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Ecology and evolution of detachable setae in dermestid beetles: implications in pest control and public health

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Padua, 27 September 2021 Enrico Ruzzier

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Contribution of Authors

The present PhD thesis is the combination of three papers published in peer-reviewed international journals. I was the first author and principal researcher for collecting and analyzing the results under the supervision of Prof. Battisti.

Prof. Kadej, Dr. Muzzi and Prof. Di Giulio were supervisors who helped collect data and supported me with the research.

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Riassunto

Le setole staccabili sono setole che possono essere facilmente rimosse attraverso una semplice stimolazione meccanica e che sono evolute quale struttura difensiva contro la predazione in almeno quattro gruppi di artropodi. Questa categoria di setole è suddivisa in due gruppi morfo-ecologici: peli urticanti in Lepidotteri (Nodotontidae: Thaumetopoeinae, Erebidae, Saturniidae, Zygenidae) e Theraphosidae (sottofamiglia Theraphosinae), e setole "ad ancora" in Polyxenidae millepiedi (Myriapoda): Polyxenidapoda e larve di Dermestidae (Insecta: Coleoptera).

Le *hastisetae* sono un gruppo specifico di setole staccabili che caratterizzano le larve dei Megatominae (Coleoptera: Dermestidae). Queste setole sono presenti sia sui tergiti toracici che addominali della larva e costituiscono una difesa primaria contro predatori invertebrati. Le *hastisetae*, singole od aggregate, fungono da trappola biomeccanica in grado di intrappolare processi cuticolari (spine e peli) ed appendici (antenne, zampe).

Si ritiene che questo sistema difensivo si sia evoluto principalmente per contrastare la predazione da parte di invertebrati, tuttavia è stato osservato come le *hastisetae* possano riguardare anche i vertebrati. Sebbene le informazioni sugli impatti verso predatori vertebrati siano limitate, le *hastisetae* si sono dimostrate essere una possibile minaccia per la salute umana in qualità di contaminanti di prodotti (cibi e tessuti), di ambienti lavorativi ed abitazioni; in particolare, l'esposizione alle *hastisetae* sembrerebbe causare reazioni allergiche e l'insorgenza di eruzioni cutanee, asma, congiuntivite ed infiammazione dell'apparato digerente.

Una revisione della letteratura passata e recente sulle larve di dermestidi ha rivelato come, nonostante le *hastisetae* siano suggerite come uno dei caratteri distintivi nell'identificazione delle specie, si sappia molto poco sulla loro ultrastruttura, evoluzione e meccanismo d'azione. Inoltre, nonostante il loro apparente interesse medico, non si conosce praticamente nulla circa la loro nocività rispetto a quanto noto per le setole urticanti di lepidotteri e tarantole.

La presente ricerca definisce lo stato dell'arte circa le conoscenze sulle *hastisetae* e si prefigge di colmare le lacune conoscitive esistenti.

La prima parte del lavoro è dedicata all'indagine della morfologia esterna e microstrutturale delle *hastisetae*; vengono descritte ed illustrate in dettaglio l'inserzione su tegumento, pedicello, fusto e parte apicale dell'*hastiseta*; inoltre, per la prima volta vengono documenti comportamenti di difesa attiva delle larve basati su *hastisetae*.

Summary

Detachable setae are true setae that can be easily removed with any kind of mechanical stimulation and that evolved as a defensive structure against predation in at least four groups of Arthropoda. The class is subdivided in two main morpho-ecological groups: urticating hairs of Lepidoptera (Nodotontidae: Thaumetopoeinae, Erebidae, Saturniidae, Zygenidae) and the spider family Theraphosidae (subfamily Theraphosinae), and anchor-like setae of Polyxenidae millipedes (Myriapoda: Polyxenida) and larvae of Dermestidae (Insecta: Coleoptera).

Hastisetae are a specific group of detachable setae characterizing the larvae of Megatominae (Coleoptera: Dermestidae). These setae are located on both thoracic and abdominal tergites and they are the primary defence of the larva against invertebrate predators.

Hastisetae, single or aggregate, function as an extremely efficient mechanical trap, based on an entangling mechanism of cuticular structures (spines and hairs) and body appendages (antennae, legs and mouthparts). It is believed that this defensive system evolved primarily to contrast predation by invertebrates, however it has been observed that hastisetae may affect vertebrates as well. Although information on the impacts of vertebrate predators of the beetles is limited, hastisetae have been shown to be a possible threat for human health as an important contaminant of stored products (food and fabric), work and living environment; in particular, the exposure to hastisetae seems to cause allergic reactions and the insurgence of skin rashes, asthma, conjunctivitis, and digestive system inflammation.

A review of past and recent literature on dermestid larvae has revealed that despite these structures are vaguely indicated as one of the distinctive characters in species identification, very little is known about their ultrastructure, evolution, and mechanism of action. Furthermore, despite their apparent medical interest almost nothing is known about their harmfulness in comparison to urticating hairs of lepidoptera and tarantulas.

The present work provides the state of knowledge on hastisetae in Dermestidae and develops a research line intended to bridge the existing knowledge gaps.

The first part of the research is dedicated to the investigation of the fine morphology of the external microstructure of the hastisetae; the insertion on integument, the pedicel, the shaft, and the apical head are described and illustrated in detail, and the first observations of active defensive behavior based on hastisetae are recorded and presented.

The second part is devoted to the ultrastructure investigation of the hastiseta with a major focus on the socket and subcuticular structure with the intent to investigate and shed a light on a possible secretory function associated with hastisetae.

Possible implications to the systematics of skin beetles are proposed based on the results of the study. Furthermore, a new morphological and functional interpretation of the hastiseta and the associated cellular is provided, suggesting possible future research developments.

Chapter 1

Introduction and aims

The cuticle plays a pivotal role in several aspects of arthropod biology, representing the interface between the living tissue and the external environment (Bereiter-Hahn et al. 1984). Thus, the cuticle displays structural specializations such as denticles, setae, setulae and spines, all with specific functions (Winterton, 2009). Correlations between structure and function are well studied especially in insects (Neville, 1975) and crustaceans (Garm, 2004a; Garm, 2004b; Garm & Watling, 2013). Setae are multicellular protuberances on the cuticle, used primarily for mechanoreception (Keil & Steinbrecht, 1984; Keil, 1997; Winterton, 2009; Barth, 2004). In all groups of arthropods, the role of setae has evolved from simple mechanoreception to various other functions, including defense (Battisti et al. 2011), locomotion (Labarque et al. 2017), prey capture (Felgenhauer et al. 1989), pheromone dispersal (Steinbrech, 1984), sexual display (Perez-Miles et al. 2005), preening (Felgenhauer et al. 1989), and camouflage (Zeledón et al.1973; Hultgren & Stachowicz, 2008).

Detachable setae are true setae characterized by the loss of the neural connection and the detachment of the base of the hair from the integument (Battisti et al. 2011). The proximal end of each seta is attached to an integument stalk or inserted into a socket and can be easily removed with any kind of mechanical stimulation. This class of hairs has evolved as a defensive structure against predation at least four times in Arthropoda. The class is subdivided in two main morpho-ecological groups: urticating hairs and anchor-like setae. Urticating hairs are typical of some Lepidoptera families such as the Nodotontidae (subfamily Thaumetopoeinae), Erebidae, Saturniidae and Zygenidae and the spider family Theraphosidae (subfamily Theraphosinae) (Battisti et al. 2011) and are described to protect from vertebrate predators (Battisti et al. 2011; Bertani & Guadanucci, 2013). Anchor-like setae are typical of some larvae of Dermestidae (Insecta: Coleoptera) and Polyxenidae (Myriapoda: Polyxenida) where they work as entangling mechanism against invertebrates (Nutting & Spangler, 1969; Eisner et al. 1996). Dermestid detachable setae (hastisetae) are used by the larvae as an active trapping system against arthropod predators (Nutting & Spangler, 1969). These specialized setae are almost exclusively prerogative of Megatominae, the most diverse group in the entire family (Háva, 2015). The mechanism of action of hastisetae and their microstructure remains largely obscure and restricted to few case studies (Nutting & Spangler, 1969; Mills & Partida, 1976);

furthermore, how the evolution of hastisetae is related to the biological success of the Megatominae remains unresolved. Although information on the impacts of hastisetae on vertebrate predators is lacking, dermestid larvae and Megatominae in particular have been documented as possible source of allergens in human (Mullen & Durden, 2009). Hastisetae and integument fragments carrying them can be contaminants of stored commodities and are present in working and living environments (Hinton, 1945). Hastisetae seem to be involved in allergic reactions through skin contact, ingestion or inhalation; symptoms can vary accordingly to exposition and consist of skin rushes, asthma, conjunctivitis and digestive system inflammation (Gorgojo et al. 2015; MacArthur et al. 2016). Correlation between the presence of hastisetae and the incidence of allergies in humans exists but the scarce and incomplete information available do not allow to consider hastisetae as a major hazard in living and working places.

The aim of this research is to synthesize the knowledge on the hastisetae of dermestid beetles and to explore hastisetae diversity, providing more information about their morphology and to investigate their possible usage in undertaking systematic and evolution of Megatominae. In particular, the research aimed to characterize the fine morphology of hastiseta, identifying those traits useful for its function against other invertebrates and to clarify if and how these setae may affect humans. Furthermore, knowing their important roles as contaminants and pests, this research focused its attention on selected genera to identify possible morphological traits useful for their identification using hastisetae Finally, future perspectives on the study of the hastisetae are envisaged.

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Chapter 2

Occurrence, ecological function and medical importance of dermestid beetle hastisetae

Survey methodology

In order to compile and then review the most exhaustive literature on hastisetae we performed a careful and reiterated research in Google Scholar and Scopus through the use of keywords such as ‘‘hastisetae’’, ‘‘Dermestidae’’, ‘‘defence’’, ‘‘larva’’, integrated by the usage the Boolean operators AND, OR, NOT and the use of ’’ ’’ for specific word combinations. The literature not available online has been recovered thanks to Network Inter-Library Document Exchange (NILDE), a web-based software for the service of Document Supply and Inter-Library Loan, managed by the Italian National Research Council. Our research has enabled the collection of more than a hundred publications, of which ninety were considered in the realization of this review. The library created was comprehensive of literature in English, German and French.

Hastisetae, structure and function

Hastisetae (or hastate setae) have been cited in several papers dealing with Dermestidae systematics (Rees, 1943; Kiselyova & McHugh, 2006), species identification (Booth et al.1990; Peacock, 1993), and product contamination (Bousquet, 1990). However, the amount of information available concerning their microstructure (Elbert, 1976; Elbert, 1978), function (Nutting & Spangler, 1969; Mills & Partida, 1976) and evolution (Zhantiev, 2000; Kiselyova & McHugh, 2006) is quite scarce. These hairs, located on the dorso-lateral surface of the tergites of larvae (Figure 1) (Rees, 1943; Beal, 1960; Kiselyova & McHugh, 2006; Kadej, 2012a; Kadej, 2012b; Kadej, Jaroszewicz & Tarnawski, 2013; Kadej & Jaroszewicz, 2013; Kadej & Guziak, 2017; Kadej, 2017; Kadej, 2018a; Kadej, 2018b), are generally quite small with an estimated length, according to the literature, between 150 and 900 µm. Density and distribution of the hastisetae vary substantially not only among genera and species but also among tergites of the same species. The hastisetae of the thoracic segments are generally scattered and in low numbers in respect to the other parts of the body. While the abdominal tergites present a wider distribution pattern, from hastisetae covering the major part the tergal disc up to proper setae fields located at the posterior corners of tergites (i.e., Reesa, Trogoderma). In some larvae, the hastisetae give origin to real tufts of hairs located on the posterior corners of the terga IV–VII (i.e., *Ctesias*) or V–VII (i.e., *Anthrenus*) (Mroczkowski, 1975; Kadej & Jaroszewicz, 2013; Kadej, 2017; Kadej, 2018a; Kadej, 2018b). The hastisetae are inserted in setal sockets on the integument and are connected to the tormogen cell trough the pedicel (Elbert, 1978). The pedicel is the breaking point of the shaft which allows the detachment of the hastiseta (Elbert, 1978). Hastisetae microstructure consists of two main parts: the shaft and the apical head (Figure 1). The shaft is long and filiform, subcylindrical in section. It is made by repeated modules, from 5 to 77, each of them constituted by one cylindrical segment provided with one wreath of spines/scales in the distal part (Elbert, 1978). These spines/scales are postero-laterally oriented and can vary in number from five to seven (Elbert, 1978). The last module of the shaft is generally bigger and thicker than the previous and can slightly vary in general shape to the others; this structure, however, has not been characterized yet. The head of the seta is a subconical anchor-like, spear-shaped structure subdivided longitudinally in sections; the apex of the head is blunt (Elbert, 1976; Elbert, 1978) (Figure 1).

The head consists of five to seven longitudinal, circularly arranged, elements separated from each other by one deep groove, connected to the stem in the upper half by cross-bracing and free in the lower part. The ‘‘anchor-like head’’, set against the thorns of the last shaft module, is involved in entangling invertebrate body parts (Nutting & Spangler, 1969), functioning as trap for antennae, legs, mouthparts, setae and spines (Mills & Partida, 1976). This structure is apparently species specific, varying in shape and length between taxa (Elbert, 1976; Kiselyova & McHugh, 2006; Kadej & Jaroszewicz, 2013; Kadej, 2017; Kadej, 2018a). The shaft allows setae to cluster together amplifying the ‘‘trapping’’ effect and the spines increases friction and entangling among hastisetae and between setae and body parts. The combined action of several hastisetae affects small predators (Nutting & Spangler, 1969) and possibly food competitors (Kokubu & Mills, 1980). These setae are hollow (Elbert, 1976; Elbert, 1978) and could potentially contain proteins or other chemicals involved in the defense, as it has been shown in Lepidoptera (Battisti et al. 2011).

Hastisetae morphology and distribution, combined together with other characters, constitute a useful tool for species identification (Rees, 1943; Beal, 1960; Peacock, 1993; Kadej, 2012a; Kadej, 2012b; Kadej & Jaroszewicz, 2013a; Kadej & Jaroszewicz, 2013; Kadej & Guziak, 2017; Kadej, 2017; Kadej, 2018a; Kadej, 2018b).

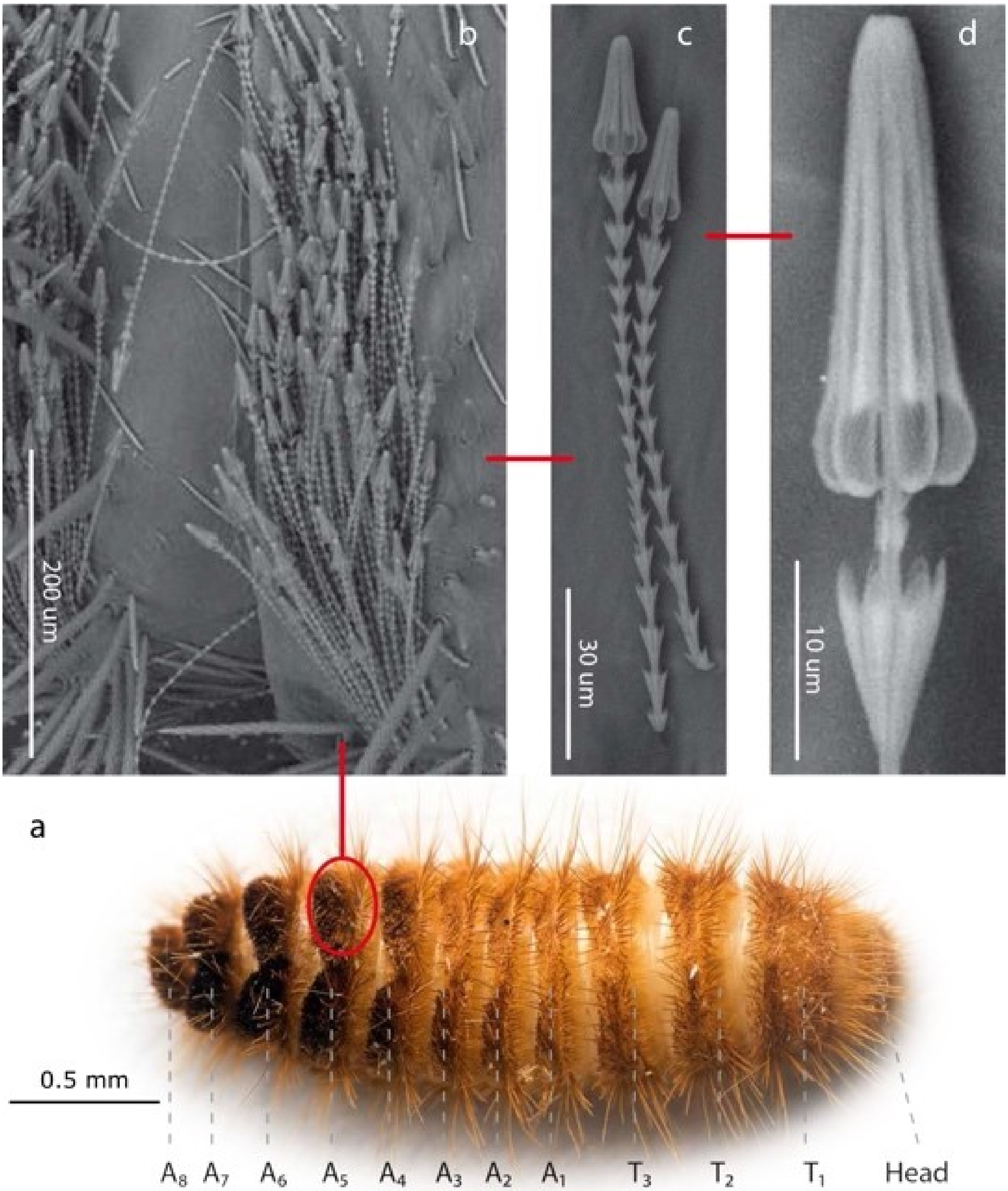

Figure 1: Hastisetae structure and distribution on Megatominae larvae (general scheme). (A). Example of Megatominae larva (*Megatoma undata* (Linnaeus, 1758)), dorsal view. T1–T3: thoracic segments; A1–A8: abdominal segments. (B). Tuft of hastisetae on abdominal segments. (C). Hastisetae, lateral view. (D). Head of the hastiseta (subconical anchor-like, spear-shaped head).

Hastisetae in the systematic and ecology of Dermestidae

Dermestidae is a cosmopolitan, comparatively small family of Coleoptera, regarded as 'a well-defined, monophyletic group' (Lawrence & Newton, 1982), consisting of six subfamilies: Orphilinae, Thorictinae, Dermestinae, Attageninae, Trinodinae and Megatominae (Háva, 2015) (Figure 2). Dermestids are homogeneous only in general appearance, hiding a complex and rich diversity in term of morphological, ecological and ethological aspects. Specific traits and evolutionary tendencies could be observed in several lineages, associated to ecological groups and niches (Zhantiev, 2009); these traits can be observed at adult (Zhantiev, 2000) and larval stage (Kiselyova & McHugh, 2006).

Orphilinae are mycetophagous, with sclerotized burrowing larvae (Lenoir et al. 2013). Thorictinae are myrmecophilous and larvae protection is provided by the associated ant species (Lenoir et al. 2013).

Dermestinae, the basal group of the ''necrophagous clade'' (*sensu* Zhantiev, 2009), have larvae feeding on fresh or relatively humid animal remains (over 15% in water content) (Zhantiev, 2009). Since Dermestinae food resource is highly perishable, the larvae develop rapidly and persist only for short periods. The oblong, sub-cylindrical and sclerotized larvae of this subfamily can dig through the feeding substrate and live in butyric fermentation condition, under animal remains. It is plausible that the absence of hastisetae on larval tergites is directly attributable to their burrowing lifestyle. Anchor-like detachable setae could be disadvantageous to move within the substrate. Hastisetae would in fact create friction and would be systematically lost, requiring an important energy expenditure necessary for their replacement. The defensive strategy in Dermestinae is based on the fast escape behaviour and the sclerotized integuments of the body. The larvae specifically require the pupation chamber to molt and they are capable to dig into soil and/or substrate in case of lacking suitable places where to hide. The pupae of this subfamily present gin-traps on the integuments, as a defensive system against predators (Hinton, 1946; Kiselyova & McHugh, 2006) (Figure 2).

Attageninae have burrowing larvae associated to wood dust, fissures of rocks and sandy environments and feed off of insects and other arthropods remains; the larvae are oblong-fusiform with integuments covered of three different kind of hairs (Zhantiev, 2000; Kiselyova & McHugh, 2006). The larvae show a fast escape behaviour, similar to Dermestinae. Attageninae prefer to pupate in hidden niches and the pupae bear gin-traps in most of the cases (Zhantiev, 2000). Trinodinae are inquiline of animals' nets: rodent borrows with larvae phoretic on mammal (Zhantiev, 2009) or larvae associated to spider nests (Beal, 1959; Kadej, 2012c).

The hastisetae, with the single exception of the genus Trinodes (Trinodinae), in which modified hastisetae are described (Kiselyova & McHugh, 2006), are prerogative of the Megatominae larvae and they are strictly associated to larval and pupal morphology and behaviour (Kiselyova & McHugh, 2006; Zhantiev, 2009) (Figure 2).

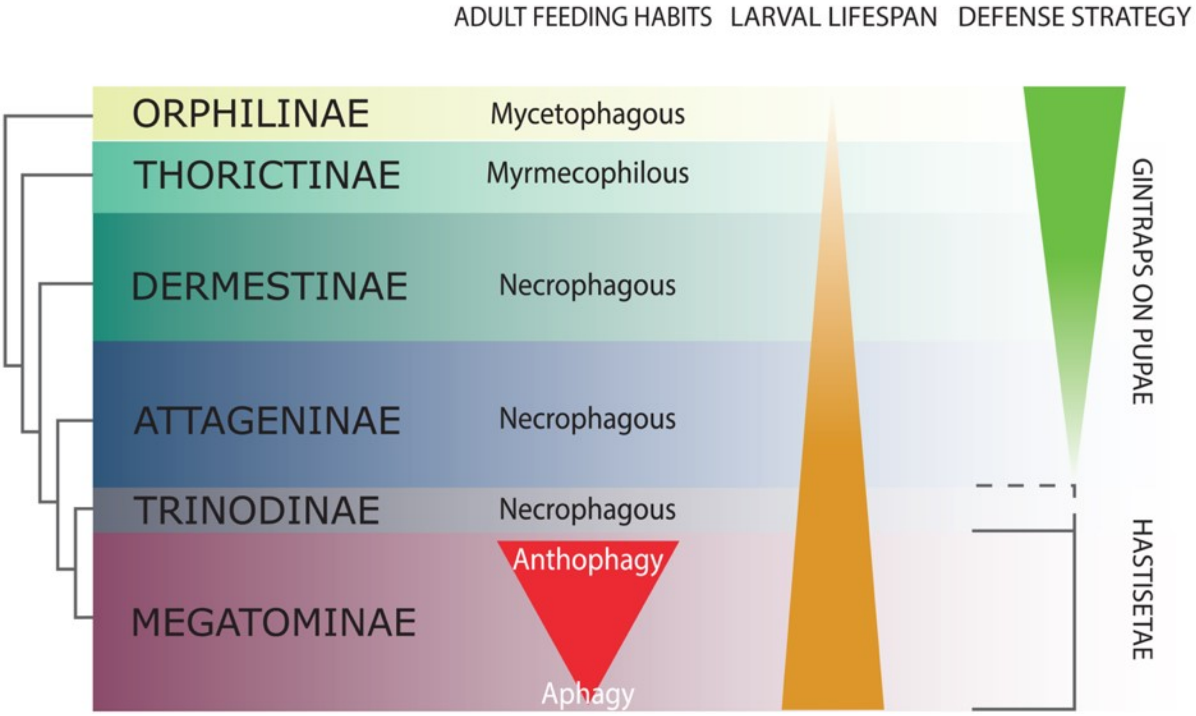

Figure 2: Schematic representation of Dermestidae phylogeny (based on Kiselyova & McHugh, 2006), with an indication of feeding habits of the adult beetles, duration of larval lifespan, and larval-pupal defensive structures. The size of the coloured bands in each subfamily is an approximated representation of the number of species.

Megatominae is the richest in species subfamily within Dermestidae and its biological success is most probably attributable to the hastisetae occurrence. Amber fossils indicate that hastisetae morphology is highly conserved and remained virtually unchanged since late Cretaceous (Poinar Jr & Poinar, 2016). This group shows a remarked investment on hastisetae as a defensive tool (Nutting & Spangler, 1969; Mills & Partida, 1976), exploiting their resistance and durability over time to protect both larvae and pupae (Kiselyova & McHugh, 2006; Zhantiev, 2009). Megatominae is the clade within the xerophilous necrophagous dermestids (*sensu* Zhantiev, 2009), which can survive on low-water food resources, especially chitinous and keratinous remains (Armes, 1990; Beal, 1998; Zhantiev, 2009). These substrates are capable to stand in the environment for a long time but the poor nutrients prolong the duration of larval development, with major implications on morphology, ethology and defensive behaviour. Lengthening of the larval phase and its persistence in the environment for a long time has promoted the evolution of morphological and ethological features in Megatominae that otherwise would have been disadvantageous in a different lifestyle.

The inability of the larvae to delve into the living substrate (Zhantiev, 2009) favoured the evolution of defensive structures (hastisetae) with low energy investment for their synthesis and to remain functional even after being dispersed in the environment. Over time, energetic investment in cuticularized integuments in larvae and gin-traps in pupae shifted to the morphology of hastisetae and its defence mechanisms.

Hastisetae provide protection in both larvae and pupae, favouring a positive energy trade-off in larval development. All the larvae of this subfamily are stout, feebly sclerotized, slow moving and present an aggressive, non-escaping defensive behaviour (Kiselyova & McHugh, 2006). In a disturbance, the larva stops moving, arches its body and spread the hastisetae, frequently from the posterior part of the body where it is densely packed with hastisetae towards the stimulus (Kiselyova & McHugh, 2006). In general, Megatominae do not make pupation chambers or hide, but simply pupate where they have been feeding. Pupae completely lack gin-traps and remain protected inside the last larval exuvia, completely covered in hastisetae (synapomorphy of Trinodinae + Megatominae) (Kiselyova & McHugh, 2006) (Figure 2). Megatominae have been able to adapt against interspecific and intraspecific competition for food resources.

A common trait associated with the evolution of the hastisetae in the dermestids is, in the necrophagous clade, the transition from scavenger habits of adults to anthophily or aphagy (Zhantiev, 2009) (Figure 2).

Hastisetae and human health

The capability to feed on a wide range of food resources scarce in water content and to resist to prolonged starvation makes Megatominae larvae the perfect candidate to inhabit working and living spaces. In addition, due to their slow movements and cryptic behaviour these larvae result difficult to detect and remove. For this reason, some species are now synanthropic and cosmopolitan (Bouchet et al. 1996; Gamarra et al. 2009), having been spread all over the world with trade. These species became serious pests, causing considerable loss and damage to stored goods of both animal and plant origin (Hinton, 1945; Burges, 1959; Kantack & Staples, 1969; Mroczkowski, 1975; Beal, 1991; Veer et al. 1991a; Veer et al. 1991b; Veer & Rao, 1995; Veer et al. 1996; Imura, 2003; Rajendran & Hajira Parveen, 2005; Lawrence & Slipinski, 2010) and to objects of organic origin in museums of cultural and natural history

(Jurecka et al. 1987; Zaitseva, 1987; Armes, 1988; Bousquet, 1990; Pinniger & Harmon, 1999; Hansen et al. 2012; Querner, 2015).

The hastisetae released by the larva throughout its entire development and abandoned in the environment in association to the exuviae are an important contaminant in dwelling, public spaces as well as food stuff (Gorham, 1979; Gorham, 1989; Burgess, 1993) and can contribute as allergens in humans (Wiseman et al. 1959; Johansson et al. 1985; Baldo & Panzani, 1988; Burgess, 1993; Pauli & Bessot, 2009; Gorgojo et al. 2015; MacArthur et al. 2016): chitin, likely the main constituent of the hastisetae, is in fact a powerful and widely recognized allergen, and its interaction with Th2 lymphocytes and human chitinases enhances the inflammation process (Brinchmann et al. 2011; Bucolo et al. 2011; Mack et al. 2015).

However, it is still unclear whether the inflammatory effect of the hastisetae is attributable to the mechanical action of the seta and its penetration through the epithelia or if it is associated to the presence of specific molecules capable to start an immunological reaction.

