a sharp tip. In *T. varians* females, structures very similar to these trichoid sensilla were identified in the distal part of the flagellum, but no microperforations were visible in the images due to the low magnification (Domenichini, 1977–1978). Sensilla similar to TS were visible in SEM images of the flagellum of *T. warreni* females (Barlin and Vinson, 1981) and of both sexes of *T. beneficus* (Ôtake, 1987), but they were not described. Within Torymidae, sensilla similar to TS were also found in flagellomeres 2–10 of both sexes of *M. sichuanensis*, and were identified as “sensillum trichoid II” (Huang et al., 2017). These sensilla were also found in all flagellomeres of both sexes of *P. jaapiellae*, and were identified as “type III sensilla trichodea” (Zhu et al., 2021).

In Encyrtidae, sensilla similar to TS were found in the pedicel and flagellum of males of *O. phongi* and were identified as “trichoid sensilla type 2”. In the same species, similar sensilla found in the flagellum of females were identified as “chaetic sensilla of type 3” (Xi et al., 2011). Sensilla similar to TS were also found in females of *Me. parasaissetiae* in all flagellomeres except the terminal one and in males in flagellomeres 5, 6 and the “first claval subsegment”. These sensilla were identified as “trichodea sensilla-2” (Zhou et al., 2013).

Within the family Eulophidae, sensilla described as “trichoid” were found in the flagellomeres of both sexes of *Tr. pupivorus*, but their similarity with TS of *T. sinensis* is unclear (da Silva et al., 2016). Sensilla similar to TS, found in the flagellum of both sexes of *S. seiceicornis*, were identified as “trichoid sensilla type-A2” (Meyhöfer et al., 1997). In both sexes of *Ta. radiata*, only one type of trichoid sensilla was described in the flagellum: it was similar to TS and identified as “multiporous sensilla trichodea” (Onagbola et al., 2009). In both sexes of *Mel. australica* sensilla similar to TS were found in the terminal part of the flagellum and described as “long thin unfluted setae” (Dahms, 1984).

In Pteromalidae, sensilla with microperforations very similar to TS were identified in *R. tutela* presumably of both sexes (Pettersson et al., 2001), in both sexes of *Pt. cerealellae*, identified as “multiporous type 3 sensilla trichodea” (Onagbola and Fadamiro, 2008), and in females of *Pt. puparum*, identified as “multiporous sensilla trichodea type 5” (Dweck, 2009).

In both sexes of *N. vitripennis* and of *Pe. discus*, sensilla similar to TS were found in the flagellum and identified in both species as “thin-walled chemoreceptors” (Miller, 1972; Wibel et al., 1984).

In Trichogrammatidae, “multiporous sensilla trichodea” very similar to TS of *T. sinensis* were described in females of *Tr. galloi* and *Tr. pretiosum* (Cònsoli et al., 1999).

From a functional point of view, an “olfactory function” was suggested in *R. tutela* (Pettersson et al., 2001).

4.5. Coeloconic sensilla

In *T. sinensis*, one or two coeloconic sensilla (COS) are found only in females in flagellomeres 8, 9 and 10. This type of sensilla has never been previously described in the genus *Torymus*. In the family Torymidae, sensilla of similar shape were found only in *P. jaapiellae* and identified as “sensilla campaniformia Sca III” (Zhu et al., 2021).

In Pteromalidae, similar sensilla were described in the flagellum of both sexes of *N. vitripennis*, and identified as “sensilla campaniformia” (Wibel et al., 1984). Although general “coeloconic sensilla” have been reported in other families, such as Trichogrammatidae (Olson and Andow, 1993; Amornsak et al., 1998; Cònsoli et al., 1999) and Agaonidae (Li et al., 2009), no clear hypothesis on the function of these sensilla has yet been advanced in Chalcidoidea.

5. Conclusions

In conclusion, the morphological investigation by SEM conducted on the antennae of both sexes of *T. sinensis* reveal the presence of five types of sensilla in the different regions, with sexual dimorphism in some of them.

The types of sensilla include chaetic sensilla of six subtypes, placoid sensilla of three subtypes, trichoid sensilla, roundish grooved tip sensilla and coeloconic sensilla. Chaetic sensilla of the first three subtypes are found in the scape and in the pedicel, and the chaetic sensilla of the other three subtypes, together with the other four types of sensilla, are found only on flagellomeres.

In the antenna, sexual dimorphism can be observed in the morphology of proper pedicel, stockier in males in comparison to females, and in the flagellum, slightly longer in males and with an increasing diameter of flagellomeres 6–8 in females. Concerning sensilla, the sexual dimorphism involves a different distribution and subtypes of placoid sensilla (PS-1, PS-2, PS-3), the presence of coeloconic sensilla (COS) only in flagellomeres 8–10 of females, and a different distribution of clusters of subtypes of chaetic sensilla (CS-5 and CS-6) in the apical flagellomeres.

The results of this investigation have been compared with those of similar studies in species of the family Torymidae and of other families of the superfamily Chalcidoidea. These data, integrated with further studies by transmission electron microscopy and electrophysiological studies, could be relevant for investigations on the roles of the different types of sensilla in host search, improving the knowledge of the relationship between the parasitoid and its host. These data could also be useful for comparative and phylogenetic studies within the superfamily Chalcidoidea.

CRedit authorship contribution statement

Marco Pezzi: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **Cristina Munari:** Investigation, Writing – review & editing. **Michele Mistri:** Investigation, Writing – review & editing. **Chiara Scapoli:** Investigation, Writing – review & editing. **Milvia Chicca:** Data curation, Writing – original draft, Writing – review & editing. **Marilena Leis:** Investigation, Writing – review & editing. **Carmen Scieuzo:** Data

curation, Writing – review & editing. **Antonio Franco:** Data curation, Writing – review & editing. **Rosanna Salvia:** Data curation, Writing – review & editing. **Chiara Ferracini:** Data curation, Writing – review & editing. **Alberto Alma:** Data curation, Writing – review & editing. **Patrizia Falabella:** Data curation, Writing – review & editing. **Davide Curci:** Data curation, Investigation, Writing – original draft, Writing – review & editing. **Teresa Bonacci:** Data curation, Investigation, Validation, Writing – review & editing.

Declaration of Competing interest

None.

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