



Antennal Fine Morphology of the Threatened Beetle *Osmoderma eremita* (Coleoptera: Scarabaeidae), Revealed by Scanning Electron Microscopy

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ABSTRACT The aim of this study was to characterize the antennal morphology of *Osmoderma eremita*, a threatened scarab beetle inhabiting tree hollows. *O. eremita* males produce a sex pheromone, (*R*)-(+)– γ -decalactone, responsible mainly for the attraction of females but also other males. Gross and fine morphology of microstructures including sensilla, microsculpture and pores were analyzed using Scanning Electron Microscopy. The antenna of *O. eremita* showed the typical lamellicorn shape of scarab beetles, with a basal scape, a pedicel, a funicle composed of five antennomeres and a club composed of three lamellae. Six different subtypes of sensilla chaetica (Ch.1 – 6), Böhm sensilla (Bo), one subtype of sensilla basiconica (Ba.1), two subtypes of sensilla coeloconica (Co.1 – 2), two subtypes of sensilla placodea (Pl.1 – 2), pores and peculiar folds were described. The two sexes did not show any significant differences in the occurrence and number of the sensilla placodea, known to be responsible for the pheromone reception. Instead, some sexual differences were found on the occurrence and topology of three different microstructures: (1) one subtype of sensillum chaeticum (Ch.2) occurring on the pedicel only in males; (2) a characteristic pore occurring on the funicle only in males; (3) a peculiar fold occurring on different antennomeres of the funicle in the two sexes, on the fourth in males and on the fifth in females. A comparison between sensilla of *O. eremita* and those of other Scarabaeoidea is provided. *Microsc. Res. Tech.* 00:000–000, 2016. © 2016 Wiley Periodicals, Inc.

INTRODUCTION

Scarab beetles have lamellate antennae with a distinctive club composed of the last three to seven antennomeres. These segments are modified into flattened plates, called lamellae, and can be expanded like a fan or folded together, to protect their inner surfaces where many sensilla occur. The inner surfaces of lamellae are the best known part of the scarab beetle antenna, considered the most interesting part of this structure, due to their olfactory function, in all the tribes of scarab beetles studied so far (Cetoniini, Dynastini, Hopliini, Melolonthini, Rutelini, Coprini, and Geotrupini) (Table 1).

Osmoderma eremita (Scopoli, 1763) (Fig. 1) is a cetoniid beetle belonging to the functional group of saproxylic organisms, that is, species that depend, during at least some parts of their life cycle, upon wounded or decaying woody material from living, weakened or dead trees (Alexander, 2008; Speight, 1989; Stokland et al., 2012). It is considered one of the most endangered inhabitants of old hollow trees in European deciduous forests (Ranius et al., 2005), being listed in

the IUCN Red List of Threatened Species (Niето and Alexander, 2010) and protected under the European Union's Habitats Directive as a priority species of community interest (Anonymous, 1992). Moreover, *O. eremita* is considered both an indicator of saproxylic species richness and a keystone species (Jönsson et al., 2004; Ranius, 2002a). The main threat affecting local populations of *O. eremita* is habitat loss, that is, the loss of veteran trees, that are particularly vulnerable mainly due to intentional removal for logging, land clearing and agricultural intensification (Lindenmayer et al., 2012).

In the last few decades, several studies have been carried out to investigate different aspects of the biology of *O. eremita*, such as the population ecology (Chiari et al., 2013a; Larsson and Svensson, 2009;

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

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TABLE 1. Occurrence of various types and subtypes of sensilla on the antennal lamellae in different tribes of superfamily Scarabaeoidea according to literature data

Tribe	Species	Coelocoinica			Placodea		References
		Co.1	Co.2	Other subtype	Pl.1	Other subtype	
Cetoniini	<i>Cotinis nitida</i>	coelocoinica	-	-			-
	<i>Pachnoda interrupta</i>	Grooved-peg	Smooth-peg	-	Smooth placodea	Grooved placodea ^a	Placodea
	<i>Pachnoda marginata</i>	Grooved-peg	Smooth-peg	-	Smooth placodea	Grooved placodea ^a	-
	<i>Pachnoda marginata</i>	Peg-shaped	Peg-shaped	-	Smooth placodea	Placodea in heterogeneous area	-
Dynastini	<i>Adoryphorus couloni</i>	Peg-like	-	-	-	Placodea with wrinkled/granu-late surface	-
	<i>Oryctes elegans</i>	-	-	-	-	Placodea without pores	-
Hopliini	<i>Oryctes rhinoceros</i>	cs1	cs2	-	SP3	SP1 with rugged plate, SP2 furrow with a ridge	-
	Different genera	-	-	-	-	PLAS I, II, III, IV, V, VI, VII	-
Melolonthini	<i>Antitrogus parvulus</i> male	-	-	-	-	With a central cup-like pore plate ^b	-
	<i>Antitrogus parvulus</i> female	-	-	-	-	-	-
Rutelini	<i>Dasylepida ishigakiensis</i>	Type IV ^b	-	Type V	-	Type I ^b , II ^b , III ^b	-
	<i>Leptotata negataria</i>	-	-	-	-	Placoid cup-shaped	-
Phylloperthini	<i>Phyllopertha anxia</i>	Coelocoinicum type I	-	Coelocoinicum type II	-	Placodeum	-
	<i>Phyllopertha obsoleta</i>	-	-	-	-	PLAS ^b	-
	<i>Phyllopertha ravida</i>	-	-	COS I, II ^b , III, IV ^b	-	PLAS I ^b , II ^b , III ^b	-
	<i>Anomala cuprea</i>	-	-	-	sm ^a , b (smooth)	ht (heterogeneous)	-
	<i>Anomala cuprea</i>	Sc	-	-	-	Pa, Pb, Pc, Pd ^a	-
	<i>Phyllopertha diversa</i>	Coelocoinica	-	-	-	-	-
Copriini	<i>Phyllopertha horticola</i>	pit-organs	Campaniform	-	Placodea ^b	-	-
	<i>Popillia japonica</i>	C/B	-	-	-	P tortoise shell-shape ^{a, b}	-
	<i>Hyamorphia elegans</i>	Sc ^b I, II, III	-	-	-	Sp in smooth surface ^b	-
Geotrupini	<i>Copris pecuarius</i>	-	-	-	-	-	-
	<i>Geotrupes auratus</i>	-	-	-	-	-	-

When the sensillum subtype in a species is homologous to one of that described for *Osmoderma eremita* in this study its name is reported in one of the relative columns, otherwise the name is set in the column of other subtype. Sensilla basicoinica and auricillica were not found in *O. eremita* but are present on the lamellae in other species. Notes on sensilla sexual dimorphism are given.

^aSensilla responsible for the pheromone reception (always placodea but with different morphological characteristics).

^bSensilla for which sexual dimorphism is reported.

Dash in a cell means that a sensillum type is not reported by the authors for the species analyzed.



Fig. 1. Male of *Osmoderma eremita*, habitus in dorso-lateral view (picture by F. Romiti). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Ranius, 2001, 2002b), the habitat preferences (Chiari et al., 2012; Ranius and Nilsson, 1997; Ranius, 2002c), the dispersal patterns (Chiari et al., 2013b; Ranius and Hedin, 2001; Svensson et al., 2011), its value as bioindicator (Ranius, 2002a), as well as the chemical ecology (Svensson and Larsson, 2008). In fact, *O. eremita* males release a sex pheromone, (*R*)-(+)- γ -decalactone, with a characteristic plum-like or peach-like scent, which attracts mainly females (Larsson et al., 2003). In *O. eremita* the antennae of males and females responded identically to (*R*)-(+)- γ -decalactone in electroantennographic (EAG) recordings, and did not show any apparent sexual dimorphism in the presence and distribution of olfactory sensilla (Larsson et al., 2003). In addition, recordings from sensilla on the inner lamellae of the antennal club (Svensson and Larsson, 2008), revealed that (*R*)-(+)- γ -decalactone sensitive olfactory receptor neurons (ORNs) are sparsely distributed over the whole surface of the antennal inner lamella in both sexes, but are mainly found in a smooth area close to the ventral edge.