Hastisetae have been directly linked to occupational diseases in working environments (Loir & Legagneux, 1922; Renaudin, 2010), especially when processing organic materials such flour, wool, silk and other commodities (Veer et al. 1996; Brito et al. 2002), or stored objects of organic origin in museums and art galleries (Siegel et al. 1991). The exposure to and inhalation of hastisetae, even in the form of dust, are reported to cause rhinoconjunctivitis (Brito et al. 2002) and asthma (Cuesta-Herranz et al. 1997; Brito et al. 2002; Bernstein et al. 2009). Megatominae are also one of the arthropod groups most commonly recorded inside houses (Gamarra et al. 2009; Bertone et al. 2016; Madden et al. 2016); the larvae persist in these environments for months, even for years, feeding on food (Gorham, 1979; Gorham, 1989; Hirao, 2000), pet food (Rudolph et al. 1981), dust, insect remains and clothes, especially wool fabric (Bouchet et al. 1996). This prolonged presence inside houses together with the persistence of the hastisetae in the environment greatly increase the possibility for the humans to come into contact and develop a sensitization to these detachable hairs (Wiseman et al. 1959; Ayres & Mihan, 1967; Kaufman et al. 1986; Burgess, 1993; Jakubas-Zawalska et al. 2016). The direct exposure of hastisetae to the skin, maybe due to contaminated bed or clothes, causes severe dermatitis (Sheldon & Johnston, 1941; Cormia & Lewis, 1948; Okumura, 1967; Ahmed et al. 1981; Alexander, 1984; Johansson et al.1985; Southcott, 1989; Horster et al. 2002; Zanca et al. 2012; Hoverson et al. 2015; MacArthur et al. 2016), while the repeated inhalation over a longer period may cause asthma (Cuesta-Herranz et al. 1997; Brito et al. 2002; Bernstein et al. 2009).

Food contamination and hastisetae ingestion has been proved to cause the inflammation of the digestive system, manifesting through nausea, fever, diarrhoea (Hirao, 2000), proctitis and perianal itching (Krause et al. 1998). Unusual, and apparently asymptomatic findings of hastisetae have been done on sputum (Johnson & Batchelor, 1989) and cervical specimens (Bechtold et al. 1985; Bryant & Maslan, 1994; Williamson et al. 2005).

The incidence of pathologies associated with Dermestidae and Megatominae in particular, seems to be considerably reduced in recent decades probably due to the increased degree of attention regarding the presence of contaminants in food and the marked improvement in the processes of conservation and storage of raw materials; the development of adequate plans for monitoring and management of pests and the general improvement in the quality of life of people associated with greater healthiness of the houses have contributed further to the imitation of the impact (Athanassiou & Arthur, 2018). However, there is also the possibility that many domestic cases of exposure to hastisetae, especially in the case of skin rushes (erythematobullous reactions) may be under-recognized and underdiagnosed, due to similar effects to attacks by other arthropods (Burgess, 1993; MacArthur et al. 2016). Furthermore, almost all the cases reported in the medical literature regard developed countries while the effect of hastisetae on human health in developing countries remains almost obscure and widely understudied.

Undoubtedly, a better knowledge of the inflammation caused by hastisetae would allow the recommendation of appropriated prevention measures and the formation of medical personnel able to provide early diagnosis and administration of appropriate therapies. Moreover, a close collaboration between occupational physicians, entomologists and immunologists could be of great help for the development of new surveillance programs and new health and safety guidelines for workers and people most at risk.

Conclusions

The scant information about the fine morphology and the ecological roles of hastisetae, and their implications in human health opens a whole horizon of research possibilities. Hastisetae morphology is undoubtedly the starting point for any future study. The characterization of hastisetae through electron microscopy and micro-CT is the basic and fundamental step to understand their functional morphology. The identification of specific morphological traits in the hastisetae will help to solve Megatominae systematics, highlighting the evolution of these structures in relation to phylogeny and biology.

A detailed knowledge of hastisetae morphology will allow us to understand the defensive mechanism and if it acts similarly in all Megatominae. Comparing reactions of different predators to hastisetae will be useful to evaluate the different effects and particularly if this defensive system is primarily directed towards invertebrates and/or to vertebrates. Are humans or other vertebrates possible targets of hastisetae, and if so what are the causes of the unpleasant side-effects in humans? Is it the penetration of these setae trough epithelia the main cause of inflammation and are there any particular substance inducing the reaction, as it has been showed in Lepidoptera? Chemical analysis of secretions can identify and characterize the compounds responsible of the inflammation in humans and clarify their possible role as adjuvants in defence against the threats. Understanding the causes of allergic responses in humans will allow the development of specific medical therapies.

Hastisetae could become an important addition in species identification, with relevant application in forensic entomology and pest management on stored products. Furthermore, the creation of a molecular fingerprint based on hastisetae content can aid in developing tools to detect insect fragments in contaminated stored products, especially food.

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Chapter 3

Entangling the Enemy: Ecological, Systematic, and Medical Implications of Dermestid Beetle Hastisetae

Introduction

Among terrestrial arthropods, only two groups have evolved specialized setae intended to entangle predators: Myriapoda: Polyxenidae (Eisner et al. 1996) and Coleoptera: Dermestidae, Megatominae Leach, 1815. Those setae are generally referred to as hastisetae or spear-like setae (Latin, *hasta*). Hastisetae are present only on larval tergites: on the thoracic segments they are generally scattered and in low numbers in respect to the other parts of the body. Abdominal tergites present a wider distribution pattern, from hastisetae covering the major parts of the tergal disc up to proper setae fields located at the posterior corners of tergites (Ruzzier et al. 2020). Aside from the hastisetae, the tergites present thorny setae, called spicisetae.

Hastisetae of Megatominae larvae constitute a mechanical trap, entangling and possibly leading predators to death (Ruzzier et al. 2020). Hastisetae, single or aggregated, are an efficient defensive mechanism aimed to entangle hairs and body appendages of invertebrates such as antennae, legs, and mouthparts. Nutting and Spangler (1969) were the first to analyse the defensive function of hastisetae against invertebrates and vertebrates. They systematically fed, in artificial conditions, different groups of arthropods such as ants, beetles, earwigs, mantids, bugs and spiders, and then amphibians (toads), reptiles (geckos and skinks), birds and mammals (mice and squirrels) with larvae of the Megatominae beetle *Trogoderma* Dejan, 1821. Although all conditions were coercive and could hardly happen in the natural environment, they proved that the hastisetae discourage, entangle, or lead arthropod predators to death. A particular interest was the discouraging effect that these setae caused in arthropods that do not commonly prey on *Trogoderma* larvae and on toads. Reptiles, birds, and mammals apparently did not show any specific response after the exposition to *Trogoderma*, with the exception of geckos, that apparently may have died of intestinal blockage caused by hastisetae (Nutting & Spangler, 1969). In 1976, Mills and Partida (Mills & Partida, 1976), and subsequently Kokubu & Mills, 1980 observed the entanglement mechanism of hastisetae, identified in the head of the hastisetae, the main structure involved in the defence mechanism.

Although the hastisetae head is well illustrated in both papers, the true mechanism of action of this structure is only briefly discussed. It is thanks to Elbert, 1976 and Elbert, 1978 that a little more is known about the general structure and ultrastructure of hastisetae, and we are now capable of linking morphology to function. Elbert firstly provided an overview of hastisetae, comparing different species, and focused his attention on the head of hastisetae (Elbert, 1976) and setal insertion on the larval integument (Elbert, 1978). He discovered that hastisetae are hollow along their entire length and that they seem to possess an opening at the apex (Elbert, 1976; Elbert, 1978). Furthermore, the author observed how the shape and microsculpture of the seta head varies among species and genera (Elbert, 1976) and provided the first and only investigation of hastisetae insertion (Elbert, 1978).

Hastisetae are also recognized as important contaminants of stored products, work and living environments, because several dermestid beetles are synanthropic (Hinton, 1945). Particularly, hastisetae seem to be involved in allergic reactions through skin contact, ingestion, or inhalation, and causing symptoms such as skin rashes, asthma, conjunctivitis, and digestive system inflammation (Gorgojo et al. 2015; MacArthur et al. 2016). Even though hastisetae and their evolution is considered one of the key factors of the biological success of Megatominae (Zhantiev, 2000; Kiselyova & McHugh, 2006; Zhantiev, 2009), the most species-rich subfamily among Dermestidae (Háva, 2015) which constitute a non-negligible threat to human health (Mullen & Durden, 2009), their structure and mechanism of action remain largely unresolved and restricted to a few cases, as summarized in Ruzzier et al. 2020.

The purpose of this contribution is to explore hastisetae diversity, providing more information about their morphology and to investigate their possible usage in undertaking systematic and evolution of Megatominae; in particular, the research aimed to characterize the fine morphology of hastiseta, identifying those traits useful for its function against other invertebrates and to clarify if and how these setae may affect humans.

Furthermore, knowing their important roles as contaminants and pests, this research focused its attention on selected genera to identify possible morphological traits useful for their identification using hastisetae.

Material and methods

Examined Materials

The genera considered were *Anthrenocerus* Arrow, 1915, *Anthrenus* Geoffroy, 1762, *Ctesias* Stephens, 1830, *Thaumaglossa* Redtenbacher, 1867, *Megatoma* Herbst, 1791 and *Trogoderma* Dejan, 1821, for a total of 19 taxa (Table 1). Megatominae larvae are generally difficult to collect; therefore, it was complex to obtain larvae in number, especially identified to a species level. The majority of the materials used here belonged to the collection of Marcin Kadej (University of Wrocław, Poland) and have been identified by the author. Those taxa with live larvae that were available at the time of this study (*Anthrenus* spp. 1, *Anthrenus* spp. 2, *Ctesias serra* (Fabricius, 1792), *Megatoma undata* (Linnaeus, 1758), *Trogoderma* spp. 1, *Trogoderma* spp. 3) were kept in ventilated containers with dead crickets and mixed cereal flakes for food. Rearing containers were maintained at room temperature (20–22 °C). If larvae were not available, exuviae were used for the analysis.

Table 1. List of the taxa included in the study.

Species	Stage	Origin	Supp. File
Anthrenocerus australis (Hope, 1843)	Larva	Australia	S1
Anthrenus (*Anthrenus*) *latefasciatus* Reitter, 1892	Exuvia	Kazakhstan	S2
Anthrenus (*Anthrenus*) *picturatus makolskii* Mroczkowski, 1950	Larva (*ex ovo*)	Poland	S3
Anthrenus (*Anthrenus*) *scrophulariae scrophulariae* (Linnaeus, 1758)	Exuvia	Poland	S4
Anthrenus (*Florilinus*) *olgae* Kalík, 1946	Larva (*ex ovo*)	Poland	S5
Anthrenus (*Helocerus*) *fuscus* Olivier, 1789	Larva (*ex ovo*)	Poland	S6
Anthrenus (*Helocerus*) *polonicus* Mroczkowski, 1950	Larva (*ex ovo*)	Poland	S7
Anthrenus spp. 1	Larva	USA (California)	S8
Anthrenus spp. 2	Exuvia	Portugal	S9
Ctesias serra (Fabricius, 1792)	Larva	Poland	S10
Megatoma (*Megatoma*) *undata* (Linnaeus, 1758)	Exuvia	Poland	S11
Thaumaglossa rufocapillata Redtenbacher, 1867	Larva	China	S12
Trogoderma granarium Everts, 1898	Larva	Czech Republic	S13
Trogoderma simplex Jayne, 1882	Larva	USA (California)	S14
Trogoderma variabile Ballion, 1878	Larva	USA (California)	S15
Trogoderma versicolor (Creutzer, 1799)	Larva (*ex ovo*)	Poland	S16
Trogoderma spp. *1*	Larva	Italy	S17
Trogoderma spp. 2	Larva	Romania	S18
Trogoderma spp. 3	Larva	Italy	S19

Microscopy

Both larvae and exuviae were mounted on scanning electron microscopy (SEM) pin stubs, fixed using SEM adhesive carbon tabs, and golden-coated using a plasma sputter coater. Specimens were observed using a JSM Jeol 6490 SEM (CEASC, Università degli Studi di Padova, Padova, Italy). Ion milling, intended to dissect hastisetae, was performed using a Dualbeam FIB/SEM mod. Helios Nanolab 600 FEI Company (LIME, Università degli Studi Roma Tre, Rome, Italy); this apparatus is capable of selectively ablating (milling process) a previously marked region of the sample by using a focused ion current from a gallium source. The milling process can be interrupted every few nanometers to take high-resolution images of the cross sections with the SEM column. To observe the inner structure of the hastiseta with transmitted light, hastisetae of *M. undata* and *Anthrenus* spp. were mounted on microscope slides using Euparal mounting medium and photographed using an Optikam B10 Digital Camera mounted on a Askania RML microscope.

Results

Hastisetae are present in both thoracic and abdominal tergites of all larvae of the studied species, with their length and density varying substantially among genera, species and body segments; on the thoracic and abdominal tergites, with the exception of the caudal tufts, the hastisetae are aggregated in tufts or distributed on bands, as already shown in Beal, 1956; Beal, 1960; Beal 1967; Peacock, 1993; Kiselyova, 2002; Kiselyova & McHugh, 2006; Kadej & Jaroszewicz, 2013; Kadej et al. 2013a; Kadej et al. 2013b; Kadej et al. 2017; Kadej & Guziak, 2017 and Kadej, 2012a; Kadej 2012b; Kadej, 2017; Kadej, 2018b; Kadej, 2018b. The quantification of the hastisetae and the relative density is complex to assess, however even at a quick glance it is possible to observe that in all species, the caudal tufts are always the richest in bristles. *Anthrenocerus*, *Megatoma*, *Thaumaglossa*, *Trogoderma* have a relatively uniform vestiture of hastisetae along their entire body, and there is only a minimum difference in length between the hastisetae of the thorax and the first segments with those on the last segments of the abdomen (Figure 1A). On the contrary, *Anthrenus* and *Ctesias* possess short hastisetae on the thoracic and first abdominal segments, while they present long hastisetae creating tufts on the posterior margins of the fourth/fifth to seventh abdominal segments (Figure 1B).

Hastiseta Morphology

The morphology of the hastiseta was highly conserved among all the genera considered in this study and three main regions could be identified: pedicel, shaft, and the apical head (Elbert, 1976; Elbert, 1978).

1) Hastiseta Insertion

The hastisetae on the thorax and first abdominal segments are located on the sclerotized parts of the tergite. Setal tufts are inserted on the lateral sides of the tergites, with the hastisetae partially recumbent and oriented from the side to the centre of the tergite. The pedicel, the most basal part of the hastiseta responsible for connecting it with the in- tegument, has cylindrical section and does not present any surface roughness or sculpture (Figure 1C). The pedicel inserts almost directly on the cuticle, as could be observed in *Thaumaglossa* and *Trogoderma* (Figure 1C), or present a larger socket, usually in the form of a papilla, as in *Anthrenus* and *Ctesias* (Figure 1D). Preliminary observations allowed observations of the chitinous sheath inside the socket, surrounding the pedicel (S10-F). The pedicel is remarkably short in comparison to the rest of the seta and its longitudinal axis is not aligned with that of the hastiseta (Figure 1C).

As a consequence, the two parts are connected at an obtuse angle, and its amplitude can vary. It is this particular type of junction which ensures that the hastisetae remain relatively recumbent on the surface of the body, all oriented in the same direction. The orientation of the pedicels and the relative density with which the hastisetae are inserted into the tergites are visible when the hastisetae are removed (Figure 1E). It is particularly interesting to note that if the hastisetae are artificially removed or the larva has lost them, they break at the level of the pedicel, thus suggesting its role as a breaking point to favour the detachment of the hastisetae. In all cases studied, the break point on the pedicel was clear, well demarcated (Figure 1F), and in a few cases occurred close to the first rosette.

In the last abdominal tergites (fourth/fifth to seventh), hastisetae are externally oriented, not recumbent, due to the short, straight pedicel, and higher in number compared to those on the previous segments. *Anthrenocerus*, *Megatoma*, *Trogoderma* present patches of hastisetae (tufts) inserted on the sclerotized part of the tergite (Figure 2A), while *Anthrenus*, *Ctesias* and *Thaumaglossa* possess hastisetae (tufts) inserted on the membranous integument at the posterior corners of the terga (Figure 2B). The number of such aggregations on tergites vary between genera. Contrary to what has been observed for the other genera, in which the insertion on the integument and the shape of the pedicel are uniform for all the hastisetae, those of *Anthrenus* and *Ctesias* showed unique features. *Anthrenus* possesses dense, circular sockets, close to each other, and apparently distributed without any peculiar pattern. Each socket has a diameter of approximately 2.5 μm and is characterized by having a well-developed collar that is raised above the surface of the integument (S2-B; S5-E). This collar partially wraps the first rosette of the hastiseta, so that the pedicel is not visible (S3-J). *Ctesias* presents insertions in the form of sockets roughly organized into rows, visible in the support file S10-H and partially illustrated in Kadej, 2018a. The distance between sockets belonging to the same row increases from ~2.5 μm, in the proximity of the spicisetae, up to ~10 μm, in the proximity of the posterior margin of the tergite. The sockets, ellipsoid in shape and with an approximated maximum width of 2.5 μm, present a slightly elevated and bordered collar. The pedicel inserts into a circular hole surrounded by the chitin sheath (S10-E, F). In both *Anthrenus* and *Ctesias*, the pedicel of the hastisetae inserted on the membranous part of the tergites is almost straight and aligned with the longitudinal axis of the hastiseta (Figure 1F; S9-M).

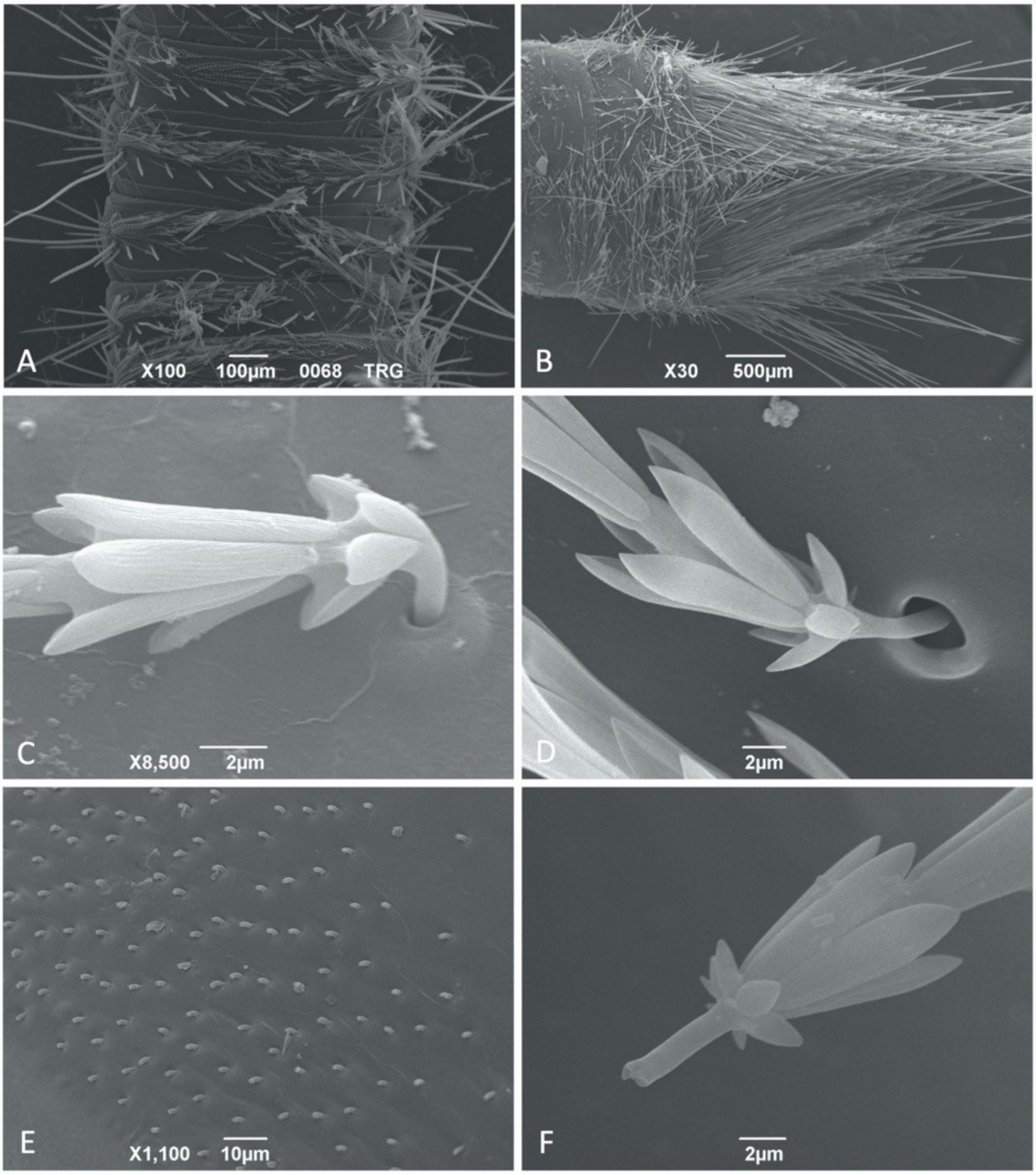

Figure 1. SEM photos of Megatominae larvae: (A) Dorsal view of the abdominal segments of *Trogoderma* spp. 3; (B) Dorsal view of the last abdominal segments of *Ctesias serra*; (C) *Trogoderma versicolor*, detail of the pedicel and insertion on the tergite; (D) *Ctesias serra*, detail of the insertion of the hastiseta on the tergite; (E) *Trogoderma* spp. 2, series of broken pedicels remaining after the detachment of one tuft of hastisetae; (F) *Ctesias serra*, detail of one detached hastiseta showing the breaking point on the pedicel.

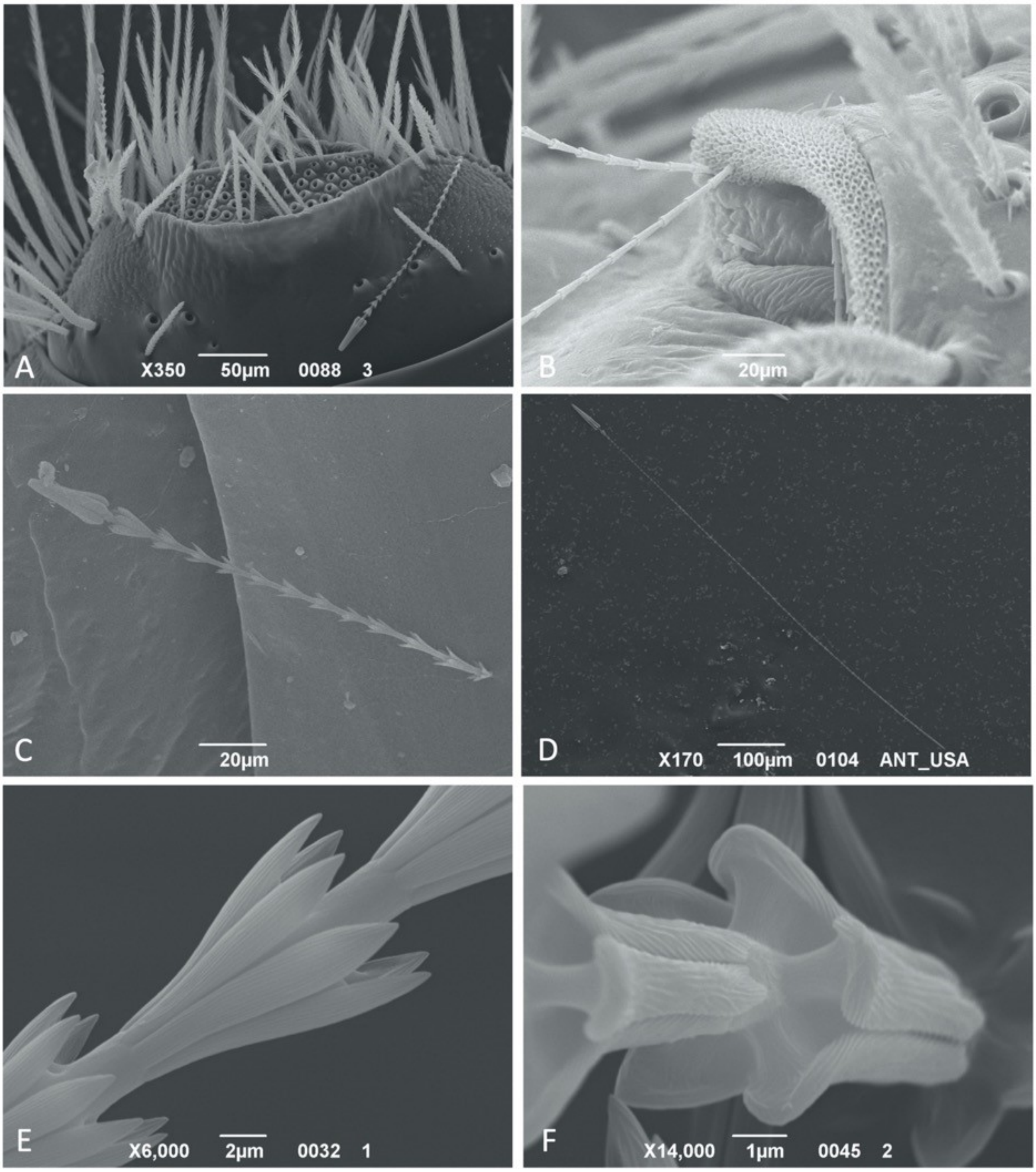

Figure 2. SEM photos of Megatominae larvae: (A) *Trogoderma* spp. 3, dorsal view of the 8th abdominal tergite showing the two patches of detached hastisetae at its corners; (B) *Anthrenus* (*Florilinus*) *olgae*, detail of the membranous part of the tergite bearing the insertion of the hastisetae; (C) Hastiseta of *Trogoderma granarium*; (D) Hastiseta from the caudal tufts of *Anthrenus* spp. 1; (E) *Megatoma undata*, lateral view of the rosettes; (F) *Trogoderma* spp. 3, anterolateral view of the rosette showing the chitin bridges supporting the scales.

2) Hastiseta Insertion and the Pedicel

The shaft dimensionally constitutes the main part of the hastiseta, varying in size from a few tens of microns, as in the thoracic hastisetae of *Trogoderma* (Figure 2C), to several hundred microns, observable in the caudal hastisetae of *Anthrenus* and *Ctesias* (Figure 2D). The constitutive unit of the shaft is the rosette, a set of scaly processes radially disposed around the hastiseta longitudinal axis and oriented externally and towards the head of the hastiseta (Figure 2C). The architecture of the rosettes was generally maintained in all the dermestids investigated, with scales varying among genera and species between five or seven, rarely nine; the only exception was observed in some *Anthrenus* in which the basal rosettes of the hastisetae on the last abdominal segments were columnar (S8-L). The length of the rosettes is not uniform and varies, even among the rosettes of the same hastiseta. Each scale is connected to the main body of the shaft through one cuticle bridge for almost its entire length (Figure 2D). This bridge is short or almost inexistent, close to the proximal part of the rosette, while it is well developed in the distal part, so that the apex of the scale is always diverging to the central axis of the hastiseta. Each scale is lanceolate, with a distal apex that can be more or less sharp, and the lateral margins slightly bent over; the profile of the scales and their length varies among genera (S2-E; S13-E), and these differences are generally difficult to appreciate and quantify at species level. The dorsal part of the scales is covered in dense longitudinal knurls for all their length (Figure 2E), while the ventral side, when visible, consists of an amorphous matrix of chitin (S11-E). The structure, shape, and size of the last rosette and the part of the shaft before the apical head were particularly interesting. Based on these features, the taxa studied can be subdivided in four morphological groups: *Trogoderma*, *Anthrenocerus* and *Megatoma* are characterized by having ultimate rosette of similar size or only slightly larger than the previous. In addition, the three genera possess a series of irregular scales on the shaft, just before the apical head. These recumbent scales are concentrated in a single point and arranged in a ring around the shaft. Each of these scales present the same knurls as rosettes (Figure 3A). *Ctesias* possesses a tulip-shaped ultimate rosette, substantially larger in size than the previous (Figure 3B). Unlike the previous two, this genus presents an enlarged shaft between the rosette and the apical head; the bulge is covered with irregular scales and resembles a bud (S10-O). *Anthrenus* presents an ultimate rosette larger but almost identical in shape to the previous rosettes (S2-C; S4-A). A peculiarity of this genus is the absence of scales or bulges on the shaft between the rosette and the apical head of the hastiseta (Figure 3C,E). *Thaumaglossa* possesses a unique combination of features, with the ultimate rosette significantly larger than the previous (Figure 3D). This rosette has a more compact appearance and is constituted by nine scales, unlike the previous, which has seven. Moreover, this taxon presents the same ring of irregular scales on the shaft as in *Trogoderma*, *Anthrenocerus* and *Megatoma*.

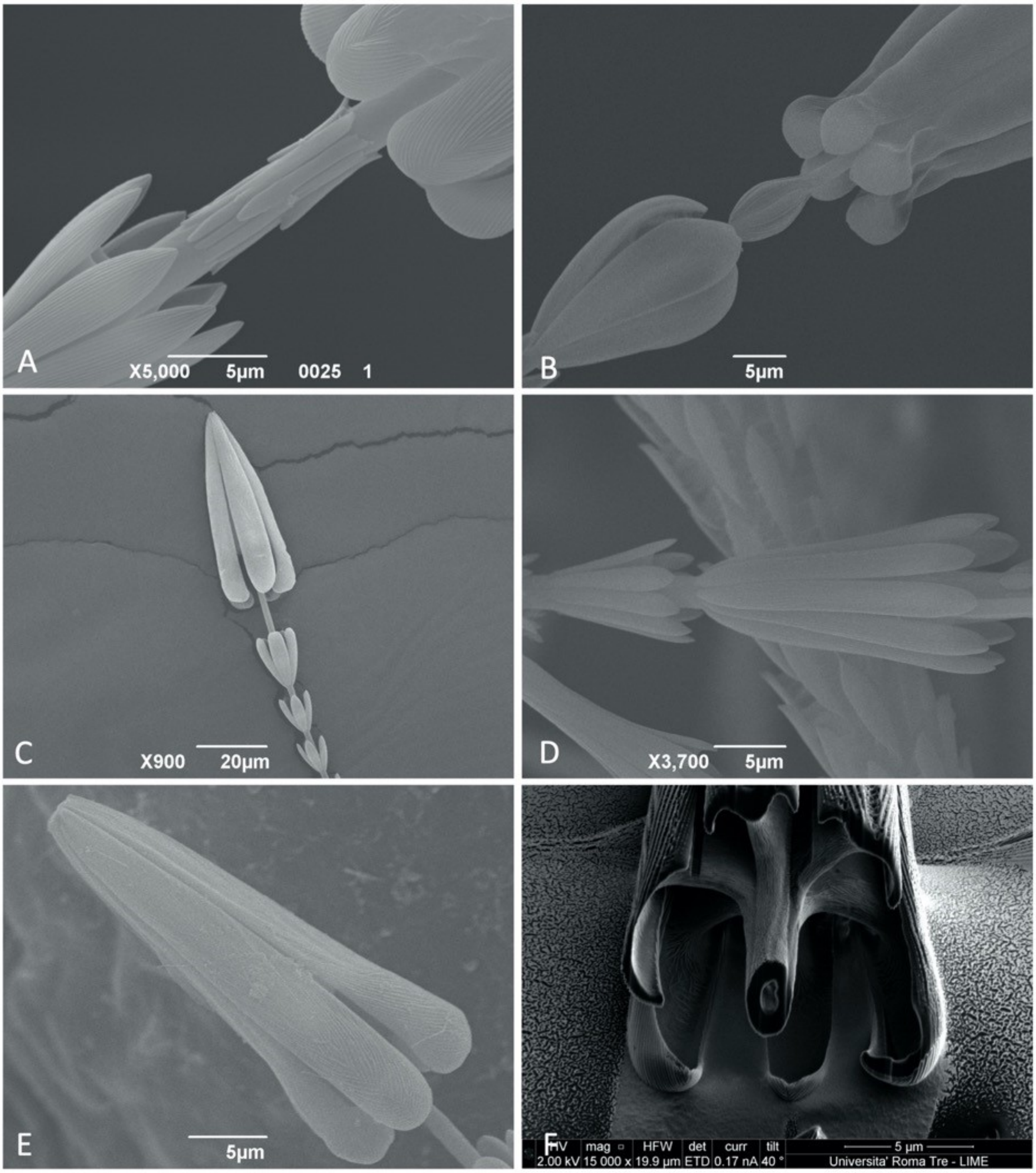

Figure 3. SEM photos of Megatominae larvae: (A) *Megatoma undata*, detail the hastiseta showing the irregular scales on the shaft; (B) *Ctesias serra*, detail of the last rosette and the bud-like modification of the shaft; (C) *Anthrenus* (*Anthrenus*) *latefasciatus*, hastiseta from the caudal tufts, detail of the apical part; (D) *Thaumaglossa rufocapillata*, size and shape comparison between the penultimate and ultimate rosette; (E) *Anthrenus* (*Florilinus*) *olgae*; head of hastiseta; (F) Diagonal section of the basal part of the head of the hastiseta of *Trogoderma variabile.*

3) Apical Head

The apical head is a truncated-cone, anchor-like structure constituted by a series of long processes posteriorly oriented with respect to the apex of the hastiseta; the number of processes varies in accordance with the number of scales on the last rosette. All the processes are identical and organized radially respect the longitudinal axis of the hastiseta (Figure 3E). Each process is constituted by one linear part starting on the apex of the head and ending distally with a crook/hook; this last part is usually convex on the outside and concave on the inside (Figure 3F). The process is connected to the shaft in its basal part throughout a chitin bridge similar to that of rosettes (Figure 3F; S20). This particular arrangement causes the processes of the hastisetal head, and in particular the distal hook, to be oriented against the last rosette. All the processes are covered for their entire length in knurls always antero-laterally oriented with respect to the apex of the hastiseta.

A common feature of the hastisetae is the blunt apex of the seta (Figure 4A); in all cases studied, at the convergence (base/origin) of the processes and exactly in correspondence to the terminal part of the shaft, the hastiseta presented one circular depression (2.5–3.0 μm in diameter). The margins of this depression can be more or less sharp and irregular depending on the incidence angle of the processes. It was impossible to define any further feature related to the circular depression, because in both larvae and exuviae it was filled with an amorphous matrix of unclear origin (Figure 4B). As for the last rosette, the head of the hastiseta presents a substantial degree of variability among genera and species. Following this variability, the studied taxa can be separated in two main morphogroups: *Anthrenocerus*, *Ctesias*, *Megatoma*, *Thaumaglossa* and *Trogoderma* are all united by having the head of the hastisetae of a relatively homogeneous shape and size on all body segments. *Anthrenus* instead presents hastisetae on the tufts on the fifth to seventh, segments in which the head substantially differs from those of the remaining abdominal and thoracic terga.

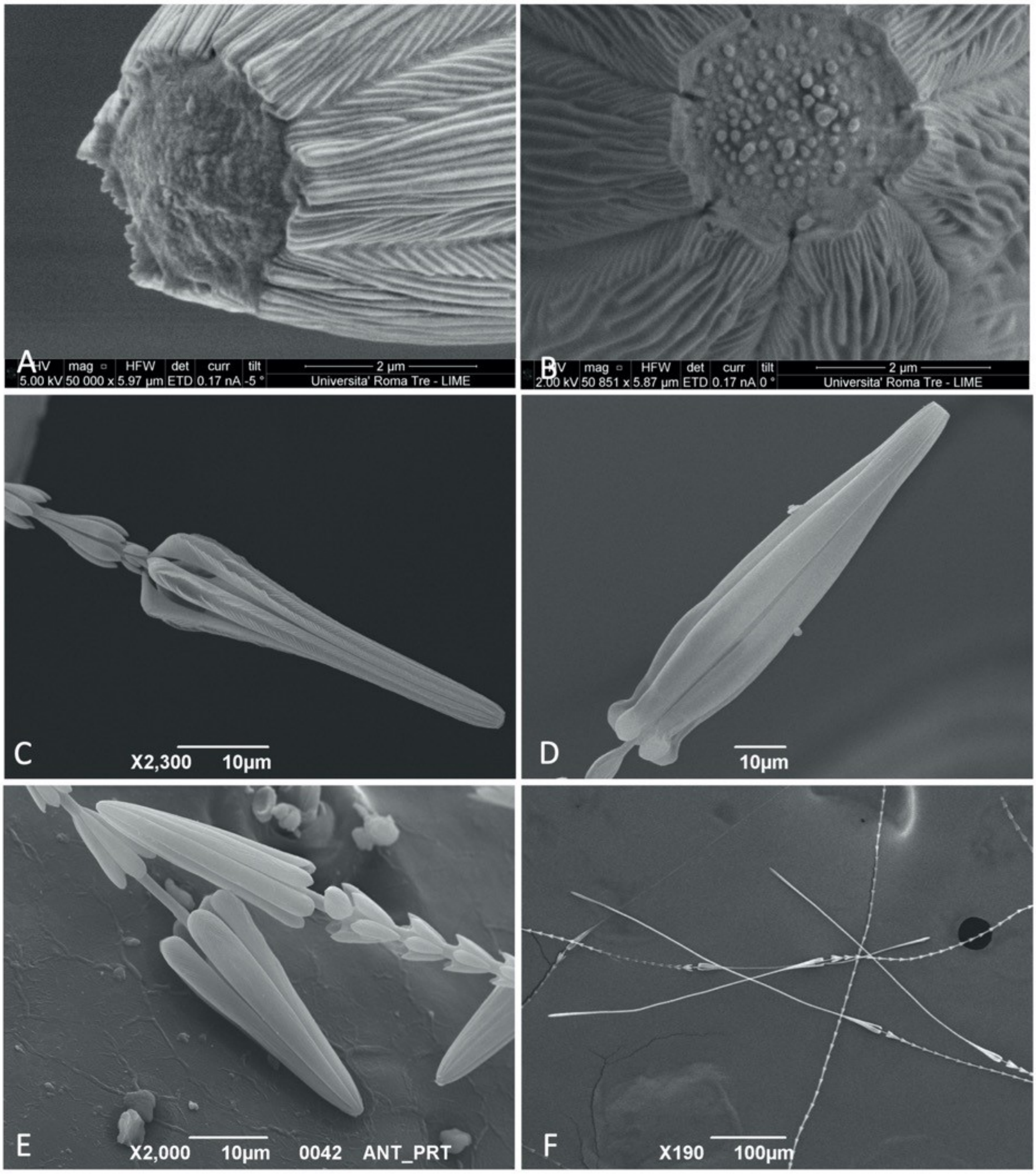

Figure 4. SEM photos of Megatominae larvae: (A) Detail of the blunt apex and the circular depression of *Trogoderma variabile*; (B) Frontal view of the circular depression on the apex of the hastiseta showing the amorphous matrix inside it; (C) Head of the hastiseta of *Trogoderma* sp. 2; (D) Head of the hastiseta of *Ctesias serra*; (E) Head of the hastiseta of *Anthrenus* sp. 2; (F) Head of the hastiseta of *Anthrenus picturatus makolskii*.

In *Trogoderma* and *Anthrenocerus*, the head of the hastiseta has a similar shape and size between tergites and caudal hastisetae. One synapomorphy of the two genera is the presence of one longitudinal depression along each process of the head of the hastiseta (Figure 4C). This depression, which can vary among species in length and depth, is defined by the convergence of the transversal or by the presence of few longitudinal ridges. In *Ctesias*, the head of the hastiseta is identical in both thoracic and caudal hastisetae and it is characterized by a unique profile, which is umbrella-shaped (Figure 4D).

This part of the hastiseta possesses a flared profile, with a bulge roughly near the distal second third of its length, and then strongly narrowed in the distal fourth. The apical bending of each longitudinal process is particularly globular, also due to the strong narrowing that precedes it. Each process of the head of the hastiseta is densely covered in fine ridges, clearly observable only at magnification >3500×.

The genus *Megatoma* possesses a head of the hastiseta that, for its proportion and in respect to the rosettes and general shape (truncated cone), resembles those of *Trogoderma* and *Anthrenocerus*. However, compared to the latter, the longitudinal processes do not present any type of depression or notch.

Thaumaglossa has homogeneous hastisetae on the whole body; however, as for the last rosette, the number of longitudinal processes that constitute the head of the hastiseta is nine, not five or seven as in the other genera. *Anthrenus* presents the most complex set of hastisetal heads compared to other genera, and some of these features have been suggested as one of the possible larval diagnostic characters in Kadej et al. 2013a, Kadej et al. 2013b and Kadej, 2018b.

Anthrenus is so far the only genus that possess two different types of head of hastiseta, one on the shortest thoracic and first abdominal segment hastisetae and a different type on the longer caudal tufts; these heads differ substantially in size or shape, with the exception of *Anthrenus* (*s. str.*) *latefasciatus* and *Anthrenus* spp. 2 (Portugal) in which the heads of the hastisetae are both conical and almost identical in size (Figure 4E). *Anthrenus* (*F.*) *olgae* and *Anthrenus* sp. (USA) are characterized by having the head of the hastisetae on the last abdominal segments conical and about 1.7 times longer than those on the previous, while those of *Anthrenus* (*H.*) *fuscus*, *Anthrenus* (*H.*) *polonicus* are 2.0× and 1.3× longer, respectively. *Anthrenus* (*s. str.*) *scrophulariae* and *Anthrenus* (*s. str.*) *picturatus makolskii* are the only species that possess oblong hastisetae heads on both thoracic and abdominal hastisetae. In both species, the longitudinal processes are relatively close to each other and not divergent from the central axis of the hastiseta. *Anthrenus* (*s. str.*) *scrophulariae* presents oblong–conical heads on the hastisetae of the thorax and first thoracic segments, while on the last tergites, the head is bottle-shaped, 3.7 times longer than those on thorax. *Anthrenus* (*s. str.*) *picturatus makolskii*, on the other hand, possesses hastisetae with an oblong, parallelepiped head on thorax; at the level of the abdominal tergites, with the exclusion of the long tufts of hastisetae, these setae are mixed with hastisetae having a more elongated head (Figure 4F). A peculiarity of this species is the hastisetae constituting the tufts on segments 5–7, in which the head of the hastiseta is remarkably stretched, about 7.7 times the length of those on the other tergites. This peculiar type of head is slightly enlarged in the proximity of the apex of the longitudinal processes, and it displays a light-clockwise torsion along its longitudinal axis.

On two different occasions during the preparation of the specimens for the SEM, two different groups of arthropods were observed while trapped by hastisetae. These two events, both natural expositions to hastisetae and not forced as in Nutting & Spangler, 1969 or Kokubu & Mills, 1980, confirmed once again the primary role of hastisetae as extremely efficient mechanical traps in entangling predators, parasitoids, and possibly competitors. The first case was regarded to some *Sitophilus* spp. (Coleoptera: Curculionidae) infesting the rearing substrate of *M. undata*. All specimens were found dead, possibly due to the effort to free themselves, with antennae, tarsi, and scales covered in hastisetae. What was particularly interesting was the fact that those beetles did not come into contact with live larvae (that were no longer present in the enclosure), but instead with loose hastisetae contaminating the substrate or larval exuviae. This condition suggests that hastisetae maintain their functionality over time, even if detached from the larval tergites. On one occasion, a *Sitophilus* remained totally stuck on a larval exuvia, providing direct proof of how synergic actions of multiple hastisetae constitute a trap almost impossible to escape (Figure 5A). In the second case, Mesostigmata mites were found entangled by *Trogoderma* hastisetae, with some of them still trapped on the dorsum of the larvae (Figure 5B). Even in this case, the trapped arthropods had tarsal claws and trichomes entangled with hastisetae but, differently from the previous case, the studied larvae were alive before the preparation for the SEM. It is plausible that these mites were predators or simply co-occurring species on the same substrate.

Figure 5. (A) SEM photo of a *Sitophilus* spp. (Curculionidae) trapped on the exuvia of *Megatoma undata*; (B) SEM photo of a Mesostigmata mite entangled by hastisetae on a larva of Trogoderma spp. 1; (C) Hastisetae of *Megatoma undata* mounted in Euparal showing the darker, empty core; (D) SEM photo of a section of the head of the hastisetae of *Trogoderma variabile* illustrating the internal cavity of the stalk filled with the amorphous matrix; (E) Larvae of *Anthrenus* spp.: left specimen in a relaxed condition and hastisetae at rest; right specimen displaying defensive behaviour with raised hastisetae.

4) Internal Structure

Both Euparal slides and ion milling revealed that in the genera considered, hastisetae are hollow, and the internal cavity extends homogeneously from the subcuticular structures up to the apex of the hastiseta (circular depression) (Figure 5C). Longitudinal sections of the hastiseta seem to indicate the presence inside the lumen of an amorphous substance of unclear origin (Figure 5D).

Response to Disturbance

Trogoderma and *Anthrenus* displayed two completely different responses to stimulation. *Trogoderma* did not show any substantial modification in body posture but stopped every activity after the stimulation in almost all trials conducted. If stimulated locally with the tip of the micropin, the larvae were capable of contracting body segments and orientating the tergites bearing hastisetae in the direction of the stimulus. Sporadically, in the case of persistent disturbance, the larva abandoned the passive defence and tried to escape.

Anthrenus instead always demonstrated a defensive behaviour of an aggressive type, which in some ways resembled that of a porcupine. At a minimum stimulus, the larvae stopped all activity, contracted the body segments, and markedly arched the last abdominal segments. This strategy determined that the tufts of hastisetae of the last abdominal tergites, usually recumbent, were raised and opened like a fan (Figure 5E); this defensive posture was held by the larva until the disturbance continued. The defensive response in *Anthrenus* was induced by both a direct stimulation on the body and a mechanical stimulation of the long tufts of supranal bristles (Ma et al. 1978).

Morphology and Biomechanics of Hastisetae

The morphological data collected here are consistent with the observations provided in Elbert, 1978, in which the pedicel extends up to the deep layer of the endocuticle. This trait indicates that hastisetae must no longer be considered true setae but instead modified setae (Battisti et al. 2011); further support to the present interpretation is given by the existence of the breaking point that permits the detachment of the hastiseta.

Observations confirmed that the head is the key structure in hooking and trapping body parts of the threatening organism that comes in contact with it. In both Mills & Partida, 1976 and Kokubu & Mills, 1980, and exactly as in the samples studied here, scales, claws, and hairs were systematically and irrecoverably trapped among the longitudinal processes of the head of the hastiseta. The unique radial symmetry, the septation, and the gaff shape of the apical part of the longitudinal processes make the head a perfect trap, capable of guaranteeing an anchorage that cannot be removed.

The head is structured in such a way that whatever organism is trapped, the more it tries to free itself the more it is stuck. An arthropod which is trying to remove the hastiseta is inclined to pull; therefore, its body parts end up more and more trapped due to the knurls present on the longitudinal processes and the reduction in the lumen between adjacent longitudinal processes of the head of the hastiseta. It should also be considered that the effort in freeing by the arthropod determines the breaking of the pedicel and the detachment of the hastisetae from larval integuments. In this way, the detached hastisetae synergically amplify the entangling effect due to the friction and trapping caused by the rosette against other rosettes and body parts.

The efficiency of trapping bristles and other structures is most likely determined by the combined action of the last rosette and the hastiseta head, in which the opposite orientation of the two parts would increase the trapping capacity of the hastiseta. It is interesting to note that Megatominae apparently solved the issue of increasing the trapping efficiency of the hastiseta at least in two different ways: *Anthrenus* evolved a particularly enlarged ultimate rosette which apparently has the function of guiding the bristles and scales towards the head trap, while *Trogoderma*-like Megatomini have developed accessory scales on the shaft as alternative structures to achieve the same result.

The capability of *Anthrenus* in rising hastisetae only when disturbed suggests a more efficient defensive strategy against threats, possibly contributing to the great success of the genus that constitutes approximately the 30% of all Megatominae. Long hastisetae are potentially more efficient in discouraging and entangling predators; however, given their size, they may be subject to involuntary detachment or to create an impediment to the larva. It is plausible that *Anthrenus* evolved hastisetae on soft teguments, able to fold down in brushes under relaxed conditions. This type of strategy is documented in *Cryptorhopalum* Guérin-Méneville, 1838 (Kiselyova & McHugh, 2006) and, even if not directly observed, may also occur in *Ctesias* and *Thaumaglossa*. On the contrary, *Anthrenocerus*, *Megatoma* and *Trogoderma* have developed a static defense that possibly bases its success on the quantity and density of hastisetae covering the larvae. The substantial difference in morphological traits and defensive behaviour may, at least among the Megatominae investigated, be attributable to the different ecological niches and environments colonized, as suggested in Zhantiev, 2009.

Among all the peculiarities emerged in the investigation, the role and origin of the circular depression on the tip of the hastiseta remains unclear. The fact that this structure is always present in all the taxa investigated and those pictured by Elbert, 1976 and Elbert, 1978, seems to suggest its involvement in the function of the hastiseta. The presence of the amorphous matrix in the circular depression and inside the lumen of hastisetae may indicate some sort of secretion; however, whether the compound has a deterrent or adhesive effect remains a future aspect to be investigated.

Systematics

The set of data collected, combined with the published information concerning the larval morphology and the phylogeny of the group, enabled identification of some features potentially helpful to define the systematics of Megatominae. *Anthrenocerus*, *Megatoma*, and *Trogoderma* are all united by possessing hastisetae located on the sclerotized part of the tergites and the presence of irregular scales on the shaft, just before the head of the hastiseta. This set of features is consistent with the lineage identified by Kiselyova & McHugh, 2006 on the base of larval morphological traits and in partial agreement with the molecular phylogeny provided by Castalanelli et al. 2012. The resemblance of the hastisetae of *Anthrenocerus* and *Trogoderma*, especially the presence of longitudinal depression on the processes of the head of the hastiseta, suggests a close relationship between the two genera, as already indicated by Kiselyova & McHugh, 2006 and Castalanelli et al. 2012.

Morphological apomorphies of *Anthrenus* (enlarged last rosette, lacking irregular scales on the shaft) indicate its belonging to a distinct lineage with respect to the aforementioned genera, as shown in Kiselyova & McHugh, 2006. *Anthrenus* apparently diverged during the early stages of Megatominae evolution (Castalanelli et al. 2012) and evolved a unique set of morphological and ethological traits. *Ctesias* and *Thaumaglossa* (Tribe Megatomini) possess a set of characteristics that suggest an intermediate position in respect to *Anthrenus* (Tribe Anthrenini) and the *Anthrenocerus–Trogoderma* lineage (Tribe Megatomini). These two genera share characteristics with *Anthrenus* hastisetae, on the membranous part of last abdominal tergites and the irregular scales/modified shaft before the apical head. *Ctesias* has a unique shape of the ultimate rosette, the presence of bud-like shaft modifications (that we may consider a proto set of scales), and the peculiar profile of the head of the hastisetae suggest a closer relation to *Anthrenus*, with which it shares elongated hastisetae on the last abdominal segments. It is interesting to note that *Ctesias* shares some similarities with Cretaceous amber fossils described by Poinar & Poinar, 2016.

The unique enlarged and modified ultimate rosette of *Thaumaglossa* agrees with the phylogeny provided by Kiselyova & McHugh, 2006, in which the genus cluster in a separate clade with respect to *Anthrenocerus*, *Megatoma* and *Trogoderma*. *Megatoma* may be considered closer to *Trogoderma*-like Megatomini (Kadej, 2017), with which its shares hastisetae similar in length on the abdominal segments, a last rosette similar in shape and size to the previous and the irregular scales on the shaft; this placement agrees with the results indicated in Beal, 1967 and Kiselyova & McHugh, 2006.

Conclusions

Hastisetae are modified setae that are released in the environment only through the mechanical rupture of the pedicel. Our observation suggests that hastisetae not only trap different subphyla of Arthropoda, but they target a broad spectrum of predator/parasite sizes; this functional plasticity provides a highly effective defensive mechanism that implies minimal energy investment in its implementation. In addition, the peculiar shape of the hastisetae suggests that they did not evolve as an offensive defensive structure against vertebrates and the effects observed, including those on humans, are fortuitous. This interpretation is supported by the shape of the hastisetae in which the blunt apex of the head renders them unable to penetrate through sclerified epithelia, such as the skin, unlike what is present on some lepidoptera and tarantulas where detachable setae are sharp (Battisti et al. 2011). Inflammation observed in humans, often affecting the soft oro/rhino-pharyngeal epithelium and conjunctiva, are most probably caused by the mechanical friction of the seta on the epithelia. Furthermore, the insurgence of the symptoms may be induced by chitin, proteins, and other compounds associated with the integument, and the way in which the inflammation manifests can be highly subjective due to the different sensitivities that each individual has towards these molecules.

In this regard of applied entomology, hastisetae may constitute a substantial help in contaminant identification, especially in the case of fragmented dermestid residues. The available information so far permits the identification at genus level of some of the most common pests and synanthropic taxa. However, in order to be able to progress and develop an identification tool at species level, it will be necessary to substantially increase the number of species to be included in the analyses and to develop a standard protocol for their observation. In fact, the way in which the seta is prepared, and its conservation state, may affect the possibility of appreciating specific, relevant characters.

Furthermore, a wider set of morphological data, inclusive of a good representation of worldwide Megatominae and *Trinodes* Dejean, 1821 (Subfam. Trinodinae; a genus that possess proto-hastisetae), can surely help in clarifying and resolving the systematic and evolution of the subfamily. Preliminary data are encouraging, and the morphological traits associated with hastisetae are consistent with the phylogeny based on larval characteristics defined by Kiselyova & McHung, 2006 and the molecular phylogeny proposed by Castalanelli et al. 2012. The increase in genetic sequences available, together with new information on the biology and ethology of the larvae, may help in clarifying whether specific hastisetal traits represent evolutionary convergence due to similar larval niches or are the result of a common origin.

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Chapter 4

Trogoderma granarium Everts, 1898 (Coleoptera: Dermestidae) - a model species to investigate hastisetae functional anatomy

Introduction

Detachable setae are defensive structures that provide both active and passive protection to the organism that possesses them (Winterton, 2009). These setae evolved independently at least in four terrestrial arthropod lineages namely Polyxenidae (Myriapoda), Theraphosidae (Arachnida), Notodontidae (subf. Thaumetopoeinae), Erebidae, Saturniidae and Zygaenidae (Insecta: Lepidoptera) and Dermestidae (subf. Megatominae) (Insecta: Coleoptera). These detachable setae possess different mechanisms of action; Polyxenidae setae target other arthropods (Eisner et al. 1996), providing a mechanical defence against possible threats, while Theraphosidae and the Lepidoptera families mentioned above present urticating hairs (true setae) targeting primarily vertebrates (Battisti et al. 2011; Bertani & Guadanucci, 2013).

What is much less clear is the role of the setae (hastisetae) of Dermestidae Megatominae. Substantial data indicate that the primary function of the hastisetae is to work as a mechanical trap against predators and competitors, similar to those on Polyxenida (Nutting & Spangler,1969; Mills & Partida, 1976). Medical literature however reports multiple cases of allergies, dermatitis and more generally, inflammatory symptoms attributable to direct or indirect contact with Megatominae, thus suggesting a possible action also against vertebrates (i.e. MacArthur et al. 2016; Gumina et al. 2021; Simon et al. 2021).

Recent studies have shown that the hastisetae cannot be “true setae” (loss of the neural connection and the detachment of the proximal end of the hair from the integument, *sensu* Battisti et al. (2011) because their release can only occur through the rupture of their basal part (pedicel). The hastisetae are apparently not capable of penetrating the sclerotized epithelia (such as the skin) because they lack a sharped, pointed apex (Ruzzier et al. 2021). The characterization of the base of the hastisetae given in Ruzzier et al. (2021) allowed us to reconsider hastisetae as detachable hairs, abandoning the interpretation of them as“true setae” (Ruzzier et al. 2020) and instead suggesting here the fit in “modified setae” (i.e. setae having a blunt base, connected to the integument and lacking the neural connection).

The discovery that the apex of the hastiseta is truncated, concave, entirely hollow, and contains an amorphous matrix, would suggest that hastisetae may have a secretory role or may be able to convey chemical compounds possibly involved with larval defence. Secretory (or glandular) setae have been recognized in mites (Di Palma et al. 2021), opiliones (Wolff et al. 2016), butterflies, true bugs (Livingstone, 1978) and biting midge larvae (Urbanek et al. 2011; Urbanek et al. 2012) and their function, despite not always fully clarified, is associated with predation and protection, self-defense included.

Given the current, albeit scarce, state of knowledge, it would seem that hastisetae are hairs combining a biomechanical and a secretory function. To match/fit the definition of modified setae, the presence of one or more secretory cells associated with the setal socket should be present and there should be no connection with the nervous system.

The aim of this paper is to characterize the insertion of the hastiseta on the larval integument and to describe the cellular structures associated. This information could clarify the putative secretory function of the hastisetae as well as shed light on possible other functions.

Materials and methods

Model species

Trogoderma granarium Everts, 1898 (Dermestidae: Megatominae: Megatomini), also known as khapra beetle, is a primary quarantine pest for several countries worldwide (Athanassiou et al. 2019). This insect, and in particular its larva, attacks stored products causing substantial economic loss due to the direct damage caused and because its remains are important contaminants of the commodities. Since *T. granarium* larvae are rich in hastisetae, it is plausible that its remains and loose hastisetae are involved in allergic and inflammatory reactions in people who consume contaminated food, especially in developing countries.

Specimen preparation - SEM fine morphological analysis

Live larvae were anaesthetised with vapours of ethyl acetate. Both larvae and exuviae were mounted on scanning electron microscopy (SEM) pin stubs, fixed using SEM adhesive carbon tabs, and golden-coated using a Q150R E plasma sputter coater (Quorum, East Sussex, UK). Specimens were observed using a JSM 6490 SEM (JEOL Ltd., Tokyo, Japan) at Centro di Analisi e Servizi per la Certificazione laboratory (CEASC, Università degli Studi di Padova, Padova, Italy).