Most antennal sensilla of *O. eremita* are specialized to receive chemical signals that play a fundamental role for inter- and intra-specific communication. Therefore, a detailed morpho-functional study of the antennae, preferably integrated with electrophysiological analyses, is a prerequisite to understand other behavioural and biological aspects of the species. Although some electrophysiological studies on *O. eremita* were carried out (Larsson et al., 2003; Svensson and

Larsson, 2008), its antennal sensilla were not described in detail.

The goal of this work was to improve the knowledge of the chemical communication system in *O. eremita* throughout a fine morphological analysis of the antennal microstructures by using Scanning Electron Microscopy (SEM). In particular, the aims of this work were the following:

1. To analyze, describe and illustrate morphology and topology of the antennal sensilla, pores and microsculpture on the whole antenna;
2. To analyze the presence of a possible sexual dimorphism in sensilla responsible for pheromone reception;
3. To compare the antennal microstructures found in *O. eremita* with homologous structures observed in other scarab beetles.

MATERIALS AND METHODS

Material Examined

This study is based on the analysis of six specimens (three males and three females) of *O. eremita* collected in Central Italy by using pitfall traps placed inside tree hollows or interception traps suspended from tree branches and baited with a neat racemic mixture of γ -decalactone (for further details on beetle collection see Chiari et al., 2013a). Only three specimens per sex, collected at the end of their activity (end of July/beginning of August), were used in order to avoid affecting the small Italian populations of this species. The material is preserved in Carpaneto G. M. and Di Giulio A. collections (Rome, Italy).

Scanning Electron Microscopy

For scanning electron microscopy (SEM) analysis, the antennae were removed from the specimens, kept overnight in a detergent water solution, cleaned by ultrasounds three times for ten seconds, rinsed in water, dehydrated in a graded ethanol series, and critical point-dried in a CPD 030 unit (Balzers Union, Fürstentum, Liechtenstein), gold coated in a K550 unit (Emitech Technologies, Kent, England), and examined with both Philips XL 30 and Dualbeam (FIB/SEM) Helios Nanolab (FEI Company, Eindhoven, The Netherlands) at the L.I.M.E. (Interdepartmental Laboratory of Electron Microscopy, University Roma Tre, Rome, Italy).

The FIB/SEM is equipped with two columns including one electron beam (SEM column) and one ion beam (FIB column), oriented at 52°, and focused on the same point of the sample (for further details on the FIB/SEM technique refer to Di Giulio et al., 2015 and Nurme et al., 2015).

The SEM micrographs obtained by using the standard XL 30 (especially for the antennal habitus) and the Dualbeam FIB/SEM (for close-up of the microstructures) were used to characterize size, number, distribution and morphological features of antennal sensilla (Table 2) by using the specific software Cell D SIS (Soft Imaging System GmbH, Münster, Germany).

We followed the terminology of Schneider (1964) and Zacharuk (1985) to classify the sensillum types, and the terminology of Harris (1979) to classify the

TABLE 2. Overview of morphological features of the antennal sensilla in male and female of *Osmoderma eremita*

Type	Sex	Length (μm) ^a	Diameter (μm) ^a	N ^b	Tip	Wall	Shape	Socket	Figures
Ch.1	♂	99.01 ± 27.90	5.68 ± 0.85	13	Sharp	Smooth	Brush-like	Wide	3A
		84.79 ± 37.42	6.91 ± 1.80	19	Sharp	Smooth	Brush-like	Wide	
Ch.2 short	♂	50.74 ± 19.85	6.99 ± 1.28	8	Frayed	Smooth/Rough	Hair-like	Wide	3B, 3C
		41.32 ± 15.02	7.08 ± 1.90	16	Frayed	Smooth/Rough	Hair-like	Wide	
Ch.2 medium	♂	160.41 ± 80.00	10.80 ± 5.78	7	Frayed	Smooth/Rough	Hair-like	Wide	3B, 3C, 6D
		156.36 ± 43.81	14.30 ± 3.16	7	Frayed	Smooth/Rough	Hair-like	Wide	
Ch.2 long	♂	410.06 ± 74.19	20.44 ± 3.55	11	Frayed	Smooth/Rough	Hair-like	Wide	3D
		349.43 ± 54.74	18.27 ± 2.40	15	Frayed	Smooth/Rough	Hair-like	Wide	
Ch.3 short	♂	202.97 ± 32.58	7.28 ± 0.44	8	Sharp	Smooth/Worn	Spine-like	Narrow	3C, 6C, 6D, 6E, 6F
		235.78 ± 23.56	7.27 ± 0.75	8	Sharp	Smooth/Worn	Spine-like	Narrow	
Ch.3 long	♂	434.56 ± 41.30	10.81 ± 1.50	9	Sharp	Smooth/Worn	Spine-like	Narrow	3C, 3D, 3E, 6C, 6D, 6E, 6F
		361.26 ± 41.77	9.85 ± 1.73	4	Sharp	Smooth/Worn	Spine-like	Narrow	
Ch.4	♂	21.91 ± 5.14	4.29 ± 0.58	11	Splitted	Smooth	Cone-like	Narrow	3F, 6C, 6D
		29.66 ± 14.19	4.48 ± 0.88	8	Splitted	Smooth	Cone-like	Narrow	
Ch.5	♂	69.67 ± 26.73	5.84 ± 1.19	22	Sharp	Smooth	Pin-like	Narrow	4A, 4B, 4C, 6A, 6B
		41.79 ± 14.53	5.74 ± 1.26	21	Sharp	Smooth	Pin-like	Narrow	
Ch.6	♂	84.54 ± 12.69	10.52 ± 1.48	12	Sharp	Smooth	Cone-like	Narrow	4B, 4D, 6A, 6B
		76.66 ± 11.88	11.17 ± 0.88	19	Sharp	Smooth	Cone-like	Narrow	
Bo	♂	23.06 ± 9.20	4.48 ± 0.55	8	Sharp	Smooth	Thorn-like	Wide	3A
		24.84 ± 7.11	4.18 ± 0.59	9	Sharp	Smooth	Thorn-like	Wide	
Ba.1	♂	3.28 ± 0.23	4.21 ± 0.14	3	Blunt	Smooth	Peg-like	Wide	3B, 4E, 4F, 6E
		3.43 ± 0.47	3.88 ± 0.73	5	Blunt	Smooth	Peg-like	Wide	
Co.1	♂	—	7.42 ± 0.83	41	—	Grooved	Onion-like	Wide	5A, 5D, 5F
		—	8.03 ± 1.03	38	—	Grooved	Onion-like	Wide	
Co.2	♂	—	6.67 ± 0.38	14	—	Smooth	Dome-like	Wide	4A, 5D, 5F
		—	6.70 ± 1.22	22	—	Smooth	Dome-like	Wide	
Pl.1	♂	—	12.05 ± 1.13	56	—	Porous	Oval	—	4A, 5A, 5B, 5C
		—	10.77 ± 1.00	65	—	Porous	Oval	—	
Pl.2	♂	—	12.07 ± 1.87	91	—	Porous	Nearly circular	—	5A, 5D, 5E
		—	11.99 ± 1.58	84	—	Porous	Nearly circular	—	

^aLength and diameter are expressed as mean ± standard deviation. Diameter was measured at the base of the sensillum.

^bNumber of sensilla measured.