Specimen preparation – FIB/SEM ultrastructural analysis

Live larvae were anaesthetised with carbon dioxide, immersed in a cold 0.1 M cacodylate buffer (pH 7.4) and cut transversely in three parts to facilitate the subsequent fixation and staining processes. Larval parts were fixed overnight in Karnovsky's solution at 4°C, washed two times in 0.1M cacodylate buffer (15 min each), post-fixed in 1% osmium tetroxide in 0.1M cacodylate buffer for 2 h at 4°C. Samples were then dehydrated to 70% ethanol and stained en bloc transversely with 2% uranyl acetate in 70% ethanol for 2 h at room temperature. After washing thoroughly, the samples were dehydrated in an ethanol series (70%, 85%, 95%, 30 min each and 100% for 2h), embedded in epoxy resin and finally polymerized for 72h at 60°C.

Resin-embedded samples were cut into sequential slices of approximately 15-20 µm thick using a glass knife on an Ultracut T ultramicrotome (Leica Microsystems, Vienna, Austria). Slices were attached to aluminium stubs by a conductive adhesive carbon disc, coated with a thin layer (30 nm) of gold using a K550 sputter coater (Emithech, Kent, UK), and analysed with a Dual Beam (FIB/SEM) Helios Nanolab 600 (FEI Company, Hillsboro, USA) at the electron microscopy laboratory of the Roma Tre University (LIME, Rome, Italy). Following the “Slice&Mill” method (Di Giulio & Muzzi, 2018), the FIB column was employed to selectively ablate the region of interest by performing sequential sectioning of the sample, while the SEM column was used to acquire micrographs of the freshly milled surface by detecting backscattered electrons in order to obtain ultrastructural data.

Results

External morphology of Trogoderma granarium hastiseta

The hastisetae of *T. granarium* present all the typical features of those of Megatomini (Ruzzier et al. 2021) (Figure 1A): setae located on the sclerotized part of the larval tergites, organized in one transversal band located in the posterior half of the tergite; in the last four abdominal segments hastisetae are aggregated in tufts located at the rear corners of the tergites. Hastisetae are oriented with their apex pointing towards the longitudinal axis of larva (Figure 1B). The pedicel (the basal part of the hastiseta) is oblique in respect to the longitudinal axis of the seta, thus determining orientation and arrangement of the seta (Figure 1C). Furthermore, the pedicel includes the breaking point responsible for the detachment of the hastiseta (1D-E). The rosettes constituting the stalk are generally oblong, uniform in size, and bear five to seven scale-like processes apically oriented (1F); the part of the stalk between the last rosette and the head of the hastiseta comprises a series of irregularly scattered, ellipsoidal scales (Figure 2A).

The head of the hastiseta is typically truncated, cone shaped, septed in seven or nine longitudinal processes (Figure 2B) and bearing multiple knurls oriented antero-laterally (Figure 2C). Furthermore, each process presents a faint longitudinal depression in its apical half (Figure 2C). The blunt apex of the head of the hastiseta presents a circular depression with a mean diameter of 2 μm filled with an amorphous matrix (Figure 2D) similar to those observed in other *Trogoderma* species (see Chapter 3 for details).

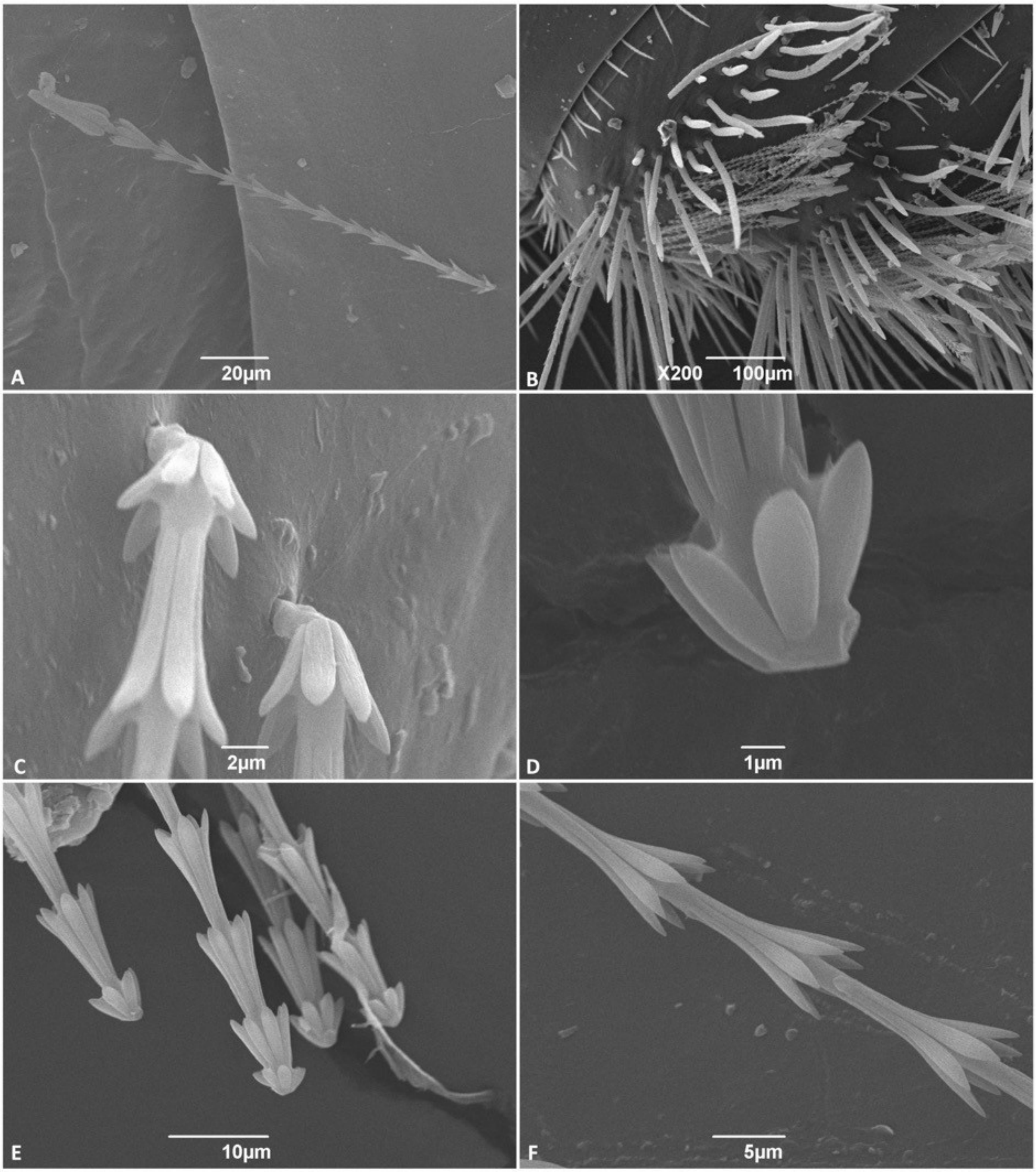

Figure 1. SEM micrographs of *Trogoderma granarium* hastisetae: (A) Hastiseta, lateral view; (B) Tufts of hastisetae on the seventh and eighth abdominal tergites of the larva; (C) Detail of the insertion of the hastiseta on the larval integuments; (D) Detail of a detached hastiseta showing the breaking point on the pedicel; (E) Group of detached hastisetae illustrating how the rupture of the hastiseta occurs exclusively at the level of the pedicel; (F) Detail of the rosettes that constitute the shaft of the hastiseta.

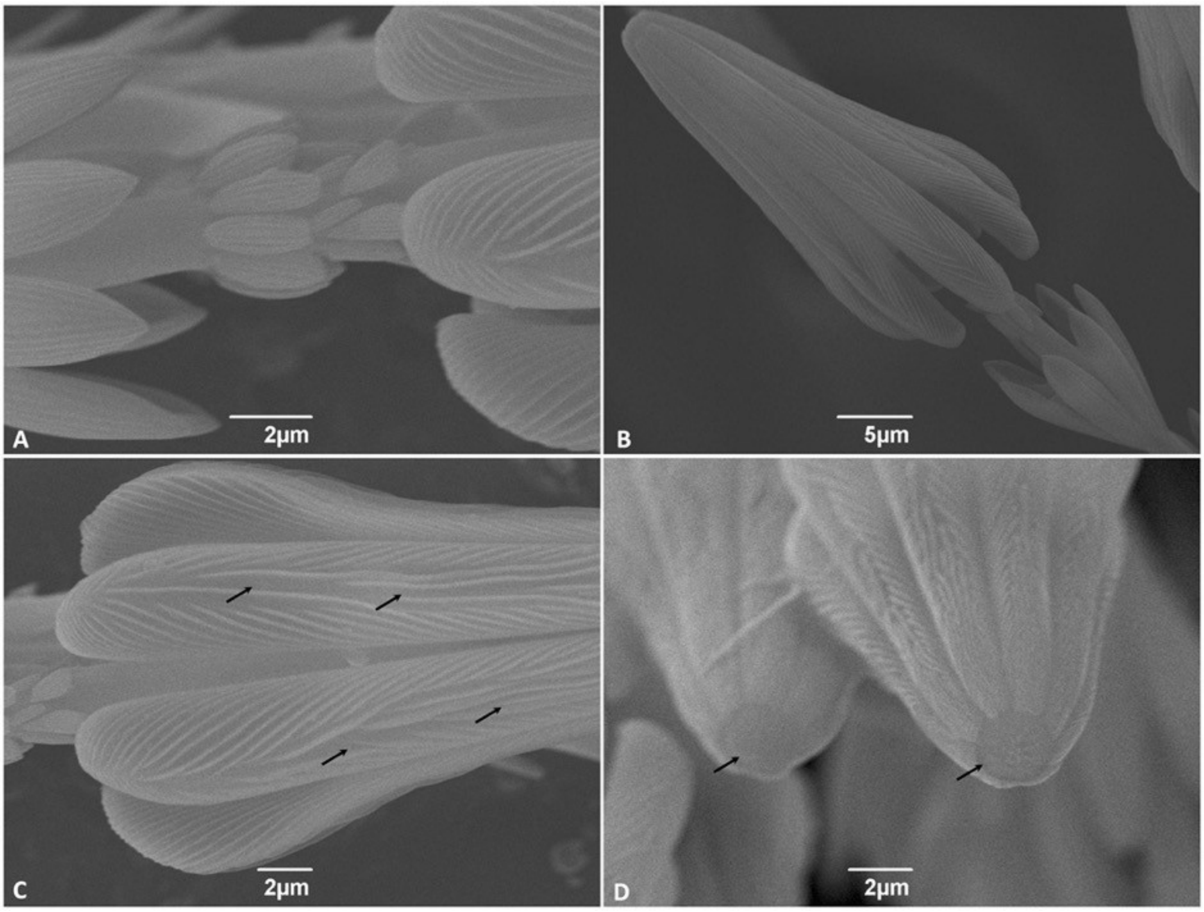

Figure 2. SEM micrographs of *Trogoderma granarium* hastisetae: (A) Detail of the shaft between the ultimate rosette and the head of the hastiseta showing the set of irregular scales; (B) Head of the hastiseta, fronto-lateral view; (C) Detail of the longitudinal processes of the head of the hastiseta showing the knurls and the longitudinal depression (black arrows); (D) Frontal view of the head the hastiseta illustrating the apical circular depression (black arrows).

Subcuticular structures

The transverse section of the cuticle showed the deep insertion of the hastisetae of *T. granarium* (Figure 3A). The basal part of the pedicel (*sensu* Ruzzier et al. 2021), and thus the insertion of the hastiseta, is deeply sunken in the integument. Observing the hastiseta insertion from the internal side of the cuticle, it is possible to observe the peculiar conical protrusion at the hastiseta insertion point (Figure 3B). This arrangement, associated with socket membrane reduction, determines that the base of the hastiseta is tightly surrounded by the cuticle, allowing only a limited lateral movement. The deep socket is lined by a thin cuticular wall that thickens towards the basal part (Figure 3A, 4A). The latter is continuous and apparently with the same structural organization of the hastiseta. This inner part of the socket possesses a sharped collar facing the sensillum accessory cells, which makes the section of the socket similar to a shape of an inverted T (Figure 4A).

The socket is surrounded by the receptor lymph space, which appears as a pale gray amorphous matrix containing darker cuticular suspension fibers (Figure 3A). The receptor lymph space is delimited by the tormogen cells, whose distal end is fringed due to the presence of several microvilli facing the extracellular space (lymphatic receptor space) (Figure 3A).

At the base of the hastiseta it is possible to observe a typical tubular body with a thin apex, penetrating the cuticle at the socket level (Figure 3A, 4A, B, D). This structure and the associated outer dendritic segment of the sensory cell are surrounded by a black dendritic sheath and have a tortuous and non-parallel course with respect to the surrounding epithelial cells, thus appearing multiple times in the same section (Figure 4D).

The tubular body is connected to the neuron, located in the deeper position of the epithelium, via the outer and inner segment of the sensory cell dendrite (Figure 4C). Between the inner and the outer segment of the dendrite, the ciliary region is clearly discernible, with a visible and strongly electrondense basal body (Figure 4C, E). From this latter, the ciliary rootlets can be seen arising and extending towards the cytoplasm of the dendrite (Figure 4E).

The dendrite presents a more lucid and less ribosome rich cytoplasm in comparison to the other cells of the epithelium and it is surrounded by the thecogen cell for its entire length (Figure 4C, E). The neuron cell body is globular in shape (Figure 5A), with a well-defined circular nucleus that is usually located centrally and immersed in a pale cytoplasm presenting a limited number of sparse organelles (Figure 5B). Perikaryon includes several free ribosomes, a few elongated mitochondria and scattered rough endoplasmic reticulum appearing in the form of flattened cisternae (Figure 5C). Golgi complex, with closely appressed cisternae, is frequently observed. While multivesicular bodies are only occasionally detected in the cytoplasmic matrix of the soma.

The epithelial cells surrounding the secretory organs are irregularly shaped, with a nucleus that varies in both shape and size (Figure 5D, E). In contrast to nerve cells, the cytoplasm of epithelial cells is more electron-dense and appears richer in organelles, abounding in elongated mitochondria and conspicuous whorls of rough endoplasmic reticulum (5D, E, F).

No glandular tissues were found in association with hastisetae, nor isolated glandular cells possessing a clear exocrine activity and releasing their products outside the cuticular layer. It is interesting, however, to note that thormogen (and thecogen?) cells exhibit small electron-lucent vesicles and numerous microvilli at the level of their apical membrane (Figure 6), features consistent with secretory activity and release of substances towards the receptor lymph cavity.

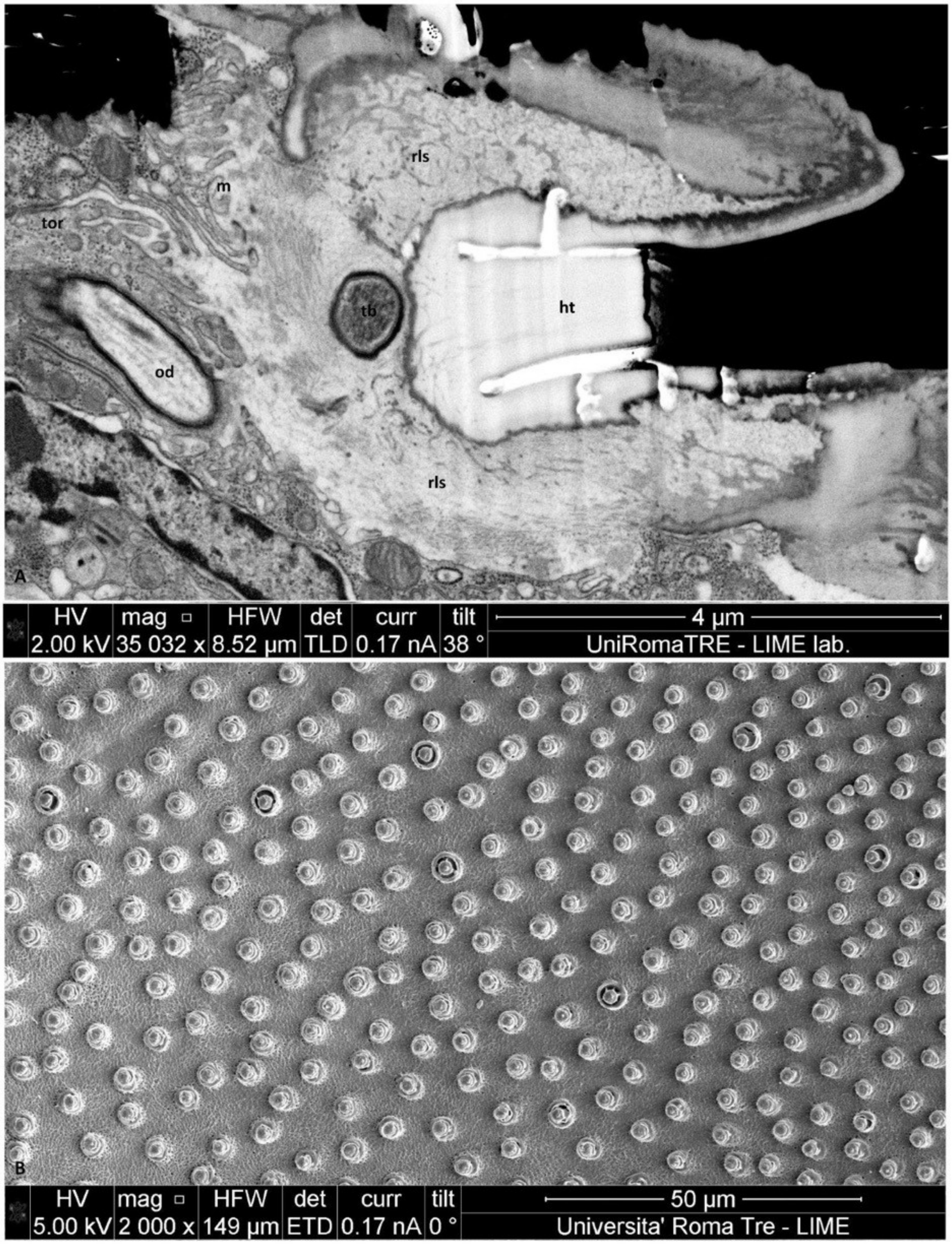

Figure 3. (A) Scanning electron micrograph of the sagittal section of the socket of the hastiseta; (B) View of the cuticle of the point of insertion of the hastisetae on the larval tergite, taken from the endocuticle perspective. ht - hastiseta (pedicel), od - outer segment of sensory cell dendrite, m - microvilli, rls - receptor lymph space, tb - tubular body, tor - tormogen cell.

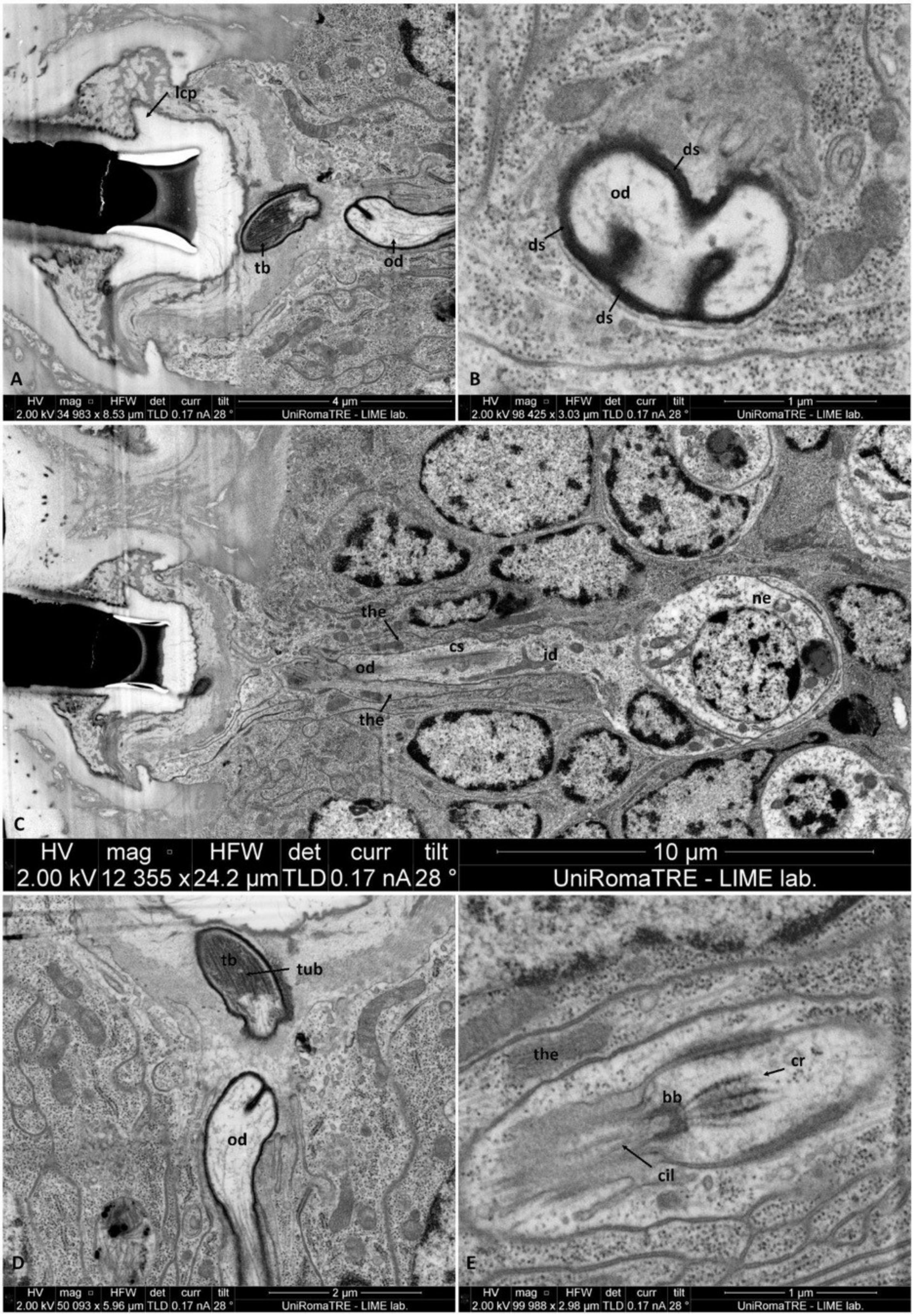

Figure 4. (A) Sagittal section of the socket of the hastiseta (pedicel missing) highlighting the lateral cuticle process probably involved in anchoring the socket; (B) Detail of a convoluted branch of the outer dendrite; (C) Sagittal section of the neurosensorial apparatus connected to the socket constituted by one neuron, its dendrite and the associated thecogen cell; (D) Section showing the tubular bundle inside the tubular body; (E) Detailed view of

the connecting cilium. bb-basal body, cil - cilia, cr - ciliary rootlet, cs - ciliary sinus, ds - dendritic sheath, id - inner segment of sensory cell dendrite, lcp - lateral chitin process, ne - neuron, od - outer segment of sensory cell dendrite, tb - tubular body, the - thecogen cell, tub - tubular bundle.

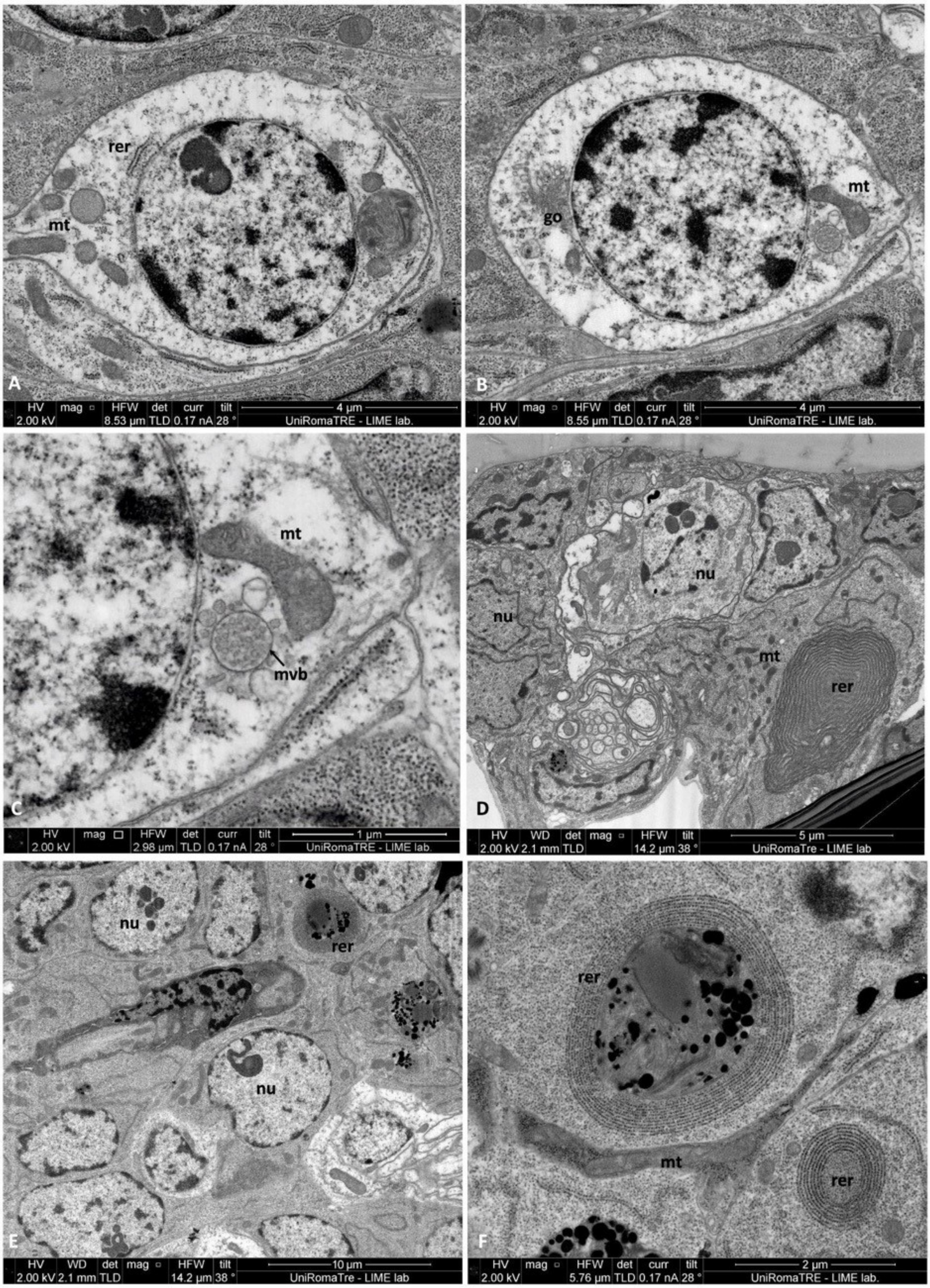

Figure 5. Ultrastructural features of soma and epithelial cells. (A-B) Sections of the soma showing the rounded and central, mitochondria, golgi complex and sparse rough endoplasmic; (C) Detail of perikaryon showed in B illustrating a multivesicular body near a mitochondrion; (D-E) Epithelial cells with irregularly shaped nucleus, note the abundance of mitochondria and the highly developed rough endoplasmic reticulum appearing as whorls; (F) Close up of an elongated mitochondrion, and rough endoplasmic reticulum. go - golgi, mt- mitochondrion, mvb- multivesicular body, nu - nucleus, rer - rough endoplasmic reticulum.

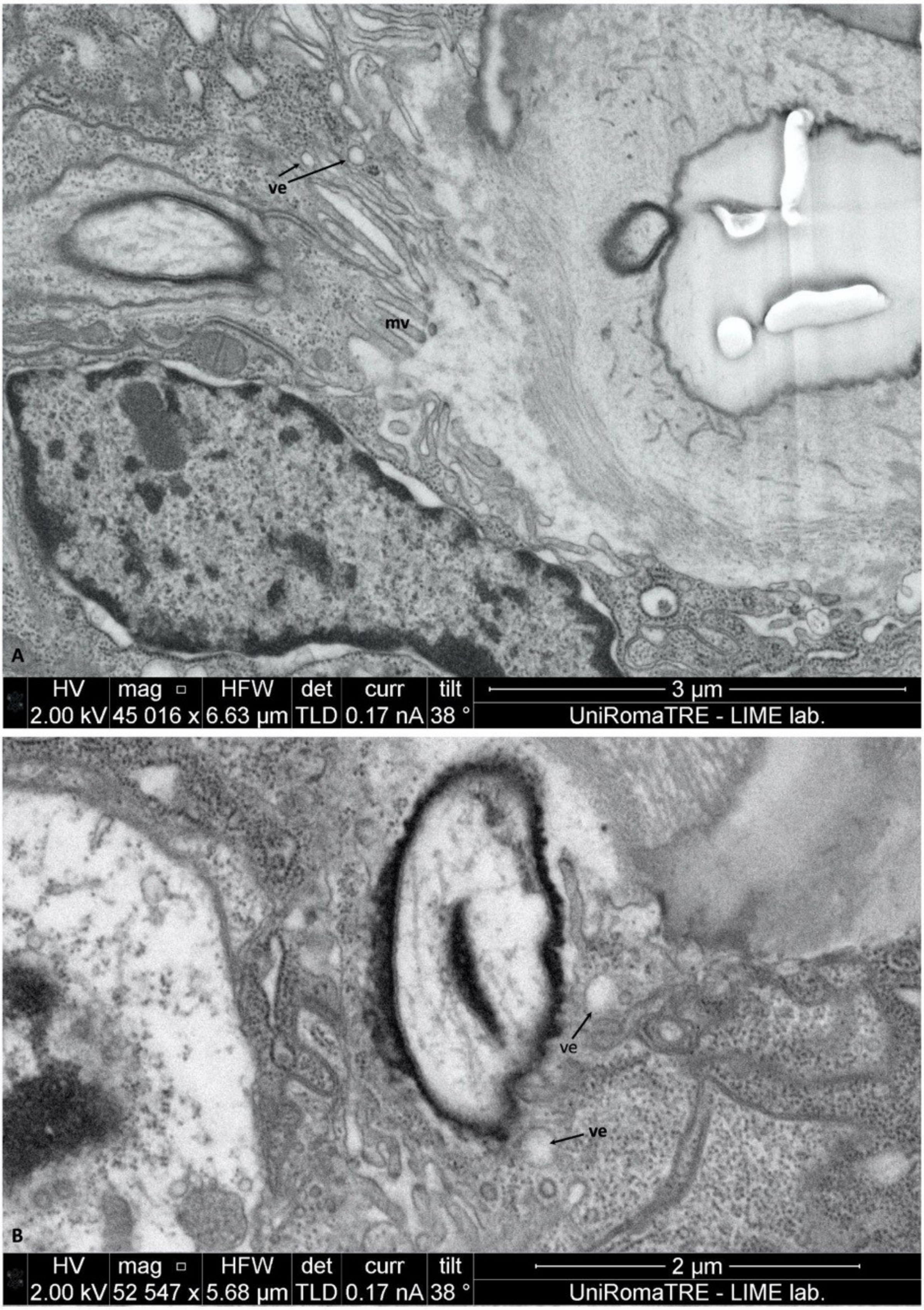

Figure 6. Scanning electron micrograph of the sagittal section of the socket of the hastiseta: A- B. Details of the apical microvillated membrane in hastisetae accessory glands. mv - microvilli, ve - vesicles.

Discussion

Unlike what was initially expected, the evidence gathered leads us to refute the initial hypothesis of hastisetae as secretory structures and to give them a different and new categorization. The interpretation of the hastisetae as modified setae is reconsidered on the base of two major morphological traits: 1) as stated in Battisti et al. (2011), modified setae have a blunt base, they are directly connected to the integument, they lack the neural connection and a cell with secretory function is connected to the seta. Our results show the existence of a well-developed sensorial component, but do not support the presence of glandular cells at the level of the epithelium. Secretory setae, as seen in Urbanek et al. (2011) and Di Palma et al. (2021), possess an empty core in continuity with the subcuticular epithelia and filled with the cellular processes of the secretory cells. On the contrary, the base of the hastisetae is continuous with the cuticle of the socket with a direct and obvious connection with the subcuticular space; furthermore, the absence of glandular structures or vesicles rich cells does not argue in favour of an secretory function. 2) the sections revealed how the general structure recalls the trichobothrium type of seta (*sensu* Keil, 1997) where the seta is connected to the cuticle via the socket membrane and it maintains the sensory function because of the presence of the neuron. These traits are in contrast with the modified seta definition.

The results indicate that although hastisetae can best be categorized among mechanoreceptors, they possess some peculiarities. Differently from the canonical trichobothria, in which the biomechanic apparatus is located in the proximity of the cuticle surface and the suspension of the seta by the socket membrane allows the seta to move and deflect, thus generating the mechanical stimulus, the hastiseta is characterized by the sinking of the socket in the cuticle and a substantial reduction of its lateral oscillation capacity. This modification is possibly associated with the specific function of the hastiseta, i.e. entangling predators (Nutting & Spangler, 1969; Mills & Partida, 1976; Ruzzier et al. 2021). In this process, the hastisetae can be detached from the integument of the larva through a mechanical traction that determines the breaking of the pedicel.

The perception of traction by the larva may have important implications since stronger the stimulus higher is the number of hastisetae detached and consequently greater is the threat to the larva. . A persistent stimulus can thus warn the larva about a concrete threat or differentiate it from an occasional encounter. Furthermore, the persisting stimulus may determine the implementation of defensive behaviours aimed to escape (as indicated in Ruzzier et al. 2021) or to maximize the possibility of detachment, and thus the trapping efficiency, of the hastisetae. As the lost hastisetae can not be regenerated until the next molt, it is also plausible that the detachment of many hastisetae, as for example after the predator attack, may induce moulting in the larva to restore the original hastisetae set; dermestid larvae are in fact capable of multiple and retrogressive molts (e.g. Beck, 1973; Klein & Beck, 1980).

The presence of the hastiseta collar (lateral chitin process) and the numerous chitin fibrils present at the base of the socket and the receptor lymph space, ensure a stable anchoring of the hastiseta that allows detachment only after a strong mechanical stimulus and in particular in case of a direct attack by the predator. This interpretation seems to be supported by the fact that, under natural conditions, *T. granarium* larvae rarely lose hastisetae due to environmental factors and due to the interaction with conspecifics (E. R. and M. K. personal observations).

Conclusions

The ultrastructure of the socket and the associated cells clearly indicate that hastisetae are a new type of defensive seta. Based on their structure and the new morphological features that have emerged, it is difficult to assign hastisetae to one of the categories of detachable bristles defined by Battisti et al. 2011. Hastisetae seem to present a hybrid condition, which can be qualified as detachable trichomes with an entangling defensive-perceptive function. This particular arrangement represents a unique case among the arthropods. It will be interesting in the near future to investigate the ultrastructure of hastisetae also in other species and genera of Megatominae in order to evaluate their diversity and possibly identify further traits associated with an active defence, such as that of *Anthrenus*, with respect to what has been shown for *Trogoderma*.

However, such a condition does not completely exclude the possibility that these setae contain substances responsible for inflammatory reactions in humans and animals. These compounds are possibly produced and accumulated in the hollow shaft during the synthesis of the hastiseta while the accessory glandular structures may have regressed after the hastiseta completion. The presence of the apical depression filled with an amorphous matrix could be a vestige of this ontogenetic process. A chemical profiling of the setae and the analysis of the transcriptome of selected synanthropic species will shed light on this still unclear nature of hastisetae.

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Conclusions

Prior to the present contribution very little was known about the diversity and the ecological role of the hastisetae. The complete review of the literature showed how the knowledge of the fine morphology of the hastisetae was substantially limited to few examples dating back to the middle of the last century (see chapter 1 and 2) and since then very little, and often superficially, has been published about. Furthermore, the importance of hastisetae as a diagnostic character useful for the identification of the larvae of Dermestidae Megatominae had never been fully addressed and generally confined to somewhat vague discussions.

The work carried out here brings back the attention to this unique type of setae, not only as regards their morphological, ecological and evolutionary aspects, but also for their implication in concrete applications, such as stored product science and human health. The morphological investigation on selected genera and species of Megatominae, some of which synanthropic, allowed to finally shed light on the great diversity of the hastisetae and how their features are important in the systematic arrangement of Megatominae, the evolution and differentiation of Dermestidae Megatominae in association to their unique feeding habits.

At the beginning of this research hastisetae were classified as detachable setae capable to be released only through the mechanical breaking of their most basal part (pedicel), differently from what was suggested in previous papers. This condition opened a new functional interpretation that led to the investigation presented in chapter 4.

The characterization of the apical part of the hastiseta, and especially the head, highlighted the great diversity of shape, sculpture and accessory structures that can be observed and allowed a deeper understanding of the biomechanical mechanism at the base of the hastisetae-based defense. The general constitutive plan of the hastiseta is generally conserved in all Megatominae, and this stability is directly linked to its primary function of mechanical trap directed against arthropod predators. The peculiar and unique radial symmetry, the septation, the microsculpture and the gaff shape of the apical part of the longitudinal processes make the head of the hastiseta a perfect trapping structure for predator’s legs, antennae, palps and setae. The single or synergic action of hastisetae as entangling mechanism may have a strong discouraging effect on predators and eventually bring them to death. This research has pointed out the great efficiency of hastisetae in targeting a wide dimensional range of predators opposed to the minimal energetic cost that the larva must sustain. This tendence perfectly agrees with the general energy saving strategy adopted by all Megatominae larvae due to their diet poor in water and nutrients.

The morphological evidence gathered, combined with behavioural observation, allowed to demonstrate how Megatominae evolved at least two completely different defensive strategies (passive or active defence). It was particularly interesting to note that the two identified classes matched with the two major lineages of Megatominae (Anthrenini and Megatomini) and that, according to amber fossils, these behaviours and morphological traits may have evolved already in the late Cretaceous, soon after the differentiation of Megatominae from the Attageninae+Trinodinae clade. The observations provided here, together with integration with new data, could thus substantially contribute to solving and understanding the systematics of this subfamily and to clarify its evolution towards the ecological niche exploited by its members (scavengers larvae adapted to feed on nutrients and water poor substrates).

The peculiarity of hastisetae in having a blunt apex filled with an amorphous matrix, in association with the hollow core, seemed to suggest, at the beginning of this project, a possible secretory function associated with hastisetae. This idea was also supported by the interpretation of hastisetae such as "modified setae" initially provided in Chapter 3.

The ultrastructure investigation (Chapter 4) completely overturned this previous interpretation, revealing instead how hastisetae belong to a type of hairs (trichomes) unique in their kind, combining simultaneously perceptive and defensive function. Hastisetae are in fact mechanoreceptors capable of perceiving traction stimuli, generally caused by interaction with a predator. This type of setae present, in fact, all the basic features of a trichome sensilla (seta connected to the integument via socket membrane and presence of a functional neuron); however, the specific modification of the base of the seta, of the socket and the associated subcuticular structures indicate that the perception of the stimulus is strictly linked to the specific function of the hastiseta to entangle and be detached. In association to the discovery of a functional sensorial apparatus, the apparent lack of secretory cells seems to support the incapability of hastisetae of actively secreting compounds involved in the ecology of the larva, as it was originally hypothesized.

The new knowledge gained constitutes an important step forward in the coverage of the biology and ecology of the larvae of Dermestidae Megatominae, although it has to be verified in other species of the group. A better understanding of the nature of the hastisetae may have important applied consequences.

Several species of Megatominae are pests on stored commodities and included in most of the quarantine lists worldwide; furthermore, their remains (hastisetae included) are important contaminants on stored food products (both raw and processed). The identification of the pest or what contaminant is present is thus crucial to avoid potential invasive species introduction or the import and marketing of good not complying the market, health and safety standards. In case of rapid and reliable detection, hastisetae may serve as an important identification tool since the characters present on the setae are very informative and allow a relatively fast identification at least at genus level. The possibility to use hastisetae is of further value especially when only insect fragments are available and molecular detection techniques are not applicable.

Hastisetae are also important contaminant of work and living environment and may constitute a substantial fraction of dust. Medical literature provides multiple observations that seem to link hastisetae to allergic or asthma-like symptoms; furthermore, direct contact and ingestion of larvae or their remnants (including hastisetae) seem to be the cause of strong inflammatory reactions. The mechanism through which the hastisetae would cause such symptoms is still unclear, but the observations conducted so far would indicate that hastisetae are not capable of penetrating the epithelia and the initiation of the inflammatory process is not similar to those caused by detachable setae of Lepidoptera or Theraphosidae spiders. It seems plausible that hastisetae are capable to vehiculate compounds, stored during the synthesis of the seta, capable of triggering the insurgence of the inflammatory symptoms. The peculiar morphology of the hastiseta seems to indicate that this unique type of detachable setae evolved primarily as defensive system against other arthropods and that those observed on humans are side effects, probably restricted to predisposed subjects.

What has been presented here represents not so much an arrival but a new starting point, especially if we consider that only a few Megatominae representatives have been studied and that the vast majority of the larvae still remain completely unknown. A better view of the real diversity that characterizes Dermestidae Megatominae, and in particular their larvae, is of fundamental importance in understanding their biological success: the evolution of the hastisetae seems in fact to have been the key factor linked to the diet of the larvae that promoted the diversification of the subfamily. In the early future, it will be important to recover and describe the larvae of as many species as possible in order to fill the knowledge gap still existing, linking morphological diversity with habits and ecological niche. Since the adults of Megatominae are highly derived and possess a general morphological convergence (they serve almost exclusively for reproduction and dispersal) only a detailed revision of larval morphological features, possibly in association with DNA based data, may provide a full systematic reassessment of the whole subfamily and redefine the evolution of the different tribes and lineages. In this context hastisetae have already proven to possess a combination of characters and apomorphies capable to provide a useful hint in splitting tribes, lineages an genera apart.

Health and safety implication remains the most unexplored and possibly the most challenging research field linked to hastisetae. Without any doubt a specific focus needs to be developed on synanthropic species to establish risk factors and defining the exposition conditions that determine the insurgence of inflammation symptoms in humans. Both morphological and ultrastructural features indicates that hastisetae are not capable to penetrate the epithelia (such as skin) nor are capable to secrete any inflammatory substance.

Consequently, two future research lines should be taken in consideration: 1) hastisetae may be vector or possess some associated cuticular compound that may work as inflammation enhancers, alone or in combination with chitin. In this case, it will be important to characterize the molecules associated to the hastisetae, both on the surface or inside the hollow core. In this sense, a full chemical profiling of the most harmful species would be fundamental to spot the attention on specific compounds, especially after a cross comparison with those belonging to not harmful taxa. Since we cannot exclude an active production of defensive substances by the larva, integrating research with a transcriptomic approach would also be of great help.

As an alternative 2) hastisetae may not be responsible for the effects caused on humans. Spicisetae are second type of setae present on dermestid larvae (not only Megatominae) that possess some features recalling the urticating hairs of Theraphosidae (new world tarantulas) and Thaumetopoeinae (processionary moths) (e.g. pointed, sharped apex and a stalk covered in barbs). This groups of setae have been neglected so far, possibly because less characteristic and less eye catching in comparison to hastisetae. This is certainly a topic that should not be overlooked and which could guarantee, if not an alternative, an important cofactor in determining the harmfulness of Megatominae to humans.

In conclusion, this study clarified the structure and function of a new type of detachable seta on arthropods and this knowledge opens the way to further exploration of the diversity of the hastisetae in the group of Dermestidae Megatominae. The analysis will have to include genomic and metabolomic approaches to understand the harmfulness of the hastisetae as environmental contaminants of food and stored products, as well as of human houses.

Supplementary material 1

Anthrenocerus australis (Hope, 1843)

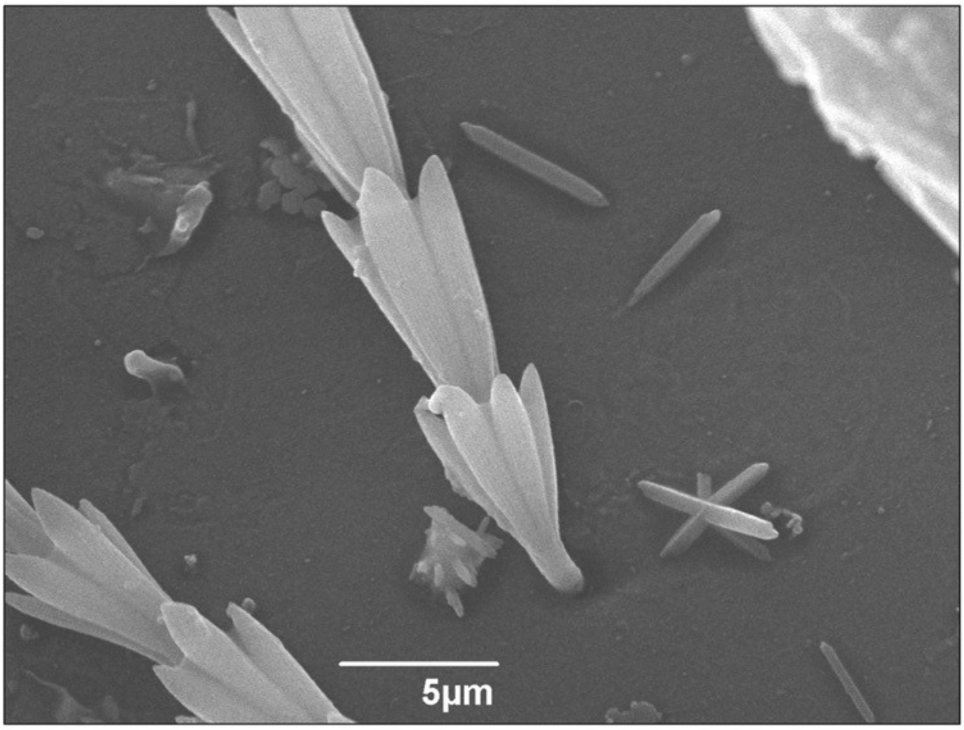

Fig. A. 4300x. Detail of the insertion of the hastiseta on the cuticle (thoracic hastiseta).

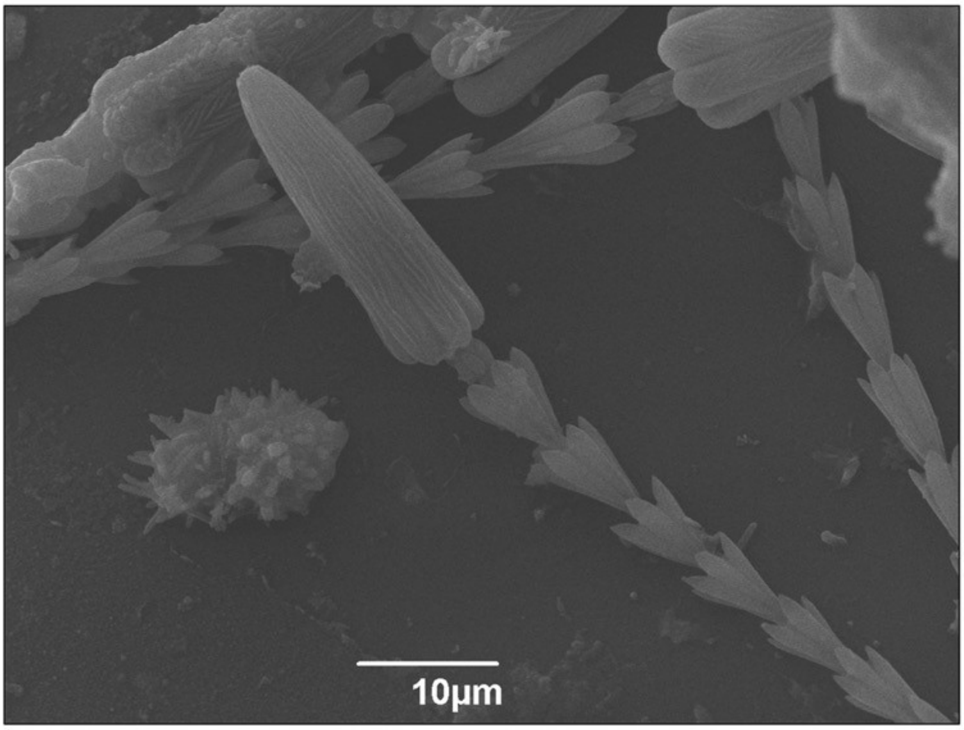

Fig. B. 1900x. Detail of the apical part of the hastiseta (thoracic hastiseta).

Anthrenocerus australis (Hope, 1843)

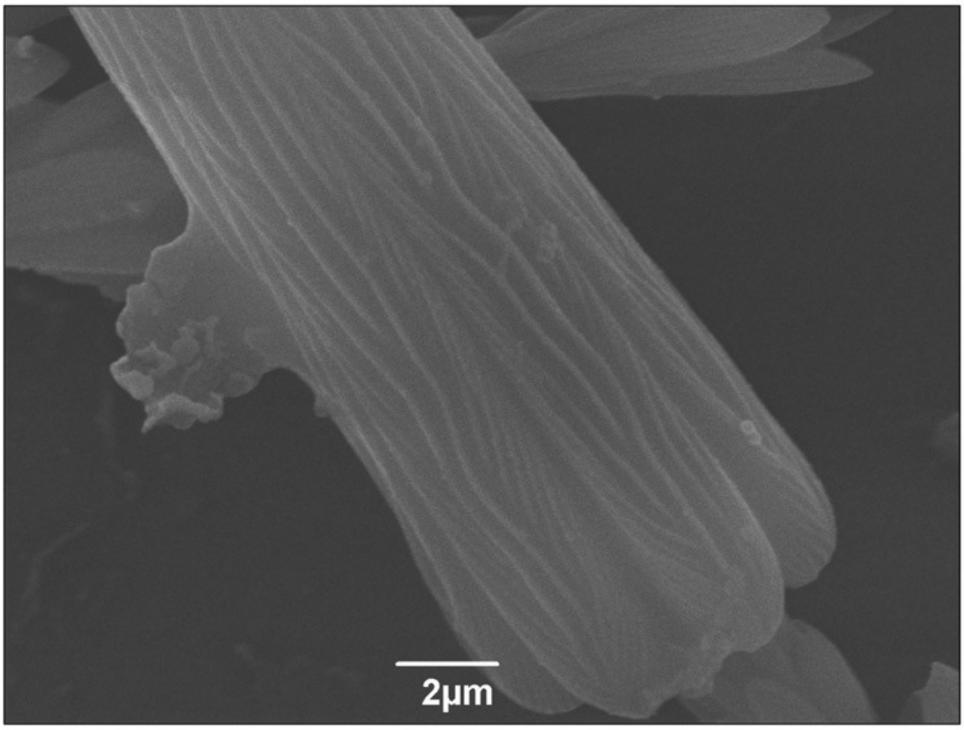

Fig. C. 7000x. Head of the hastiseta, detail of the knurls on the longitudinal processes.

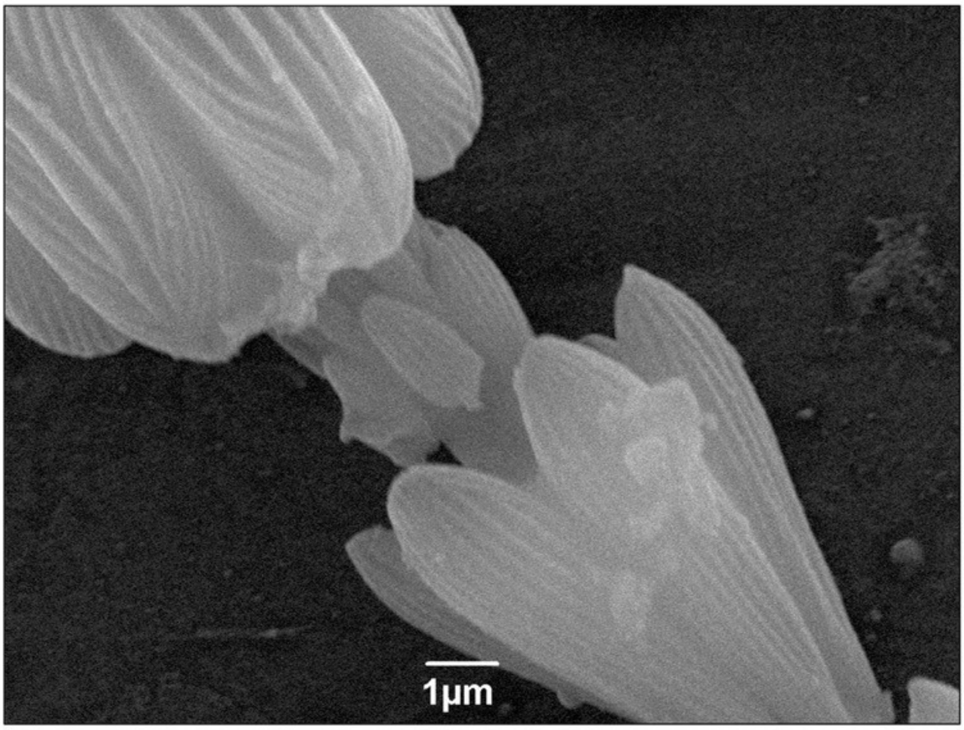

Fig. D. 10000x. Detail of the ultimate rosette and the set of scales on the shaft.

Anthrenocerus australis (Hope, 1843)

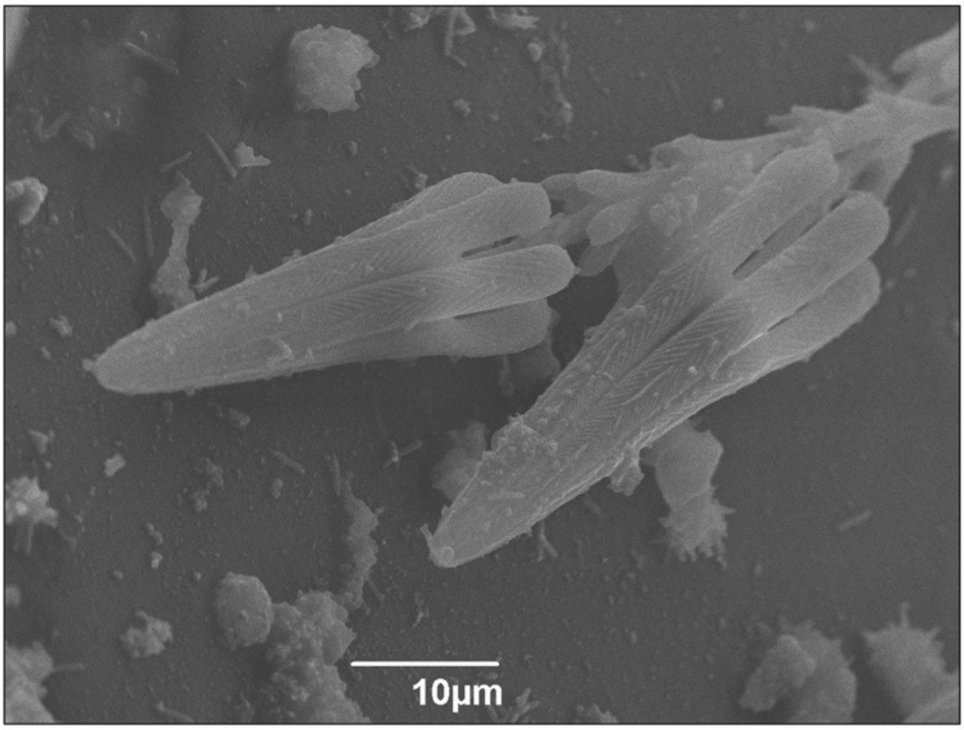

Fig. E. 2000x. Head of the hastiseta belonging to the tufts on the last abdominal segments.

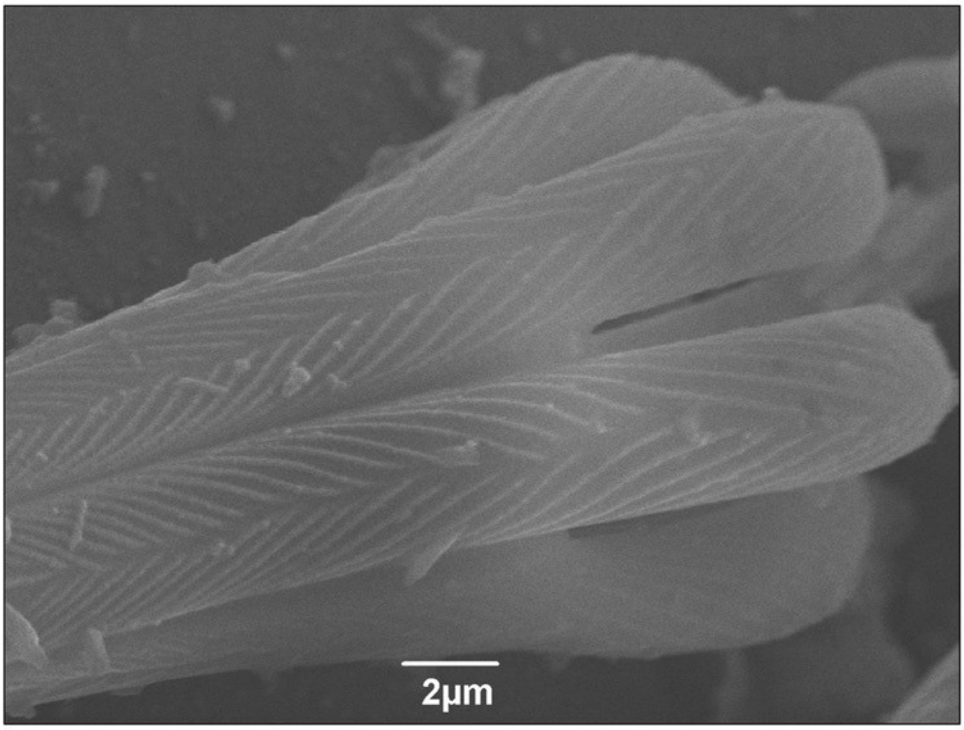

Fig. F. 6500x. Detail of the knurls covering the longitudinal processes.