TABLE 3. Values of the morphological traits for the two sexes and results of the Wilcoxon rank-sum test to investigate whether the sample means between sexes in *Osmoderma eremita* are significantly different

Morphological trait	Male	Female	W	p
	(mean ± SD)	(mean ± SD)		
Elytron length (mm)	18.27 ± 1.03	17.25 ± 0.43	1	0.20
Surface L1 (mm ²)	1.26 ± 0.09	1.00 ± 0.05	0	0.10
Surface Pl.1 (L1) (mm ²)	0.24 ± 0.01	0.20 ± 0.04	1	0.20
N. of sensilla Pl.1 (L1)	727 ± 38	789 ± 151	6	0.70
Surface Pl.2 (L1) (mm ²)	0.44 ± 0.02	0.40 ± 0.03	1	0.20
N. of sensilla Pl.2 (L1)	2324 ± 124	1227 ± 81	0	0.10
Surface L2 (mm ²)	1.07 ± 0.09	0.97 ± 0.11	2	0.40
Surface Pl.1 (L2) (mm ²)	0.20 ± 0.02	0.22 ± 0.09	5	1.00
N. of sensilla Pl.1 (L2)	614 ± 75	864 ± 369	6	0.70
Surface Pl.2 (L2) (mm ²)	0.40 ± 0.03	0.40 ± 0.06	3	0.70
N. of sensilla Pl.2 (L2)	2105 ± 159	1221 ± 169	0	0.10
Surface L3 (mm ²)	0.92 ± 0.07	0.84 ± 0.11	2	0.40
Surface Pl.1 (L3) (mm ²)	0.18 ± 0.01	0.16 ± 0.04	3	0.66
N. of sensilla Pl.1 (L3)	532 ± 6	622 ± 151	6	0.70
Surface Pl.2 (L3) (mm ²)	0.41 ± 0.01	0.41 ± 0.03	3	0.70
N. of sensilla Pl.2 (L3)	2161 ± 8	1245 ± 106	0	0.10

W is the value of the Wilcoxon statistic and p is the probability value for each test. None of the comparisons resulted statistically significant ($p < 0.05$).

L1: first lamella of the antennal club; L2: second lamella of the antennal club; L3: third lamella of the antennal club.

Pl.1: sensillum placodeum subtype 1; Pl.2: sensillum placodeum subtype 2.

types of microsculptures. We also referred to classifications reported in other papers on antennal sensilla of Coleoptera Scarabaeidae (Ågren, 1985; Kim and Leal, 2000; Leal and Mochizuki, 1993; Renou et al., 1998).

Data Analysis

Morphological traits measured and used in the analyses were the elytron length (measured with an

electronic calliper, precision 0.05 mm) as a proxy of the body size, the surface of the three lamellae, the surface occupied by sensilla placodea on each lamella and the number of sensilla placodea for each lamella (Table 3). To compare these morphological traits between males and females the Wilcoxon rank-sum test was applied, instead to investigate significant differences in the number of sensilla placodea between the three lamellae and within each sex the Kruskal-Wallis ANOVA test was applied. Analyses were performed using the program R version 3.1.2 (R Core Team, 2014).

RESULTS

Gross Morphology of the *Osmoderma eremita* Antenna

The antenna of *O. eremita* shows a lamellicorn shape (Figs. 2A and 2B) typical of the Scarabaeiformia (Meinecke, 1975) and it is composed of ten antennomeres (Figs. 2A and 2B), modified and arranged in four distinctive functional parts from base to apex: scape (= scapus), pedicel (= pedicellum), funicle (= funiculum) and a lamellate club (= clava). The latter is formed by the last three antennomeres, modified into three plates (= lamellae) that form a fan-like sensorial structure.

Scape (SC) (length 1.40 ± 0.01 mm, both sexes; Figs. 2A and 2B): a twisted, pipe-like antennomere, expanded at base and apex, and sub-basally constricted, articulated basally with the antennal fossa by a globular condyle.

Pedicel (P) (length 400 ± 1 μm , in male; 440 ± 10 μm in female; Figs. 2A, 2B, 6C, and 6D): a mushroom-shaped antennomere, characterized by a bulged,

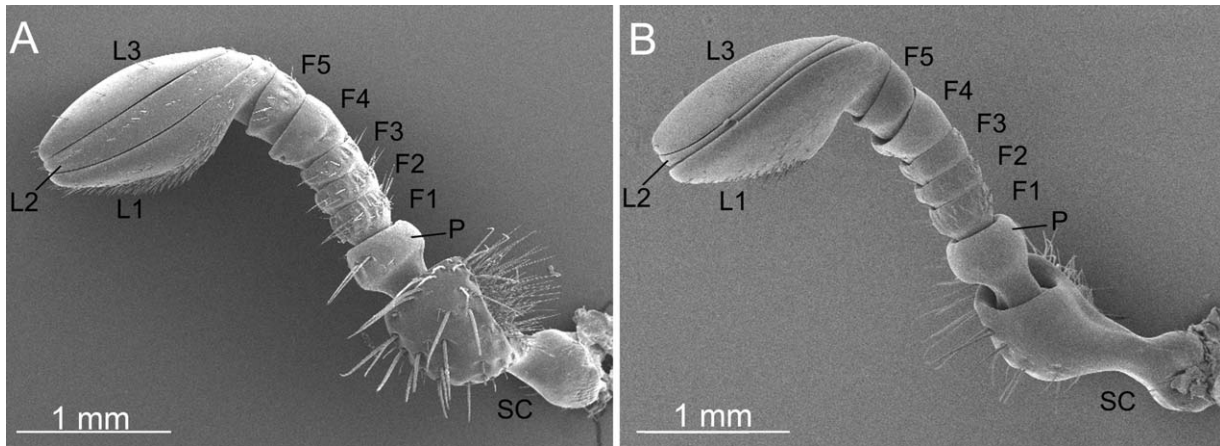


Fig. 2. SEM micrographs of male *Osmoderma eremita* general antennal shape: (A) right antenna, dorsal view; (B) left antenna, ventral view. SC: scape; P: pedicel; F1–F5: antennomeres of the funicle; L1–L3: lamellae of the antennal club.

cylindrical distal part, and a thin elongate base, loosely inserted inside the scape, giving a high mobility to the articulation between scape and pedicel.

Funicle (F1–F5) (length 1.30 ± 0.03 mm in male, 1.30 ± 0.07 mm in female; Figs. 2A, 2B, 6C, and 6D): composed of five cup-shaped antennomeres which give support and mobility to the terminal club; F1–F3 are small, cylindrical, subequal, tightly stuck to one another; F4–F5 are larger, widely transverse, asymmetrical, the anterior part being wider than the posterior.

Lamellate club (L1–L3) (maximum length 1.70 ± 0.10 mm in male, 1.30 ± 0.05 mm in female; Figs. 2A, 2B, 4A, 4B, 5A, 6A, and 6B): formed by three elongated and plate-like antennomeres; outer surface of L1 and L3 strongly convex; inner surface of L1 and L3, and both surfaces of L2 slightly concave or flattened; L2 very thin on ventral side, slightly but distinctly thicker on dorsal side. In resting position, the lamellae are closely pressed to one another, well protecting their inner sensory surfaces.

The cuticular microsculpture varies significantly between different portions of the antennae. Most of the antennal surface is covered by a more or less deeply reticulate microsculpture, composed of polygonal, partly imbricate meshes. The condyle of the scape shows a spinulate sculpture with appendages of cuticular plates (ACP) (Fig. 3A), while its sub-basal portion is glabrous and smooth. On the edges of the lamellae, the reticulate microsculpture presents transverse and elongated wrinkles (Fig. 4A), while both inner and outer surfaces of the lamellae are glabrous and smooth among the sensilla placodea (Figs. 5A and 5F).

Fine Structure and Distribution of Sensilla

We identified six different subtypes of sensilla chaetica (Ch.1 – 6), Bohm sensilla (Bo), one subtype of sensilla basiconica (Ba.1), two subtypes of sensilla coeloconica (Co.1 – 2) and two subtypes of sensilla placodea (Pl.1 – 2).