Anthrenocerus australis (Hope, 1843)

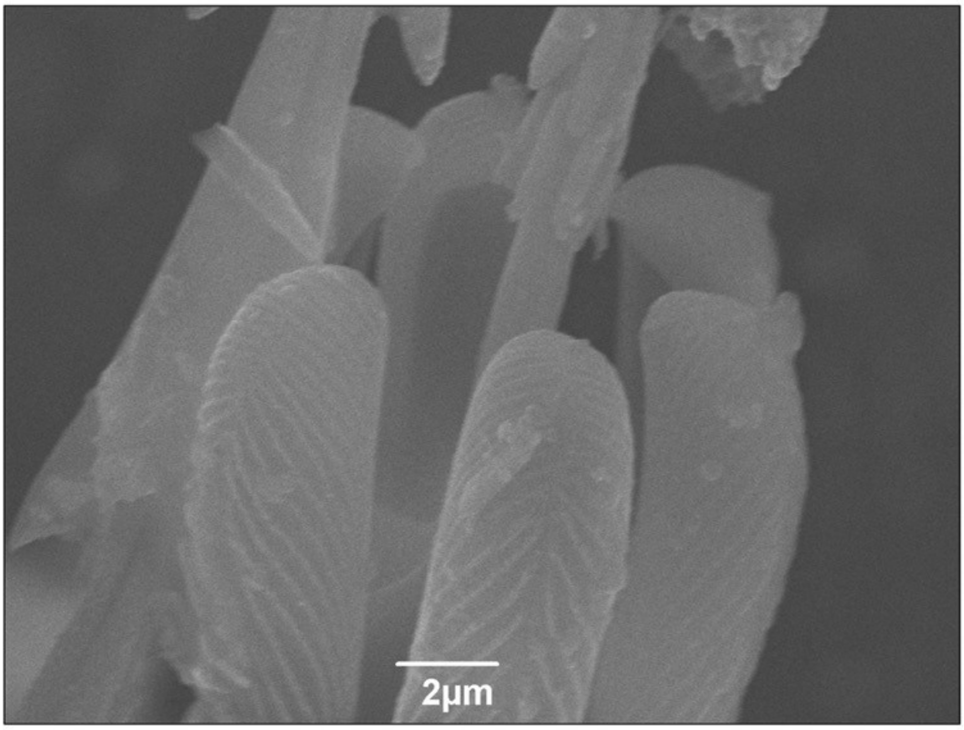

Fig. G. 7000x. Detail of the apical processes of the head of the hastiseta showing the concave inner part

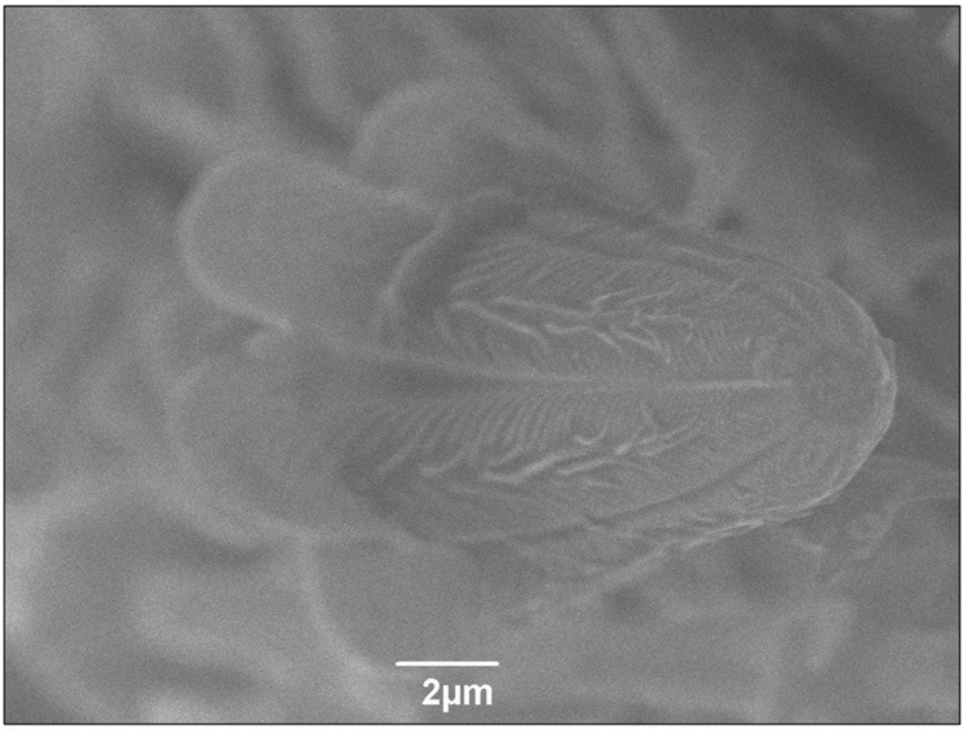

Fig. H. 7000x. Apex of the head of the hastiseta showing the circular depression

Supplementary material 2

Anthrenus (*Anthrenus*) *latefasciatus* Reitter, 1892

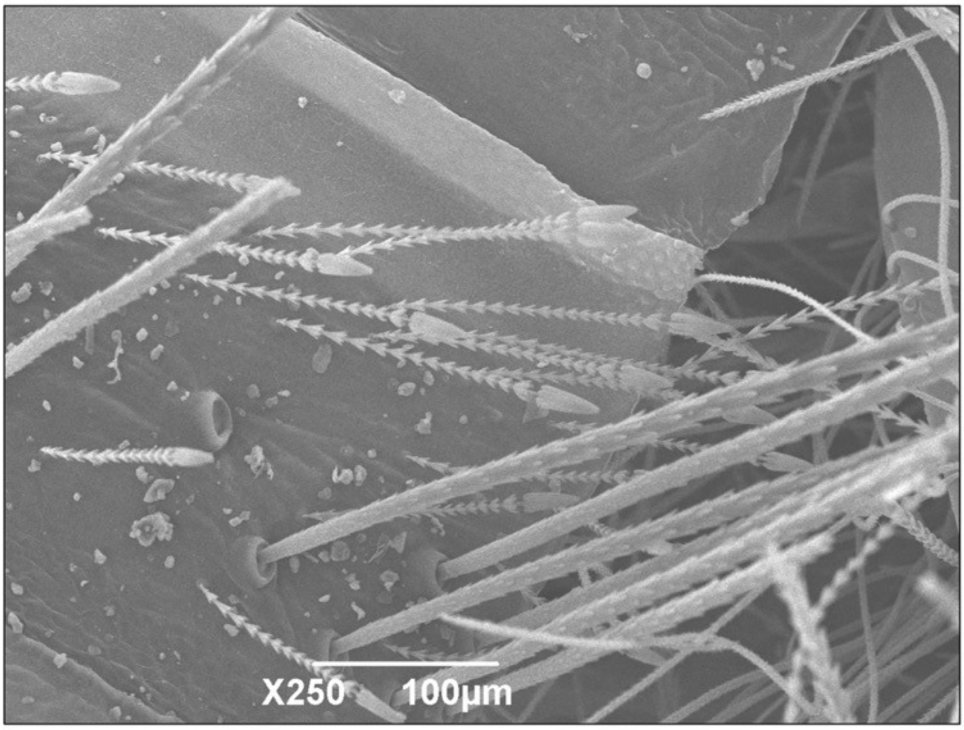

Fig. A. 250x. Detail of the hastisetae on first abdominal tergites.

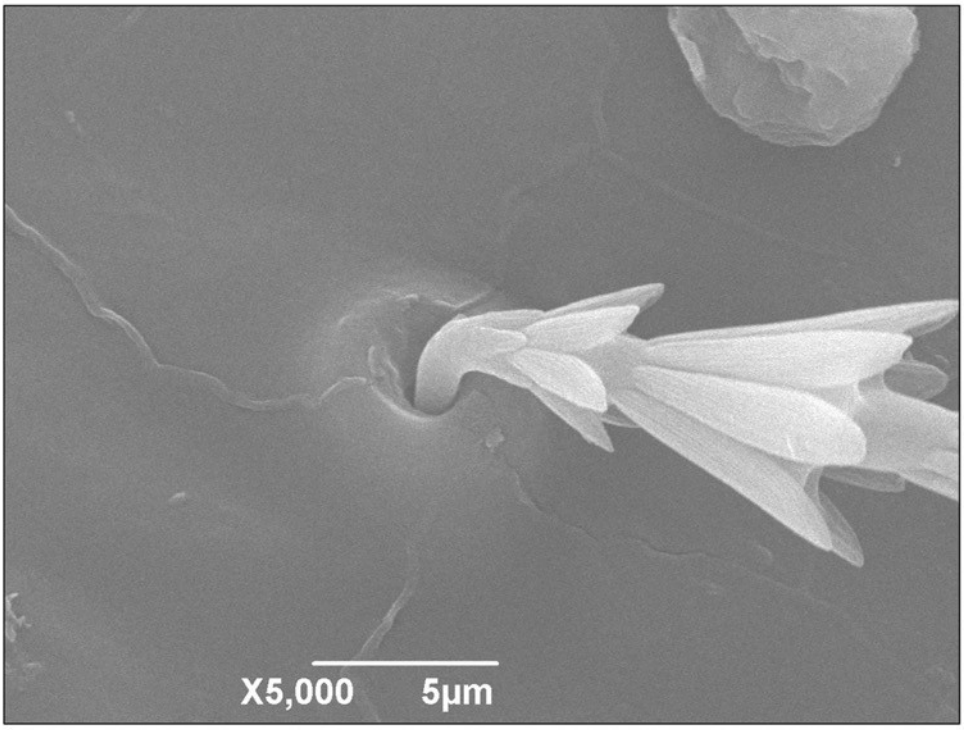

Fig. B. 5000x. Detail of the insertion of the hastiseta on first abdominal tergites

Anthrenus (*Anthrenus*) *latefasciatus* Reitter, 1892

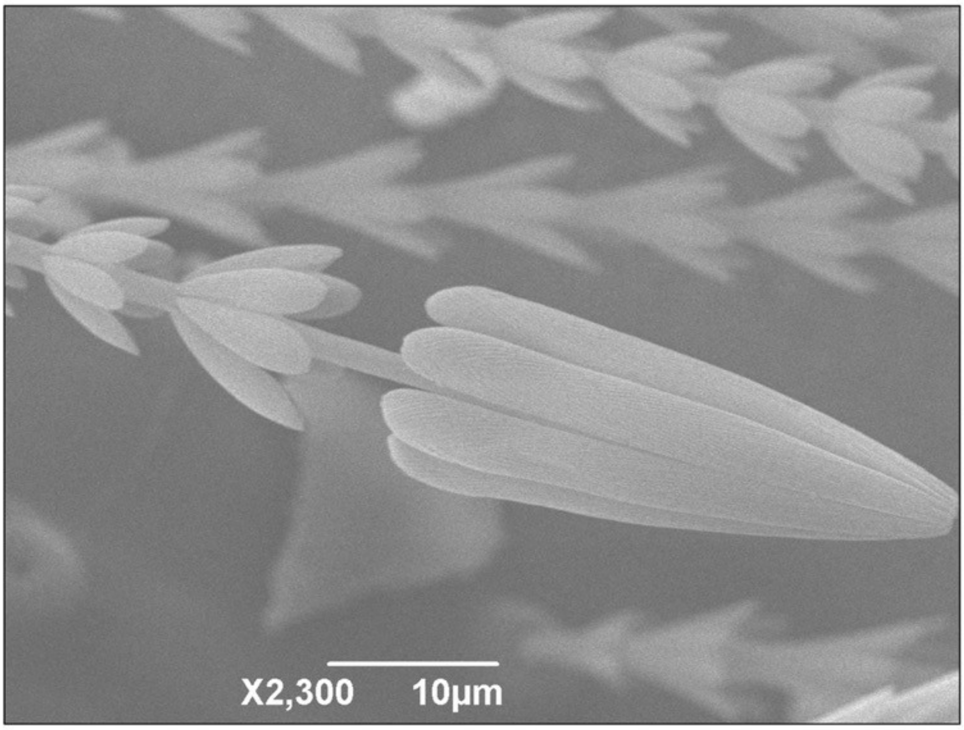

Fig. C. 2300x. Detail of the hastisetae on first abdominal tergites.

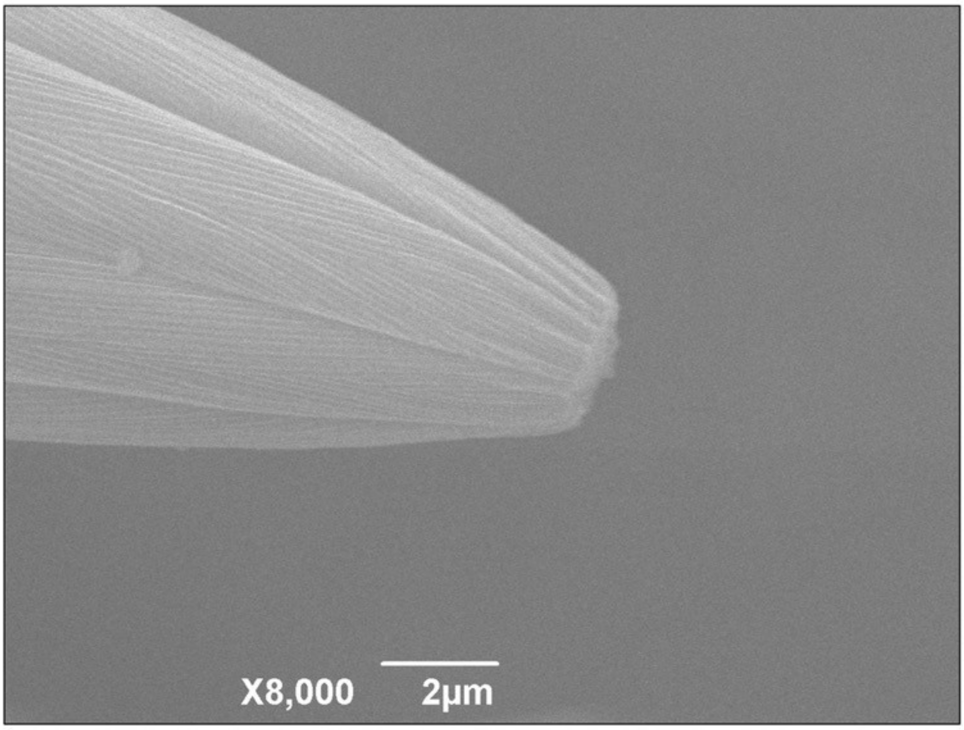

Fig. D. 8000x. Detail of the apex of the head of the hastiseta, lateral view

Anthrenus (*Anthrenus*) *latefasciatus* Reitter, 1892

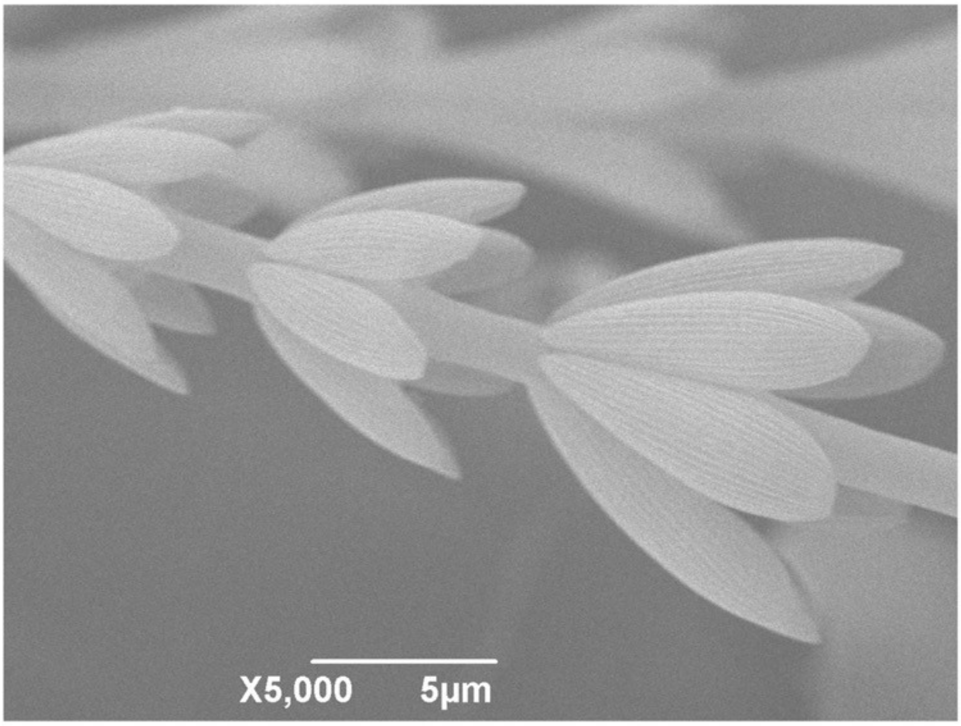

Fig. E. 5000x. Last three rosettes of an hastiseta, detail.

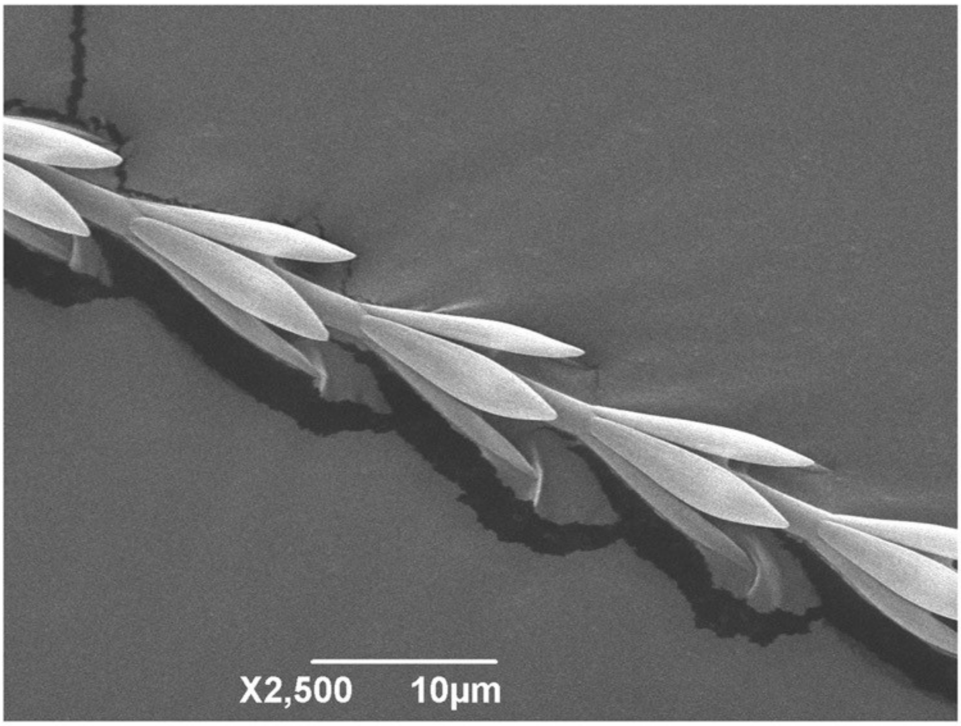

Fig. F. 2500x. Series of rosettes constituting the central section of the stalk.

Anthrenus (*Anthrenus*) *latefasciatus* Reitter, 1892

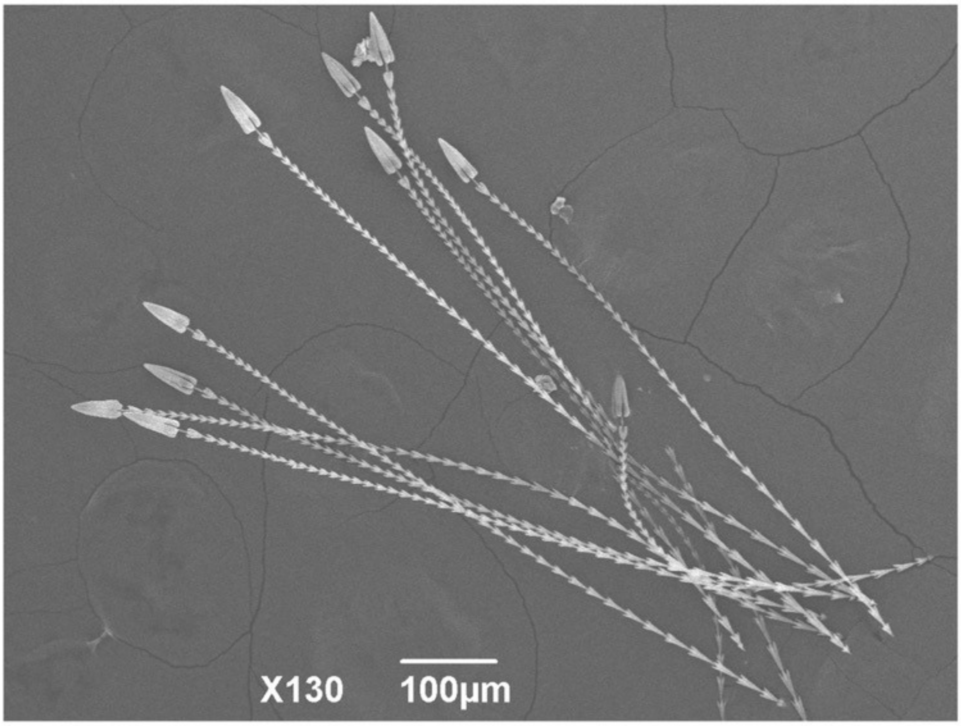

Fig. G. 5000x. Detached hastisetae belonging to the tufts on abdominal segments 5th-7th.

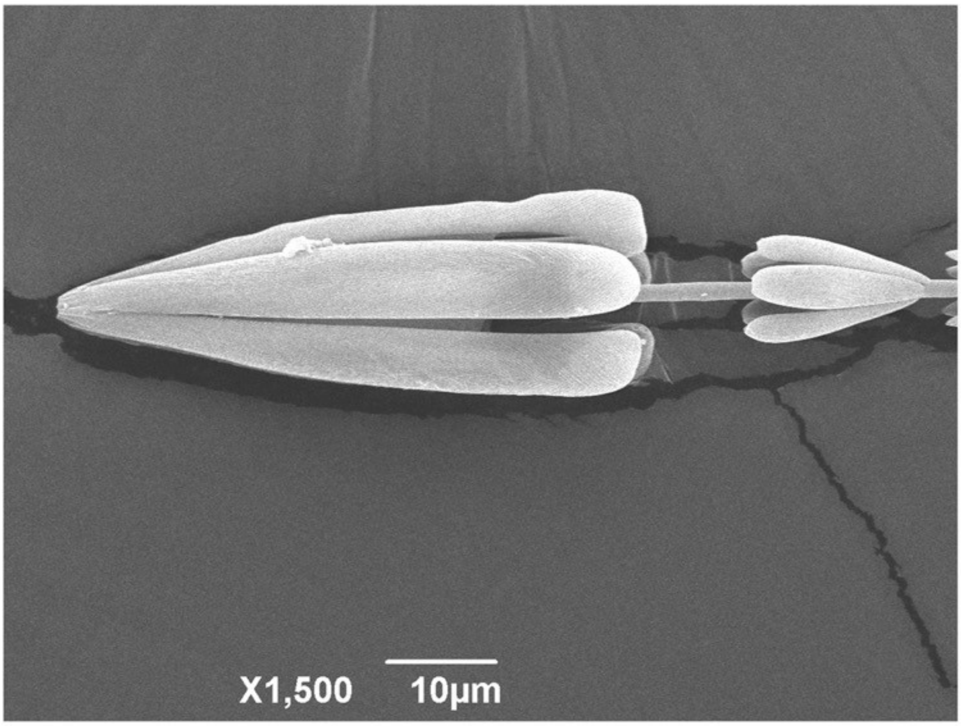

Fig. H. 2500x. Ultimate rosette and head of the hastiseta on 5th-7th segments, lateral view.

Anthrenus (*Anthrenus*) *latefasciatus* Reitter, 1892

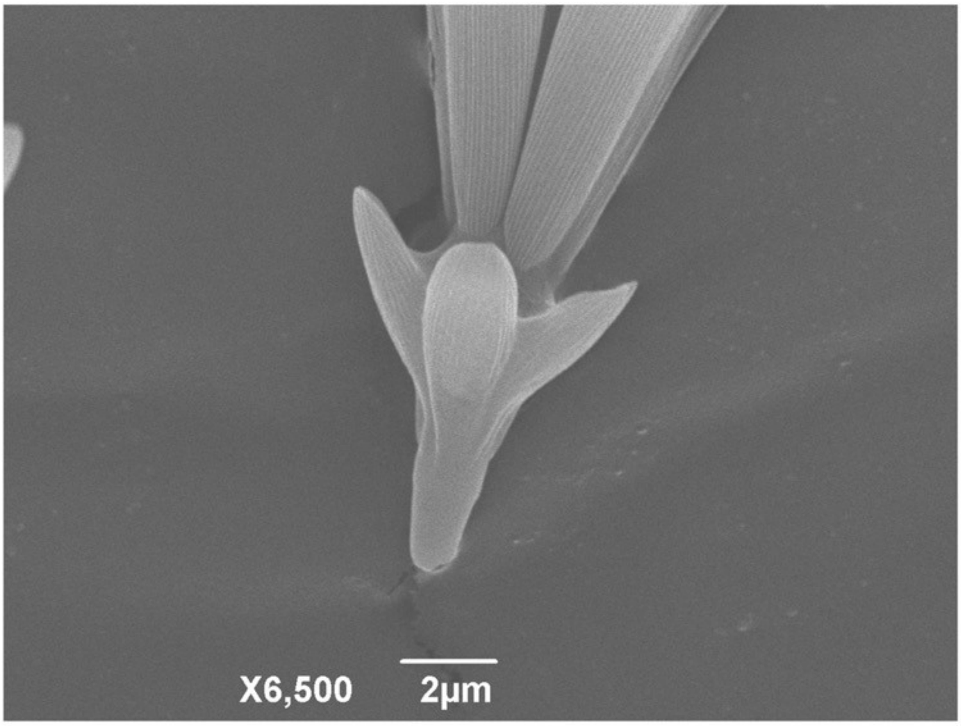

Fig. I. 5000x. Detail of the peduncle of one hastisetae belonging to 5th-7th segments.

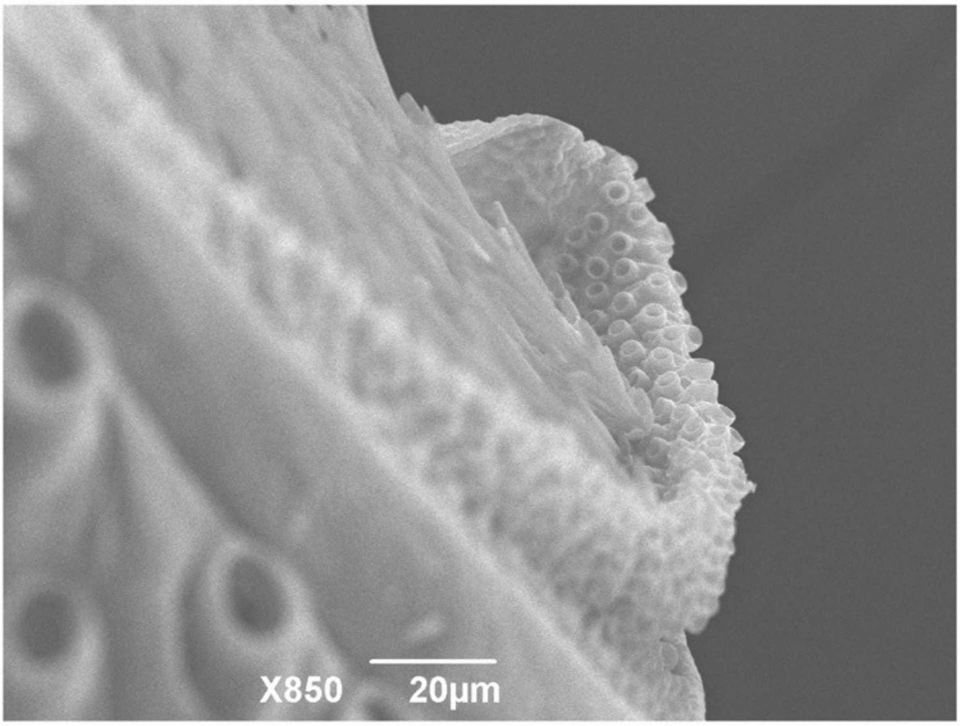

Fig. J. 2500x. Detail of the membranous part of the 6th abdominal tergite showing the insertion of the hastisetae.