None of the considered morphological traits (the elytron length, the surface of the three lamellae, the occupied surface and the number of sensilla placodea on

the three lamellae) resulted to be significantly different between sexes (Table 3). Moreover, the mean number of sensilla placodea of both subtypes (Pl.1 and Pl.2) do not differ significantly on the three lamellae within each sexes (Table 4), meaning that their number is similar on the three lamellae in both sexes (Table 4).

Sensilla Chaetica Subtype 1 (Ch.1)

Short and stout, brush-like sensilla (Fig. 3A; Table 2), with the basal part of the stem almost straight or slightly curved, medial and apical part irregularly frayed by thin projections with sharp tips. The whole surface of the sensillum is smooth. These bristles appear prostrate toward the antennal surface and are inserted in a deep wide socket, generally with one cuticular pore close to the base. They are distributed on the condyle of the scape, dorsally and ventrally, among the Bo (Fig. 3A).

Sensilla Chaetica Subtype 2 (Ch.2)

Hair-like sensilla (Figs. 3B–3D and 6D; Table 2), elongated, flattened, and tapered towards the apex, which can be straight or curved but frayed at tip. The basal stem is smooth and stout inserted in a wide socket with one cuticular pore associated, and emerging at about $60\text{--}90^\circ$ from the antennal surface. The medial and distal portion of the hair is characterized by an irregularly worn, rough microsculpture. These hairs are inserted in pits of the antennal cuticle. Though the general shape is quite constant, the length and bearing of such hairs varies significantly between different positions, enabling recognition of three size classes (short, medium-sized, long) (Table 2). There is a tendency for increasing hair length towards the apex of the scape. These hairs are distributed on the dorsal sub-apical portion of the scape, among the Ba.1 (Fig. 3B), and on the pedicel (Fig. 6D). The pedicel of the females has no sensilla chaetica (Fig. 6C), while that of males shows medium-sized Ch.2, single or in couple, located in the dorso-lateral surface (Fig. 6D).

Sensilla Chaetica Subtype 3 (Ch.3)

Spine-like sensilla (Figs. 3C, 3E, and 6C–6F; Table 2), straight and tapered towards the apical part, which is thin and sharp, with the base inserted in a shallow pits with a narrow socket. The stem is smooth basally and worn-looking on the rest of the surface. These sensilla project from the antennal surface at 45°–90°. They are arranged in a crowded group of about 50 sensilla, and located on the lateral side of the scape. These bristles are also spread dorsally and laterally on the funicle F1–F5 (Figs. 6C–6F).

Sensilla Chaetica Subtype 4 (Ch.4)

Cone-like sensilla (Figs. 3F, 6C, and 6D; Table 2), set in a narrow socket and associated with one cuticular pore. Their stem is basally smooth and apically provided with small and short pointed projections, tapered toward the apex. They are inserted in pits of the antennal cuticle, emerging at 90° from the antennal surface, distributed through the dorsal and lateral sides of the funicle, mostly on F1 and F2 (Figs. 6C and 6D), and scattered on F3–F5.

Sensilla Chaetica Subtype 5 (Ch.5)

Pin-like sensilla (Figs. 4A–4C, 6A, and 6B; Table 2), straight and tapered toward the apex, sharp-tipped, almost smooth-walled emerging at 90° from the antennal surface. Their base is tightly set in a deep socket, sometimes with one pore associated. These sensilla often occur in pairs and are distributed dorsally on the external edges of the three lamellae.

Sensilla Chaetica Subtype 6 (Ch.6)

Cone-like sensilla (Figs. 4B, 4D, 6A, and 6B; Table 2), relatively short, strong, smooth-walled and sharp-tipped sensilla. They are densely arranged in one group (about 60–70 sensilla) on the outer surface of the first lamella, forming altogether a brush-like structure. The shape of each sensillum is conical or spiniform, emerging 45–60° from the cuticular surface, distinctly bent distally towards the surface, and abruptly tapered at the sharp apex. The base is stout, inserted in a tight, adherent socket. One cuticular pore is associated with some of these sensilla.

Böhm Sensilla (Bo)

Thorn-like bristles (Fig. 3A; Table 2), smooth-walled, sharp-tipped, straight or slightly curved, emerging almost at 90° from the antennal surface, set in wide socket. They are present around the base of scape and pedicel.

Sensilla Basiconica Subtype 1 (Ba.1)

Thick and short conical pegs (Figs. 3B, 4E, 4F, and 6E; Table 2), smooth-walled, deeply inserted in a wide round socket and showing an apical pore. They are present in both sexes on the dorsal sub-apical portion of the scape, scattered among the Ch.2. In females they are also present dorsally on the fourth antennomere of the funicle interspersed with pores (Fig. 6E).

Sensilla Coeloconica Subtype 1 (Co.1)

Short onion-like pegs (Figs. 5A, 5D, and 5F; Table 2), sunken in a wide circular socket. The cuticular

walls of the sensillum present longitudinal grooves. They occur exclusively on the inner surface of all lamellae, in both sexes and are frequently associated with Co.2, both scattered among the Pl.2 (Fig. 5D), and contribute to create a heterogeneous region in the chemo-sensorial areas on the inner surface of lamellae (Fig. 5A).

Sensilla Coeloconica Subtype 2 (Co.2)

Dome-like pegs (Figs. 4A, 5D, and 5F; Table 2), sunken in a circular socket. They are distributed exclusively on the inner surface of all lamellae where they are associated with Co.1. Both are scattered among Pl.2 and contribute to create a heterogeneous region in the chemo-sensorial areas (Fig. 5A). They are also present in pairs on the borders of the outer surface of L3, scattered between Pl.1.

Sensilla Placodea Subtype 1 (Pl.1)

Oval plate sensilla (Figs. 4A and 5A–5C; Table 2), with sensory cuticle nearly smooth and flat, at the same level of the antennal surface, without a surrounding furrow. Their cuticular surface presents a small central pore and a series of radially aligned pores. They are densely distributed (about $44 \cdot 10^2$ sensilla/mm²), arranged in a homogenous chemo-sensorial area (Fig. 5A) on the inner surface of L1 and L3, and on both surfaces of L2. In L3 they also occur on the outer surface. On the inner surfaces of all the lamellae in the medial part, Pl.1 are sharply substituted by Pl.2 (Fig. 5A). Only some of them are scattered in the heterogeneous area among the Pl.2.

Sensilla Placodea Subtype 2 (Pl.2)

Nearly circular plate sensilla (Figs. 5A, 5D, and 5E; Table 2), surrounded by a furrow. The flat surface shows some secretion most probably extruding from fine pores occurring on it (Fig. 5E). They occur in both sexes and are densely distributed (about $44 \cdot 10^2$ sensilla/mm²) in the heterogeneous chemo-sensorial area on the inner surface of L1 and L3, and of both surfaces of L2.

Cuticular Pores and Folds

Cuticular pores occur at the base or close to various types of sensilla mainly chaetica (Ch.1–2, Ch.4–6) but also basiconica (Ba.1) (Figs. 3A, 3F, 4A, 4D, and 4E). On the lamellae both on the inner surfaces and on their outer edge the pores present diameters vary from 0.70 to 2.50 µm (Figs. 4A and 4D). The latest structures are more abundant on the inner surfaces of the all lamellae in the heterogeneous area, scattered among Co.1, Co.2, and Pl.2 (Fig. 5D). In males, a big pore (about 16 µm diameter) (Fig. 6D) occurs on the ventral portion of F4. Instead in females, on F4 a group of four pores of different diameters (2.0–4.5 µm) occur on the dorsal surface (Fig. 6E). In both sexes, peculiar folds formed by invaginated cuticle are present on the lateral surface of the antennomeres, F4 in male and F5 in female (Figs. 6E and 6F).

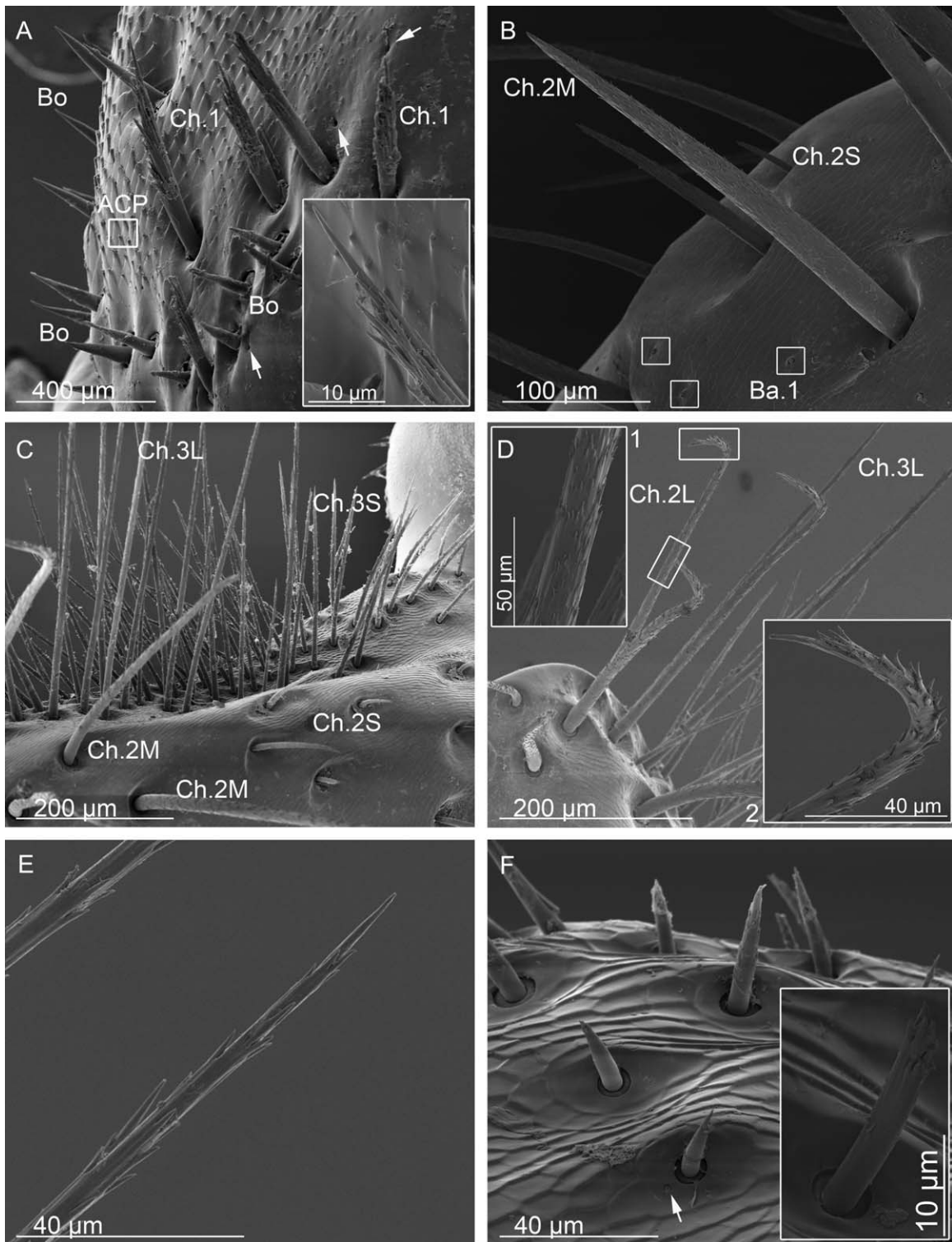


Fig. 3. SEM micrographs of *Osmoderma eremita* antennal sensilla occurring on the scape, pedicel and funicle: (A) condyle of the scape showing appendages of cuticular plates (ACP), sensilla chaetica Ch.1 and Böhm sensilla Bo interspersed with pores (white arrows), the insert shows the sharp tip of sensillum Ch.1; (B) dorsal view of the distal part of the scape showing the reticulate microsculpture, sensilla chaetica Ch.2 of short (Ch.2S) and medium (Ch.2M) size and sensilla basiconica Ba.1; (C) dorso-lateral view of the apical portion of

the scape showing sensilla chaetica Ch.2 of short (Ch.2S) and medium (Ch.2M) size and Ch.3 of short (Ch.3S) and long (Ch.3L) size; (D) dorso-lateral view of the apical portion of the scape showing long sensilla chaetica Ch.3L and long sensilla chaetica Ch.2L having rough walls (insert 1) and curved tips (insert 2); (E) detail of long sensilla chaetica Ch.3; (F) lateral margin of antennomere F1 showing sensilla chaetica Ch.4 magnified as insert and a pore occurring at base (white arrow).