Supplementary material 3

Anthrenus (*Anthrenus*) *picturatus makolskii* Mroczkowski, 1950

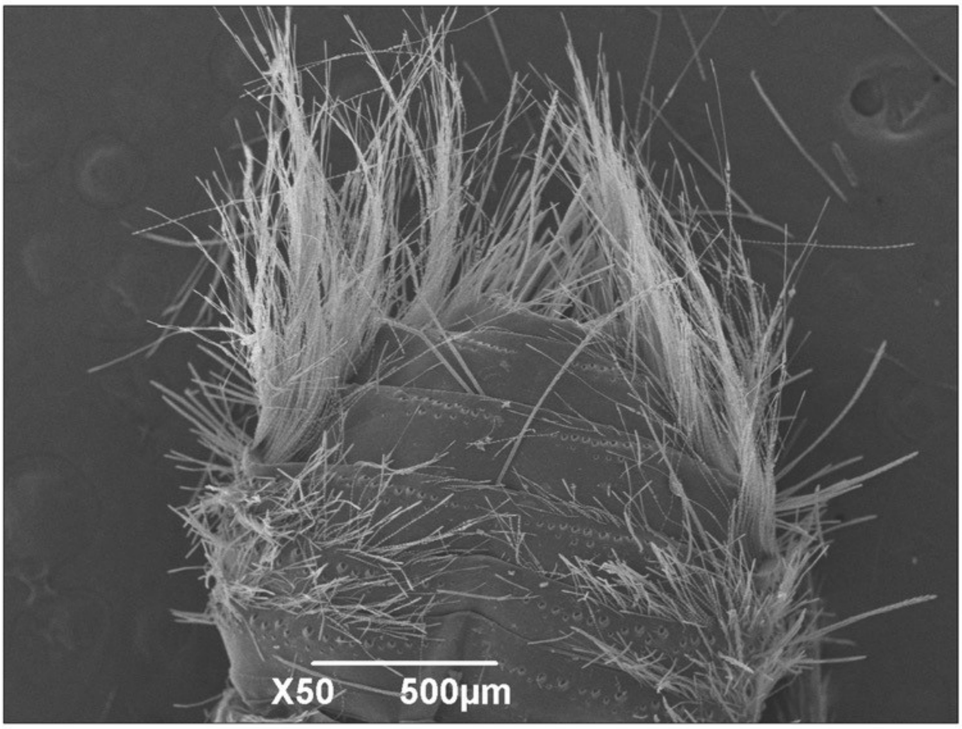

Fig. A. 50x. Last abdominal segments of a mature larva, dorsal view.

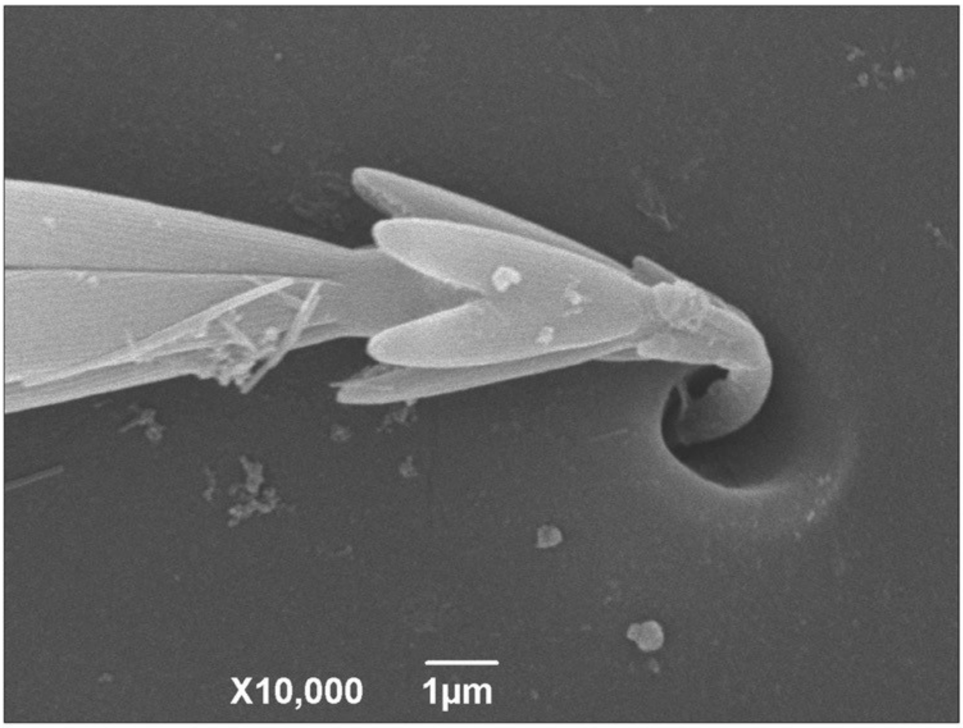

Fig. B. 10000x. Detail of the insertion of an hastiseta on a thoracic tergite

Anthrenus (*Anthrenus*) *picturatus makolskii* Mroczkowski, 1950

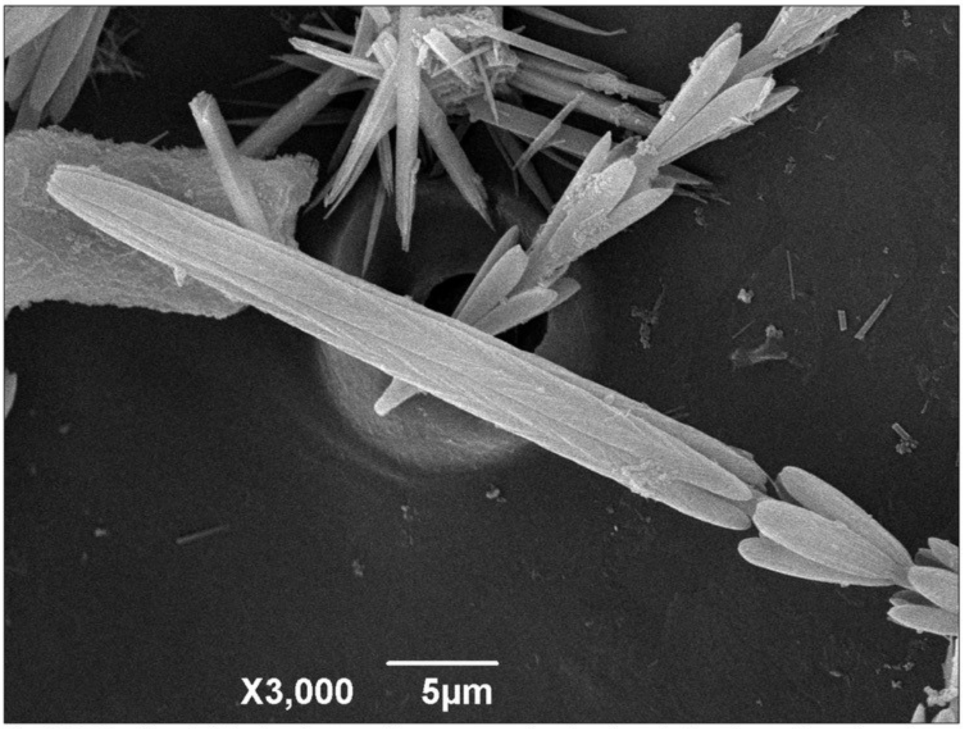

Fig. C. 3000x. Head of the hastiseta and last rosette of an hastiseta belonging to thoracic and first abdominal segments.

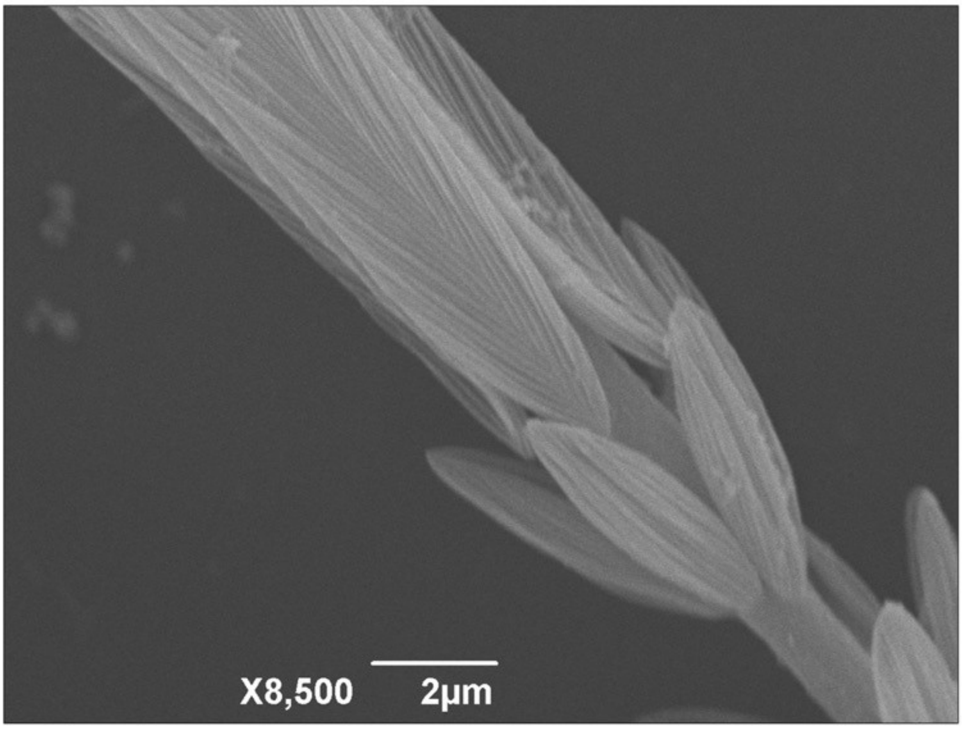

Fig. D. 10000x. Detail of the last rosette of an hastiseta belonging to thoracic and first abdominal segments.

Anthrenus (*Anthrenus*) *picturatus makolskii* Mroczkowski, 1950

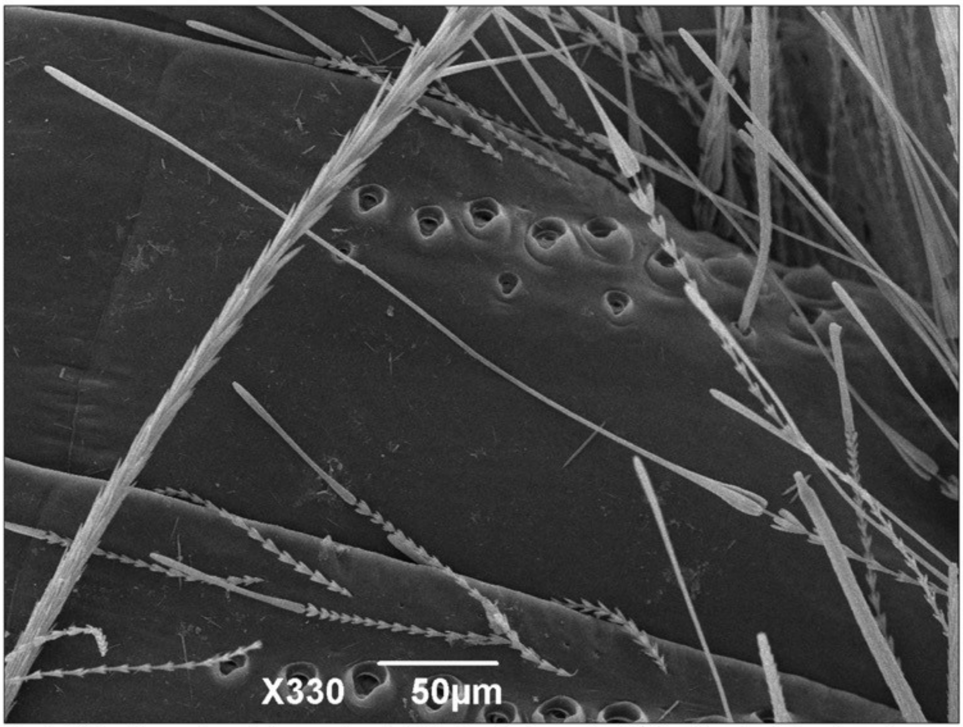

Fig. E. 330x. Comparison between head of the hastiseta on the 6th tergite: short heads - setae attached on sclerotized part of the tergite; long heads – setae on the membranaceous tergite.

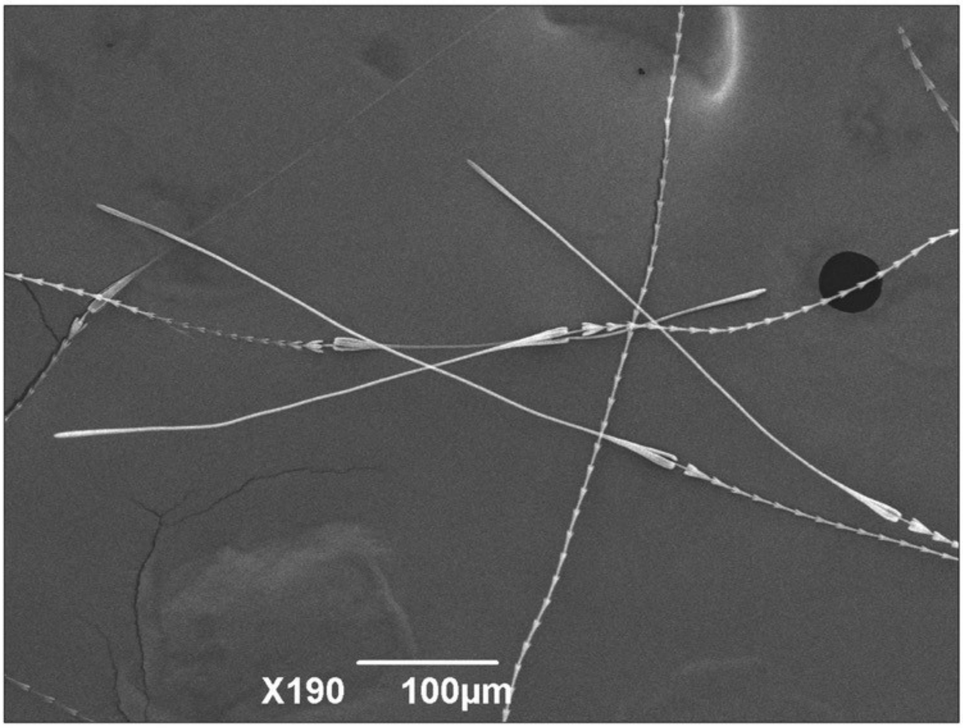

Fig. F. 190x. Head of the hastisetae belonging to the tufts on the last abdominal tergites.

Anthrenus (*Anthrenus*) *picturatus makolskii* Mroczkowski, 1950

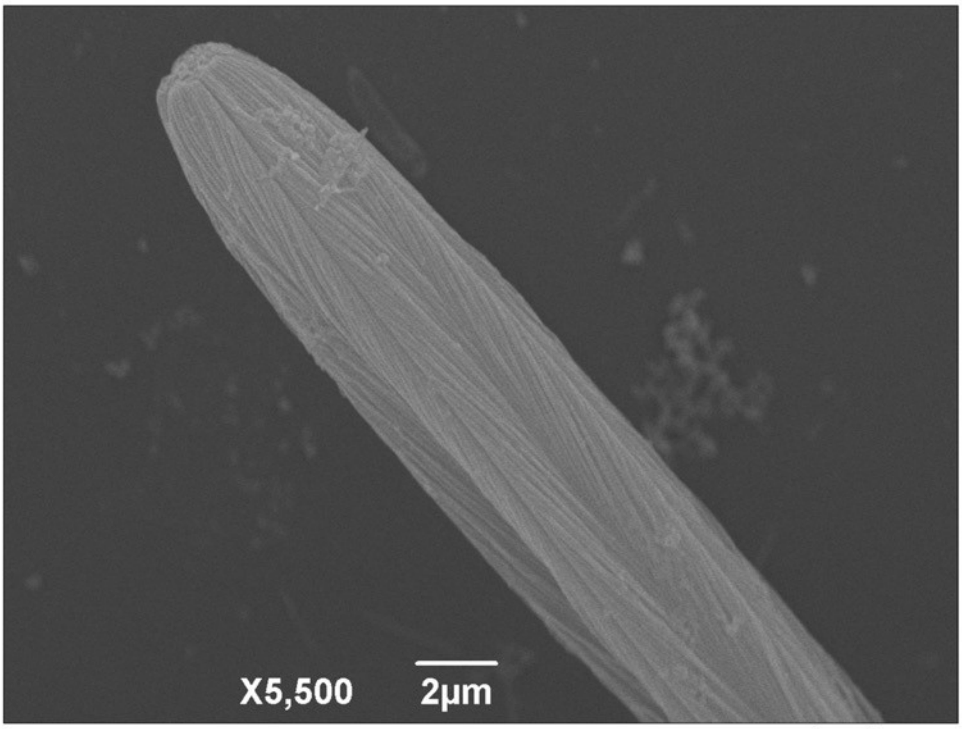

Fig. G. 5500x. Detail of the apex of the head of hastiseta of the tufts on segment 5th to 7th.

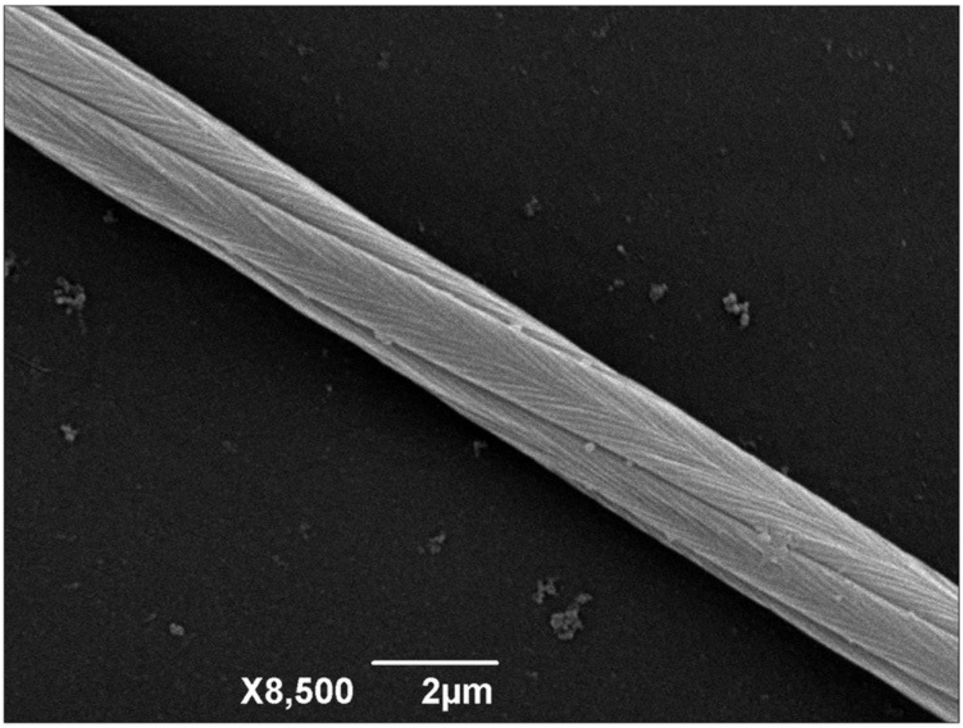

Fig. H. 8500x. Detail of the head of the hastiseta showing its left-handed torsion and knurls orientation.

Anthrenus (*Anthrenus*) *picturatus makolskii* Mroczkowski, 1950

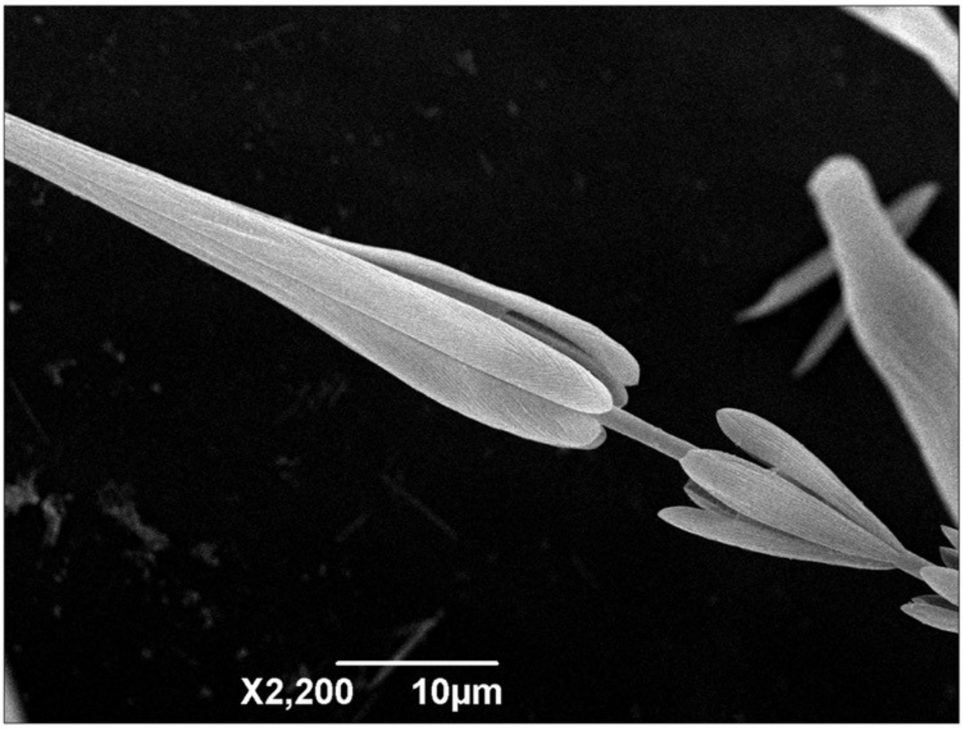

Fig. I. 2200x. Detail of the apex of the longitudinal process of the head of the hastiseta and the last rosette.

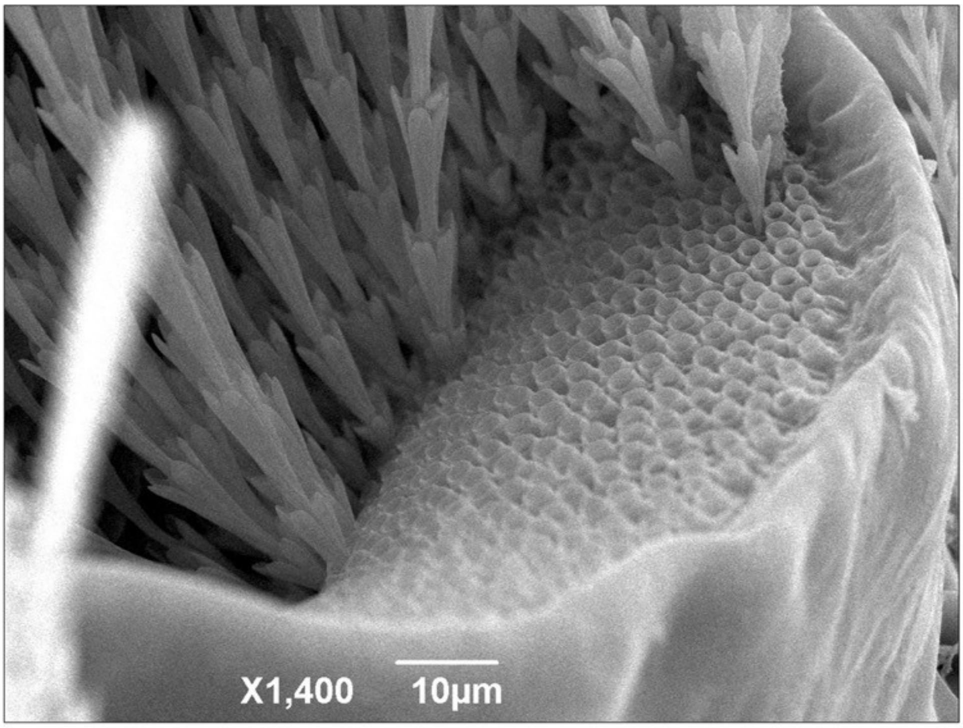

Fig. J. 10000x. Insertion of the hastisetae on the membranaceous part of the 5th abdominal tergite.

Supplementary material 4

Anthrenus (*Anthrenus*) *scrophulariae scrophulariae* (Linnaeus, 1758)

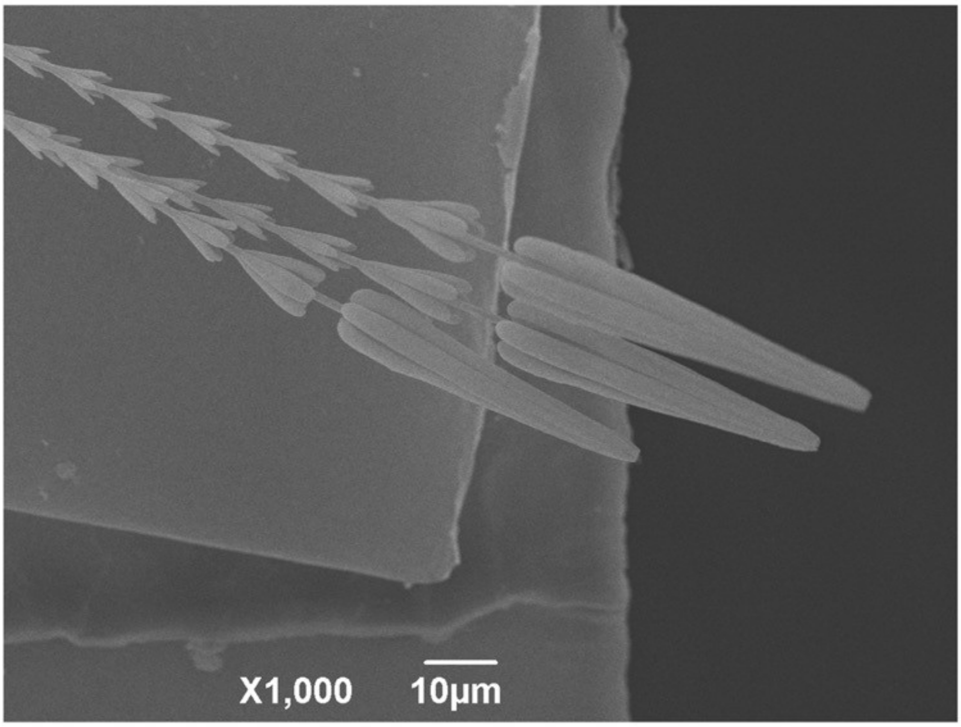

Fig. A. 35x. Apex of the hastisetae on thoracic and first abdominal tergites.

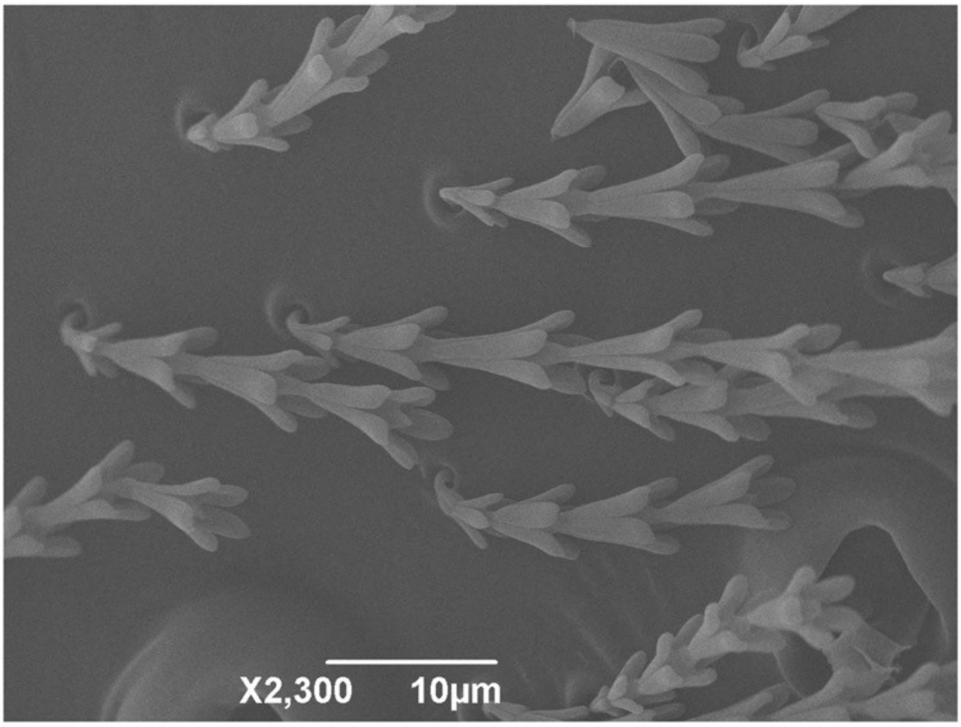

Fig. B. 130x. Detail of the insertions of the hastisetae on thoracic and first abdominal tergites..