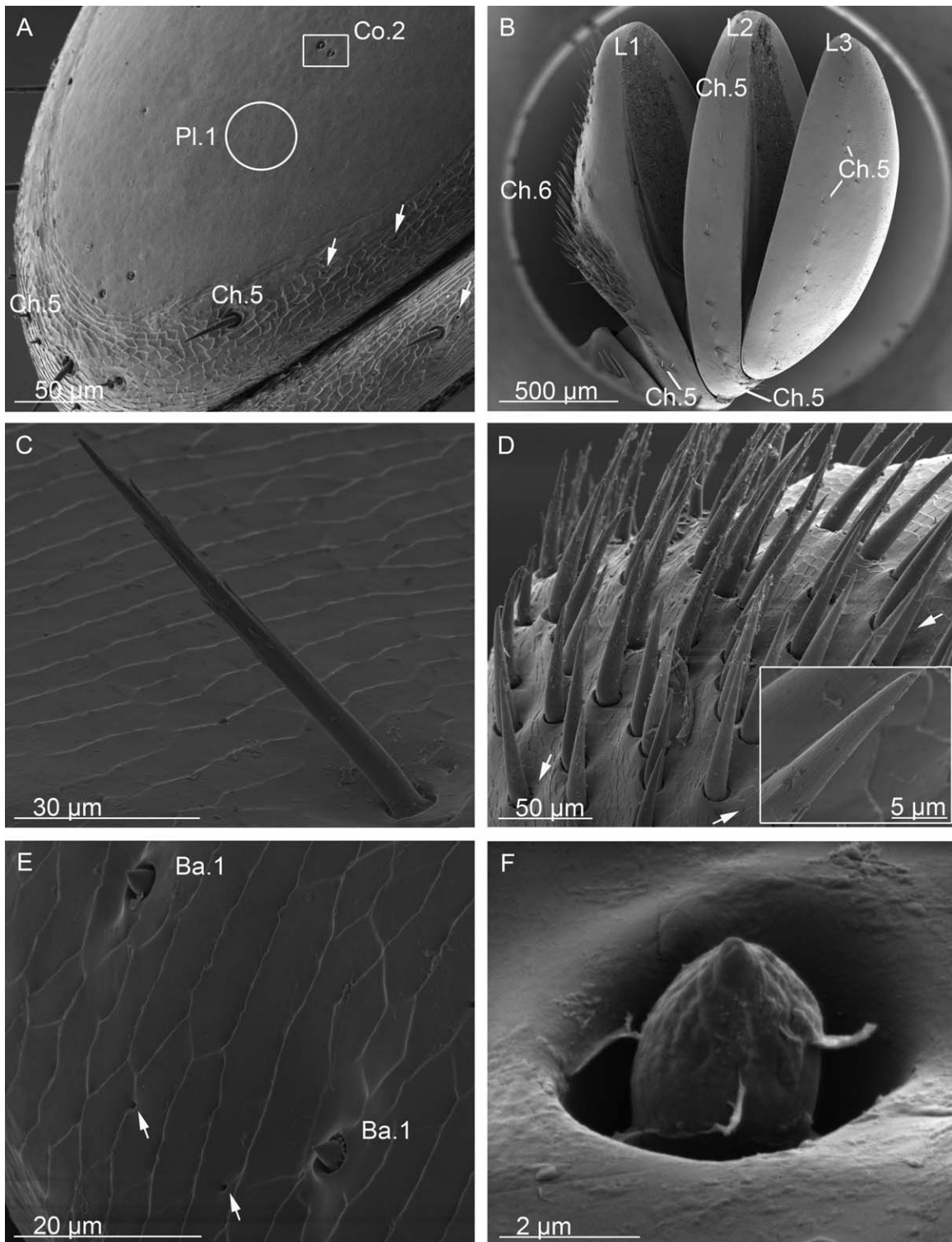


Fig. 4. SEM micrographs of *Osmoderma eremita* sensilla occurring on the antennal club and scape: (A) outer surface of the third lamella (L3) in ventral view showing on the edge the reticulate microsculpture with transverse and elongated wrinkles, sensilla chaetica Ch.5 and pores (white arrows). On the central portion of the lamella are present sensilla placodea Pl.1 and some sensilla coeloconica Co.2, in this portion the surface is smooth; (B) general view of the female right antennal club composed by three lamellae (L1-L3), on dorsal

view, showing on the outer surface of L1 sensilla chaetica Ch.6 and on the external edges of L1-L3 sensilla chaetica Ch.5; (C) detail of sensillum chaeticum Ch.5; (D) outer surface of first lamella (L1) of the antennal club showing sensilla chaetica Ch.6, densely arranged in a brush-like structure, with interspersed pores (white arrows). The insert shows a magnified sensillum chaeticum Ch.6; (E) sensilla basiconica Ba.1 and pores (white arrows) on apical part of the scape; (F) magnified sensillum basiconicum Ba.1.

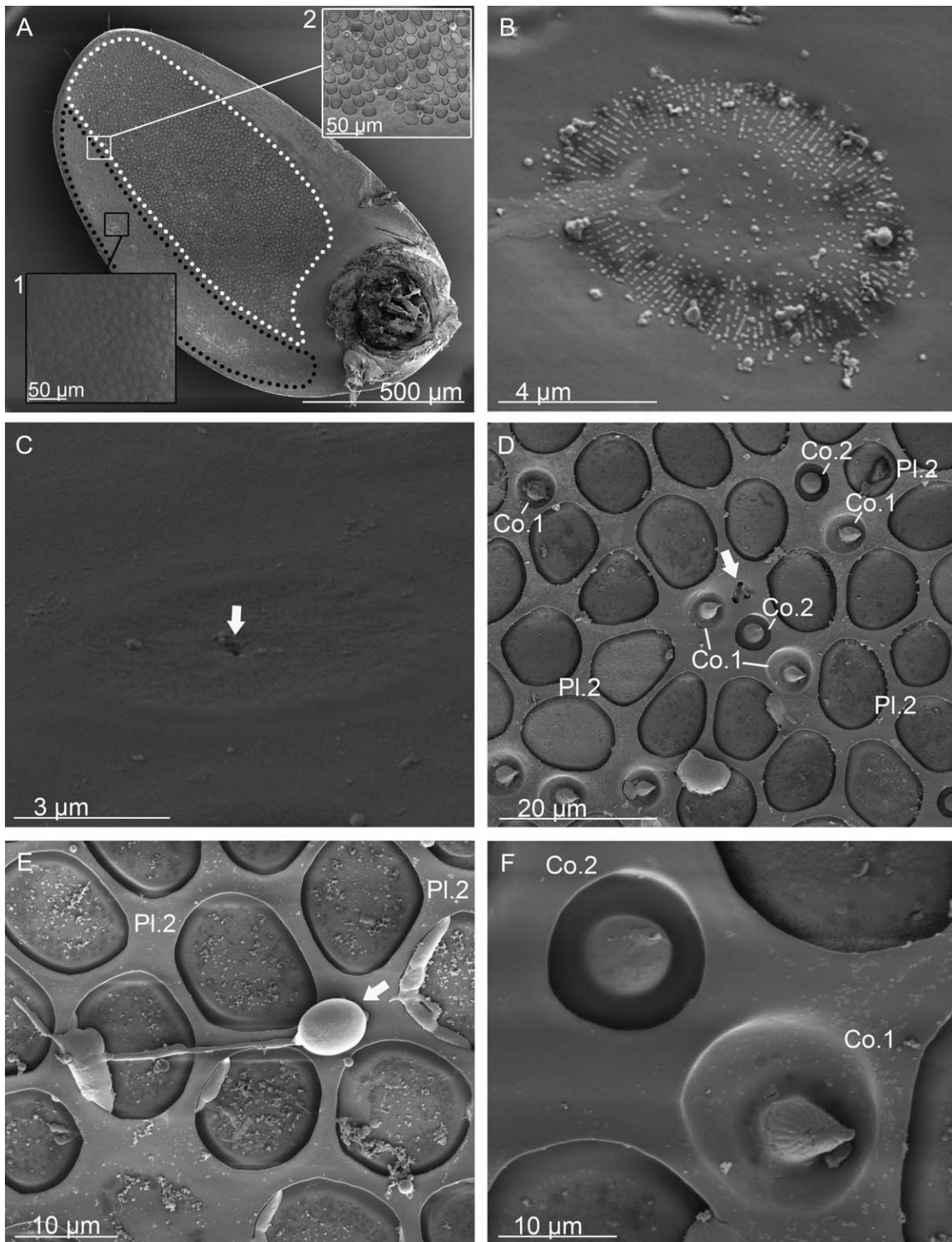


Fig. 5. SEM micrographs of *Osmoderma eremita* sensilla occurring on the antennal club: (A) inner surface of the lamella L1, where it is possible to identify two areas: a homogenous one, more ventral, with sensilla placodea Pl.1 (insert 1) and a heterogeneous one, more dorsal, comprising mainly sensilla placodea Pl.2 and some scattered Pl.1, sensilla coeloconica Co.1, Co.2 and pores (insert 2); (B) sensillum placodeum Pl.1 covered with secretion extruding from pores on its surface; (C) a sensillum placodeum Pl.1 from the inner surface of lamella

L1, its surface is smooth and flat, a pore occurring at its center is shown (white arrow); (D) heterogeneous sensorial area on the inner surface of L2 with sensilla placodea Pl.2, sensilla coeloconica Co.1, Co.2 and pores (white arrow); (E) sensilla placodea Pl.2 with secretions extruding from fine pores occurring on their surface; other secretions coming out from pores located among Pl.2 are showed (white arrow); (F) detail of sensilla coeloconica Co.1 and Co.2, from the inner surface of lamella L2.

TABLE 4. Results of the Kruskal-Wallis ANOVA test for differences in the number of sensilla placodea subtype 1 (Pl.1) and subtype 2 (Pl.2) between the three lamellae (L1, L2, L3) within each sex in *Osmoderma eremita*

Sensilla placodea subtype	Sex	F	p
Pl.1	Male	11.97	0.10
	Female	0.76	0.51
Pl.2	Male	2.85	0.14
	Female	0.03	0.97

F is the value of the Kruskal-Wallis ANOVA statistic and p is the probability value for each test. None of the comparisons resulted statistically significant ($p < 0.05$).

DISCUSSION

Gross Morphology

The antenna of *O. eremita* is very similar in male and female. The inner surfaces of L1 and L3 are not flat but a little concave forming two narrow chambers between them and L2, as was observed also by Ågren (1958), in the sagittal section of the male antennal club of *Phyllopertha horticola*.

In both sexes, L2 shows a dorso-ventral asymmetry being very thin in ventral view. A similar asymmetry on the club was observed by using a stereo microscope (Olympus SZ11, maximum magnification 110x), in other European Cetoniinae of the genera *Valgus*, *Gnorimus*, *Trichius*, *Tropinota*, *Oxythyrea*, *Cetonia*, *Protætia*, and *Aethiessa* (G.M. Carpaneto, Personal communication). Another trait shared by *O. eremita* and the previously cited genera is the chaetotaxy strongly reduced over the ventral surface of the antenna, which is almost glabrous. Therefore, the reception of external stimuli should mainly occur in the dorsal side of the antenna. A third trait shared by these beetles is the high density of bristles forming an irregular fringe on the outer lateral surface of the scape.

Fine Morphology

Sensory bristles (Ch. 1–6) are the most widespread sensilla, more or less densely occurring in all the antennomeres of *O. eremita*. They do not present deep external modification from the typical bristles described by Schneider (1964). The most modified bristles are Ch.1, present at the base of the scape, interspersed among the Bo, with thin projections extruding from their surface. We can infer from their position that Ch.1 co-operate with the Bo to mechanical proprioception. Up to now, these sensilla were never described in scarab beetles. Concerning the Ch.2 we found a wide variability in length. The absence of TEM data prevents us from making hypothesis on their function, although the involvement in the reception of mechanical stimuli is very likely.

The third subtype of sensory bristle, Ch.3, occur on the lateral side of the scape and on the funicle of *O. eremita*. A dense group of straight and long bristles, as a fringe on the lateral side of the scape occurs in Dynastini (*Oryctes rhinoceros*), Melolonthini (*Antitrogus parvulus* and *Lepidiota negatoria*) and Rutelini (*Popillia japonica* and *Phyllopertha horticola*) (Ågren, 1985; Allsopp, 1990; Kim and Leal, 2000; Renou et al., 1998). These bristles are likely proprioceptors providing the beetles with information about the position of the

antenna with respect to the head such as when the antennae are brought toward the side these bristles can come in contact with an area near the eye. The fourth subtype of sensory bristle, Ch.4, the smallest subtype detected, is widespread on the funicle of *O. eremita* and similar bristles were not observed in other scarab beetles. The sensory bristles Ch.5, are spread on the external edges of lamellae in *O. eremita*. Observing the micrographs published in the literature, similar sensory bristles occur on the edges of lamellae in many species: *Pachnoda interrupta* and *P. marginata* (Bengtsson et al., 2011; Stensmyr et al., 2001), *Adoryphorus couloni* (McQuillan and Semmens, 1990), *Oryctes elegans* (Al-Dorsay, 2009), *Oryctes rhinoceros* (Renou et al., 1998), *Amorphochelus retusus* (Romero-López et al., 2013), *Antitrogus parvulus* (Allsopp, 1990), *Dasylepida ishigakiensis* (Tanaka et al., 2006), *Lepidiota negatoria* (Allsopp, 1990), *Anomala cuprea* (Leal and Mochizuki, 1993), *Phyllopertha diversa* (Hansson et al., 1999), *Phyllopertha horticola* (Ågren, 1985), *Popillia japonica* (Kim and Leal, 2000). The occurrence of these sensory bristles in several groups of Scarabaeidae throughout the continents led us to suggest that this bristle type has a very important function. Their function may involve mechanoreception, but somehow linked to the mechanism deputed to open lamellae exposing their inner surface to olfactory cues. The bristles Ch.6 form a brush-like structure on the outer surface of L1, densely clumped as Ch.2 on the scape. Similar bristles occur also in *Oryctes elegans* (Al-Dorsay, 2009), *Oryctes rhinoceros* (Renou et al., 1998) and *Phyllopertha horticola* (Ågren, 1985) in the same antennal area.

Böhm sensilla, described for the first time in Lepidoptera (Böhm, 1911) are typical bristles found at the base of the scape and at the base of the pedicel, and most probably are homologous in all insects (Schneider, 1964). In *O. eremita* Bo are distributed both at the base of the scape and the pedicel as expected. This distribution pattern of Böhm bristles is common in all beetles with flagellar antennae such as in Carabidae (Merivee et al., 2000, 2001, 2002) and Elateridae (Merivee, 1992; Merivee et al., 1998, 1999).

Only one subtype of sensilla basiconica, Ba.1, occurs on the antenna of *O. eremita*, commonly in association with Ch.2 on the apex of the scape in both sexes, and also with pores arranged in a transverse row across F5 only in females. Structures homologous to Ba.1 were found in ground beetles (Carabidae), in *Paussus favieri* (Di Giulio et al., 2012) and in *Platynus dorsalis* (Merivee et al., 2001). Unfortunately, the lack of ultrastructural data in the mentioned studies makes it difficult to infer about their function. In contrast with other studies regarding scarab beetles, in which sensilla basiconica have been found on the inner surface of the lamellae (Table 1), in *O. eremita* these sensilla are completely absent from the club. Sensilla coeloconica and placodea, are found only on the lamellate club of *O. eremita*. In other studies regarding Cetoniini, Hopliini, Melolonthini, Coprini and Geotrupini on the lamellae have also been found sensilla basiconica and similarly in Hopliini and Melolonthini sensilla auricillica have been described (Table 1). Overall, the lamellate club seems to be a successful structure in the evolutionary history of scarab beetles, showing a

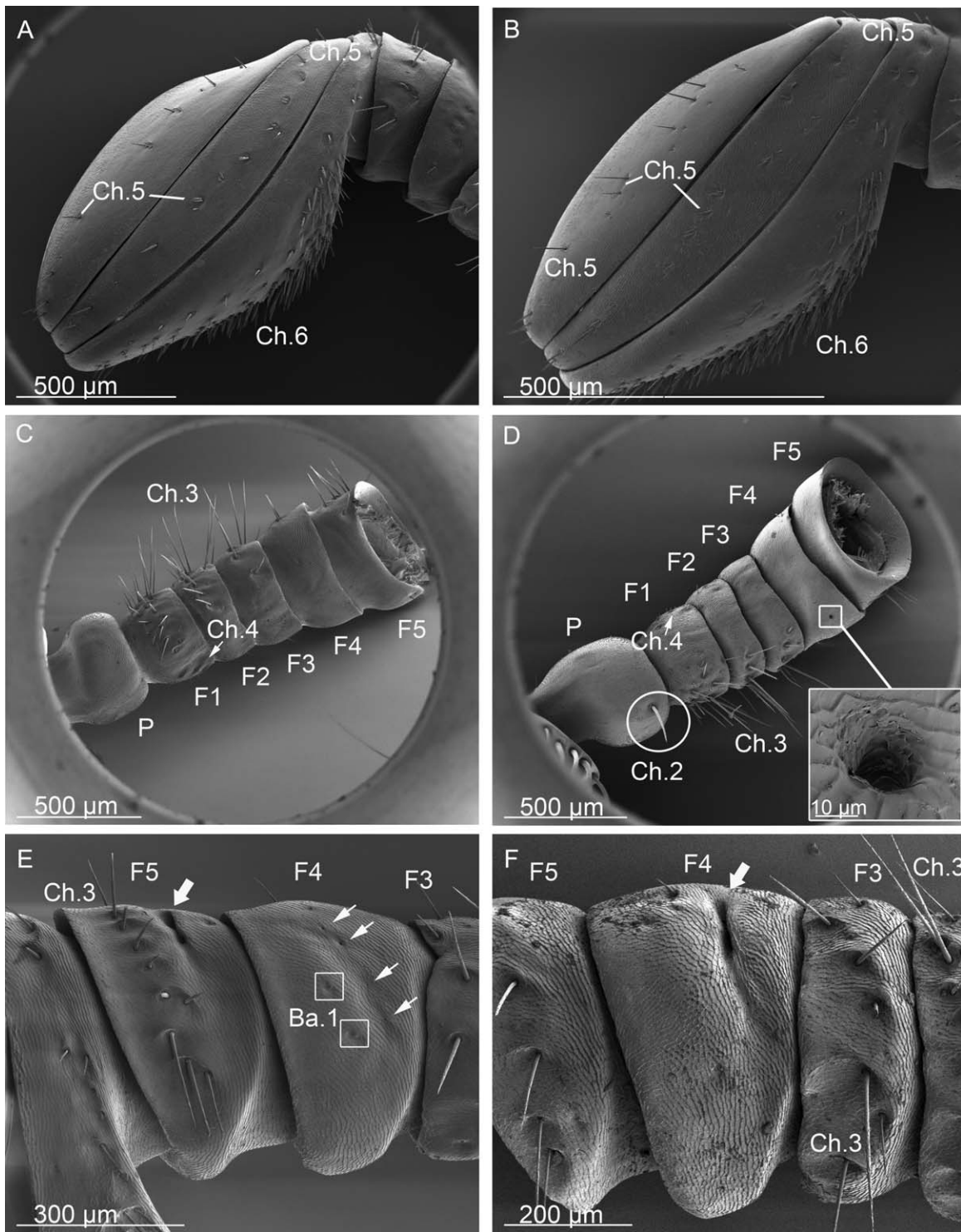


Fig. 6. SEM micrographs showing a comparison of characters between sexes of *Osmoderma eremita* (A, C, E female; B, D, F male): (A) female antennal club in dorsal view showing sensilla chaetica Ch.5 on the external edge of the lamellae L1-L3, and Ch.6 grouped on L1; (B) male antennal club in dorsal view showing sensilla chaetica Ch.5 on the external edge of the lamellae L1-L3, and Ch.6 grouped on L1; (C) female pedicel without any elongated sensilla chaetica Ch.2 and antennomeres of the funicle (F1-F5) in ventro-lateral view, showing the reduced number of sensilla in ventral portion; (D) male

pedicel and antennomeres F1-F5 in ventro-lateral view showing a Ch.2, a reduced number of sensilla in ventral portion and a big pore (about 16 μm diameter) (white rectangle) on F4, magnified as insert; (E) female antennomeres F3-F5 in dorso-lateral view showing sensilla chaetica Ch.3, a group of four pores (small arrows), two sensilla basiconica Ba.1 on F4 (white rectangles), and a peculiar dorsal fold (big arrow) on F5; (F) male antennomeres F3-F5 in dorso-lateral view showing sensilla chaetica Ch.3 and a peculiar dorsal fold on F4 (white arrow).

steady structure with minor changes in some apomorphic versions.

Sensilla Co.1 and Co.2 in *O. eremita* occur in low number on the inner surfaces of lamellae, in close association with the sensilla placodea. These peg-like sensilla are very similar to those described in other scarab as in Cetoniini, Dynastini, Melolonthini, and Rutelini (Table 1). In other scarab beetles it was suggested an olfactory function for these pegs (Ochieng et al., 2002), even if Kim and Leal (2000) excluded the possibility of being pheromone receptors.

Pl.1 and Pl.2 are probably homologous to those described in other scarab beetles of different subfamilies and tribes. As revealed by the study of Svensson and Larsson (2008) in *O. eremita*, as well as in other scarab beetles (Table 1), these sensilla placodea are pheromone receptors. In particular for *O. eremita* an electrophysiological study on L2 (Svensson and Larsson, 2008) showed that γ -decalactone-sensitive ORNs occur mainly in the smooth area close to the ventral edge of the lamella, corresponding to the area where there is the maximum concentration of Pl.1. Also in other species, sensilla homologous to Pl.1 are mainly concentrated in the ventral portion of each lamella, as in Cetoniini, that is, *Pachnoda interrupta* and *P. marginata* (Stensmyr et al., 2001; Bengtsson et al., 2011), and Rutelini, that is, *Anomala cuprea*, *Phyllopertha diversa*, *Phyllopertha horticola*, and *Popillia japonica* (Ågren, 1985; Hansson et al., 1999; Kim and Leal, 2000; Larsson et al., 2001; Leal and Mochizuki, 1993).

The sensorial areas located in the inner lamella surfaces are protected, because these beetles are able to fold the lamellae forming a compact club at rest. However the ability to perceive olfactory stimuli is not entirely lost when the club is closed, because the outer surface of L3 with Pl.1 is still exposed (Ågren, 1985).

In most scarab beetles, as in the generality of insects, sex pheromones are released by the females to attract males, and therefore the number of pheromone-sensitive sensilla (placodea, in case of scarab beetles) is much greater in males (Kim and Leal, 2000; Leal, 1998). A similar sexual dimorphism seems to be a rule in Rutelini (Ågren, 1985; Kim and Leal, 2000; Mutis et al., 2014) and Melolonthini (Allsopp, 1990; Romero-López et al., 2004, 2010) (Table 1), but is not observed in *O. eremita*. Different density and distribution of olfactory sensilla in various species may reflect different ways in the use of sexual pheromones for intraspecific communication. In general, as reported by some authors (Allsopp, 1990; McQuillan and Semmens, 1990), the long-range attraction of pheromones is correlated with a strong sexual dimorphism in both density and diversity of sensilla involved in pheromone reception. On the contrary, the lack of sexual dimorphism is associated with short-range attraction and to close-range sexual encounters, for example at feeding sites where individuals congregate (Allsopp, 1990; McQuillan and Semmens, 1990). Larsson et al. (2003) hypothesized that the γ -decalactone of *O. eremita* could function as an intraspecific long-distance odour signal, attracting both males and females, but the range of attraction and the mating behaviour are still to be clarified.

Traits of sexual dimorphism in *O. eremita* are mainly in pedicel and funicle being not directly

involved in the pheromone reception. In the males the pedicel shows one or two Ch.2 while F4 has a wide pore in ventro-lateral position. These traits are absent in females where instead a series of four smaller pores were found in dorso-lateral position of F4. A big pore (16 μ m) as that found in male F4 has never been described in scarab beetle antennae. Even if the main function of the insect antennae is sensorial, glandular activity has been described for some species and in some cases identified as the site of the pheromone secretion (Bartlett et al., 1994; Bin and Vinson, 1986; Bin et al., 1999; Di Giulio et al., 2009; Isidoro et al., 1999; Romani et al., 2005; Skilbeck and Anderson, 1994). At present, abdominal pheromone glands in scarab beetles have only been described from three taxonomic groups (Rutelinae, Melolonthinae, Dynastinae) (Hoyt et al., 1971; Kim and Leal, 2000; Leal, 1998; Rochat et al., 2000; Romero-López et al., 2009; 2011; Tada and Leal, 1997). The possible connection of these large antennal pores with antennal glands and the potential significance of the secretions should be object of further TEM investigations.

Another type of pores was observed in *O. eremita* at the base of Ch.1, near the proximal end of the scape; some authors inferred that their secretion may serve to reduce friction between sclerotized parts (Martin, 1977; Skilbeck and Anderson, 1994).

To conclude, *O. eremita* do not show any sexual dimorphism for the sensilla placodea, that previous studies demonstrated to be responsible for the pheromone reception, and this match the fact that both sexes are attracted by the pheromonal compound (*R*)-(+)- γ -decalactone (Larsson et al., 2003). Even if the low number of specimens examined (three for each sex) cannot give a reliable description of individual variation, it was however possible to describe new structures for scarab beetles, that is, two subtypes of sensilla chaetica (Ch.1 and Ch.4), a big pore on the antenna of males, and peculiar folds on the funicle. Finally, this study on the fine morphology of *O. eremita* antennae helps to illustrate the connection between structures and behavior in a rare and protected species.

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