Anthrenus (*Anthrenus*) *scrophulariae scrophulariae* (Linnaeus, 1758)

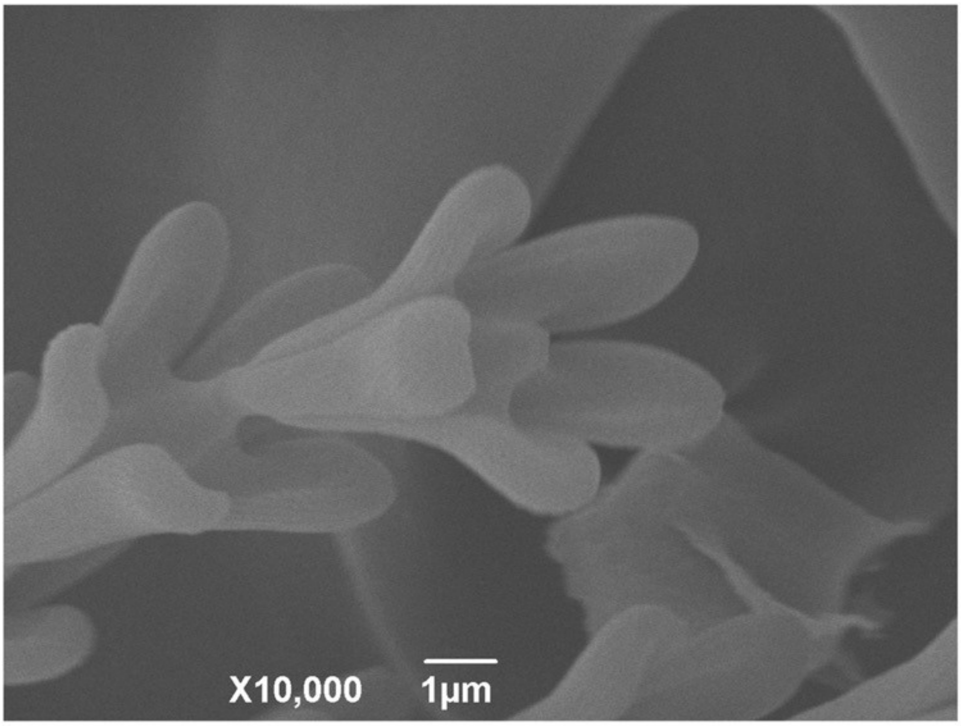

Fig. C. 35x. Detail of a broken hastiseta showing the general structure of one rosette.

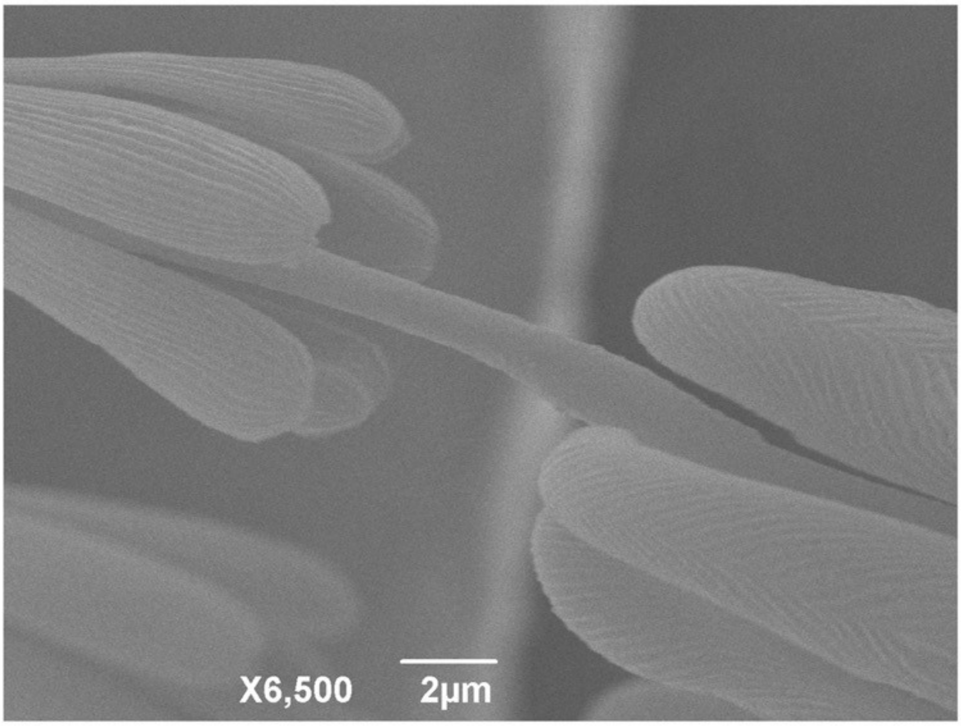

Fig. D. 130x. Detail of the last rosette and the stalk before the head of the hastiseta.

Anthrenus (*Anthrenus*) *scrophulariae scrophulariae* (Linnaeus, 1758)

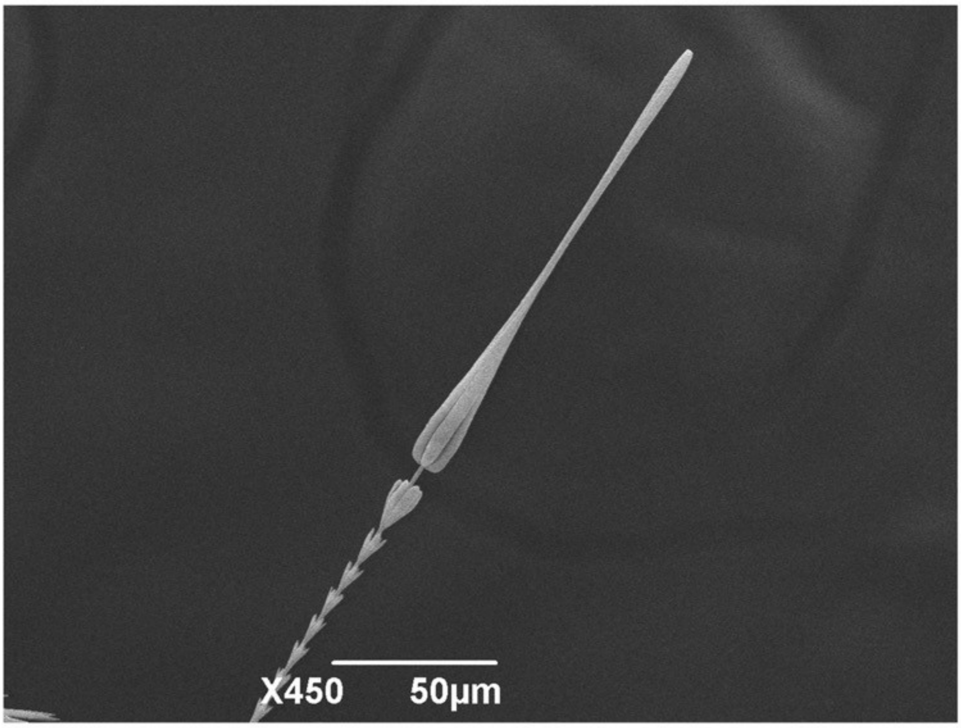

Fig. E. 35x. Apex of an hastiseta belonging to the tufts on the last abdominal tergites..

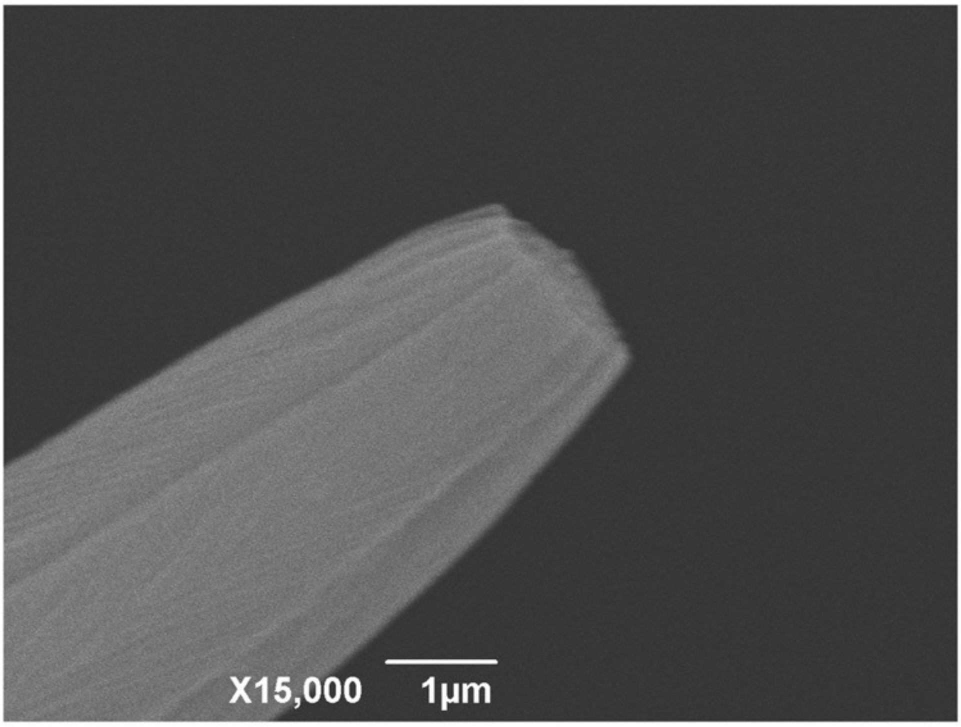

Fig. F. 130x. Detail of the of the head of the hastiseta, lateral view..

Anthrenus (*Anthrenus*) *scrophulariae scrophulariae* (Linnaeus, 1758)

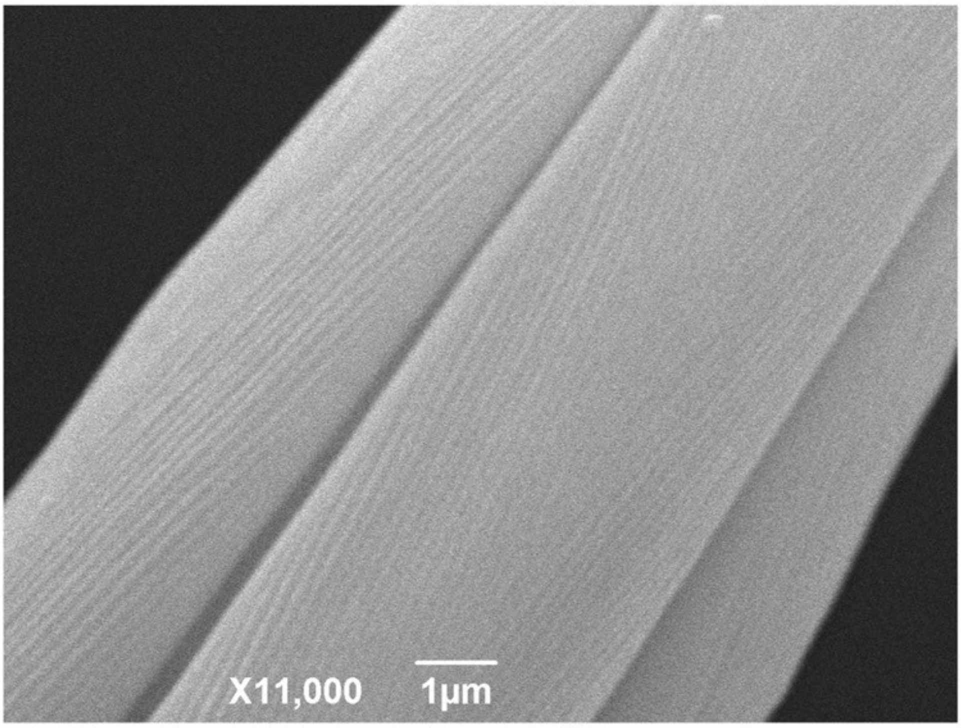

Fig. G. 35x. Detail of the knurls covering the longitudinal processed of the head.

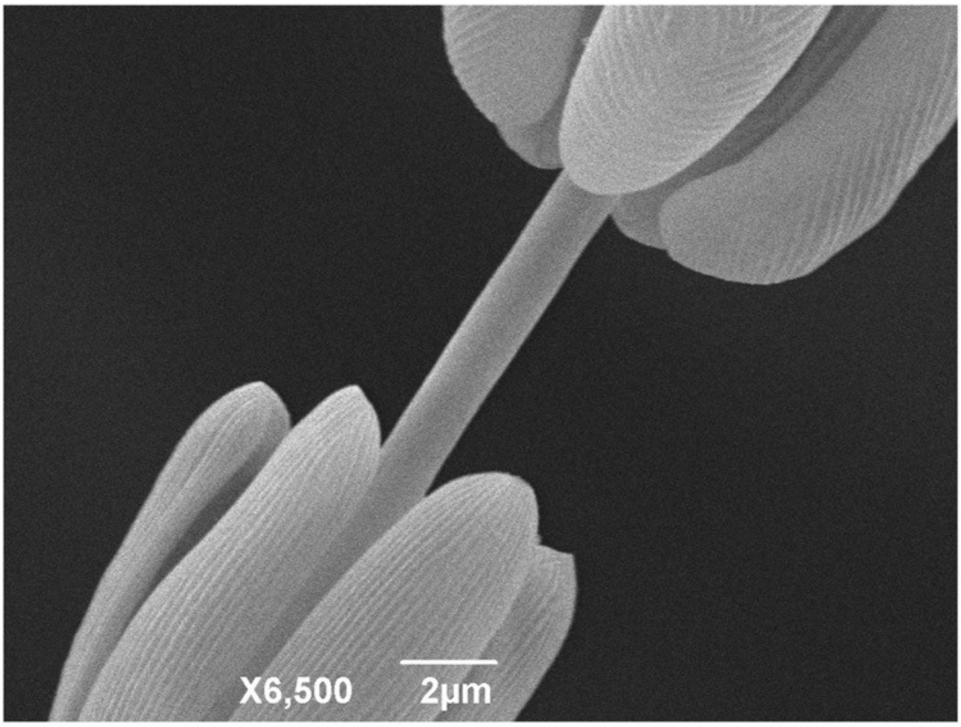

Fig. H. 130x. Detail of the stalk prior to the head of the hastiseta..

Supplementary material 5

Anthrenus (*Florilinus*) *olgae* Kalík, 1946

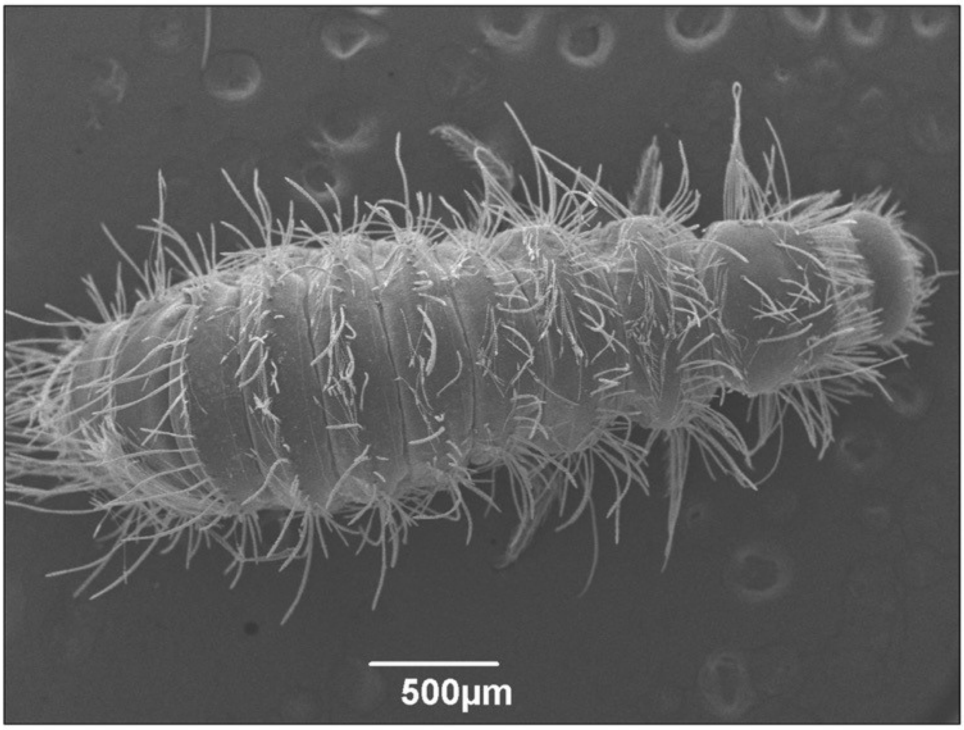

Fig. A. 35x. Mature larva, dorsal view.

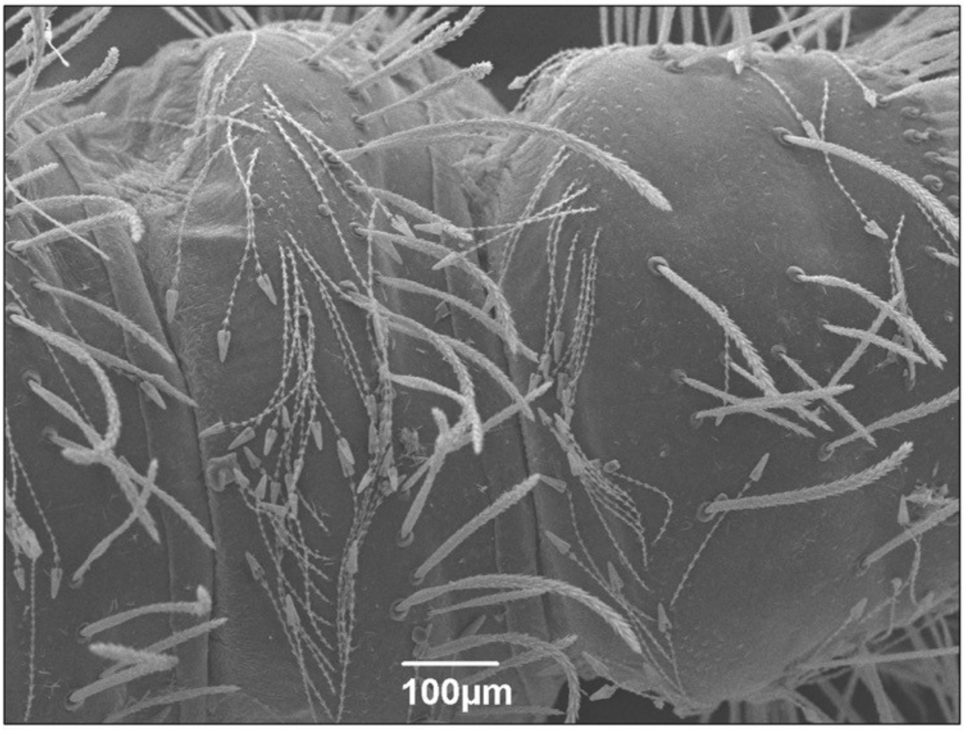

Fig. B. 130x. Detail of the first and second thoracic tergite, dorsal view.

Anthrenus (*Florilinus*) *olgae* Kalík, 1946

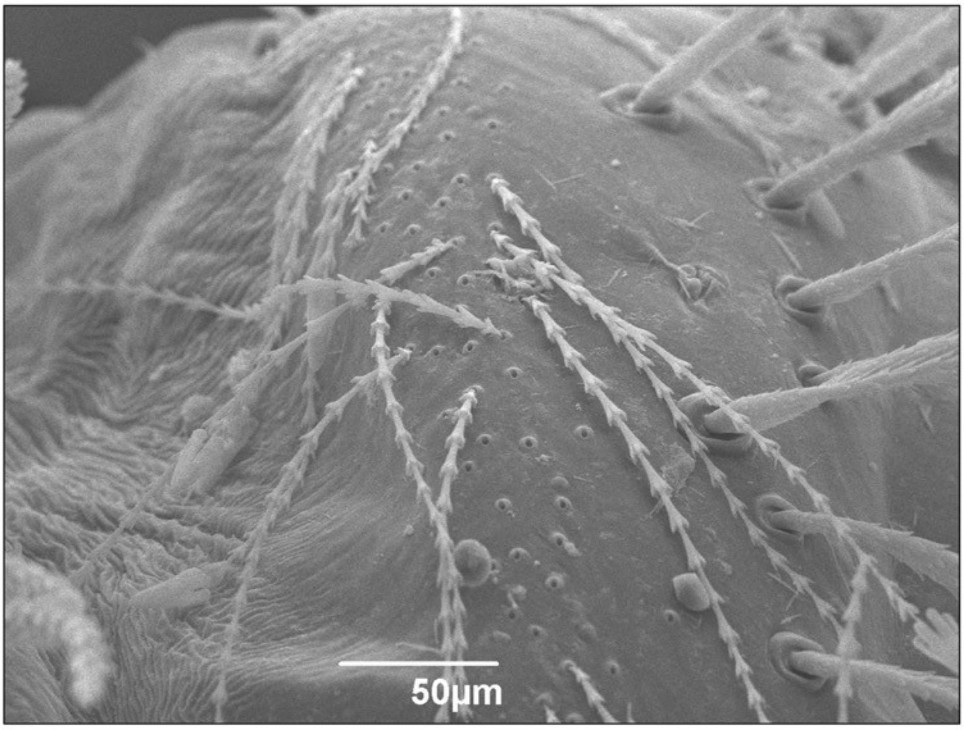

Fig. C. 430x. Band of hastisetae on the posterior margin of the second thoracic tergite

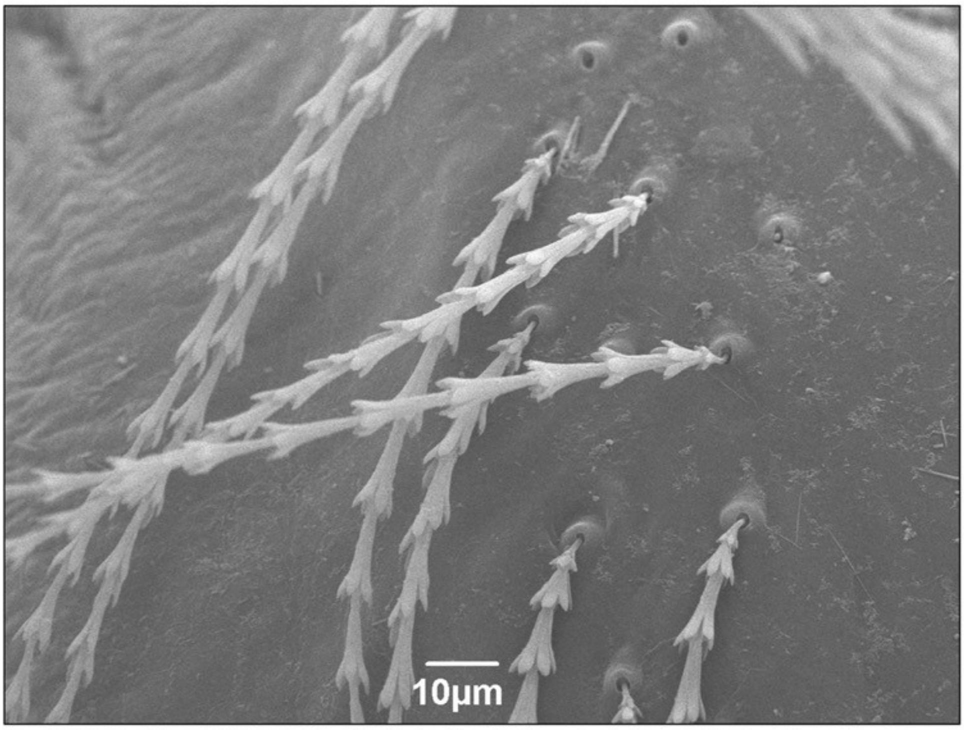

Fig. D. 1000x. Group of hastisetae displaying the similar orientation on the tergite

Anthrenus (*Florilinus*) *olgae* Kalík, 1946

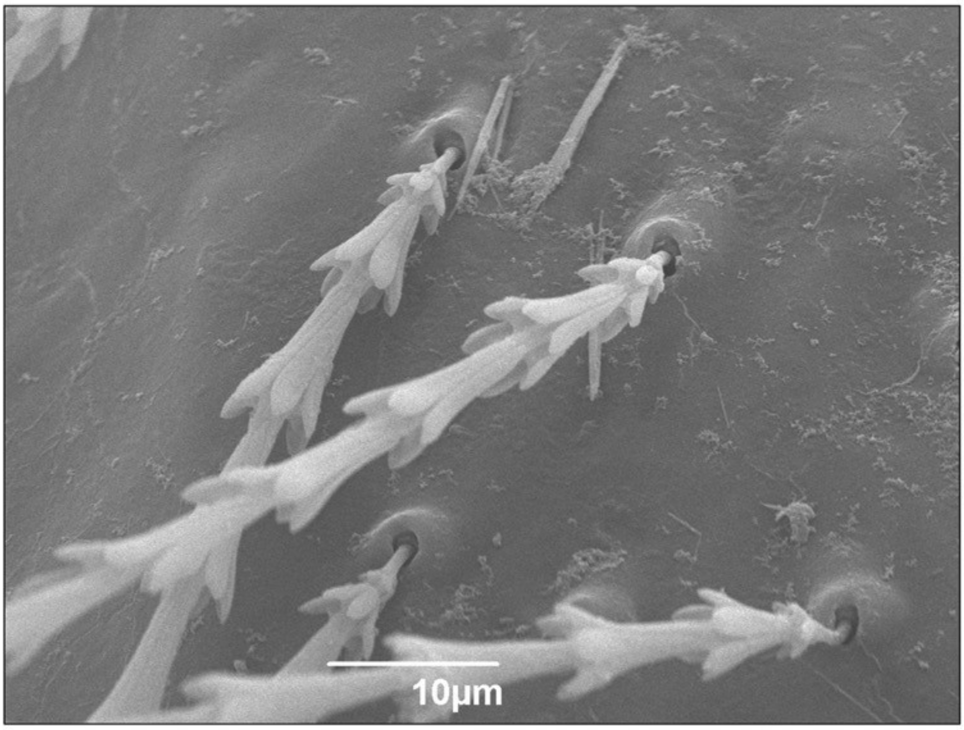

Fig. E. 2300x. Insertion of the hastisetae on the integument, highlighting the papilla

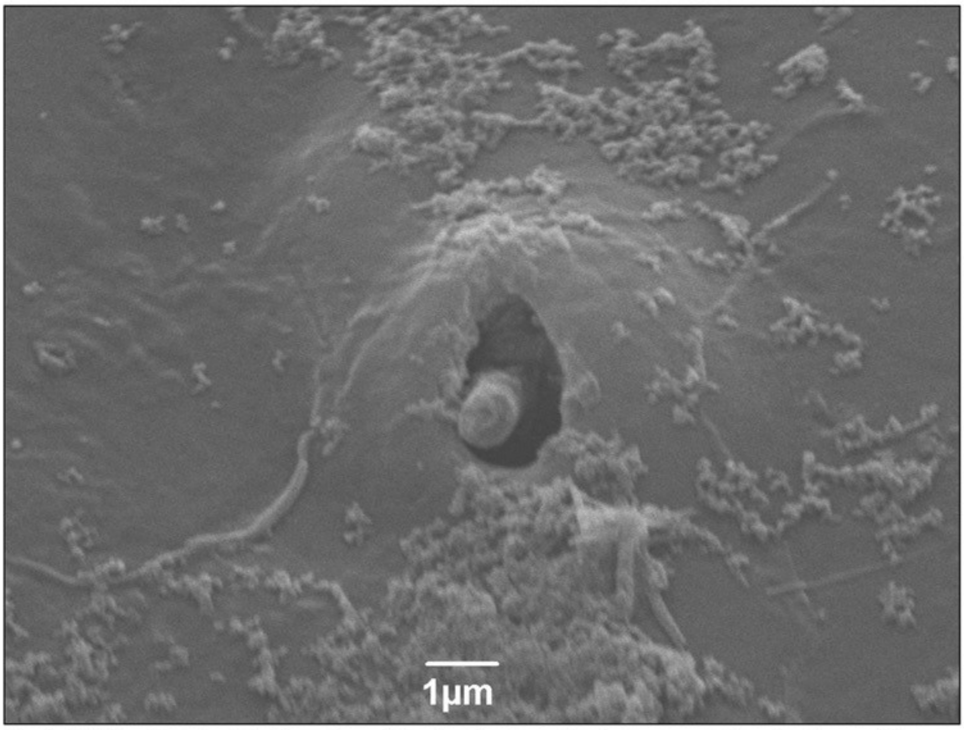

Fig. F. 10000x. Detail of the insertion of the hastiseta on the integument showing the papilla and the remaining part of the pedicel after the seta detachment

Anthrenus (*Florilinus*) *olgae* Kalík, 1946

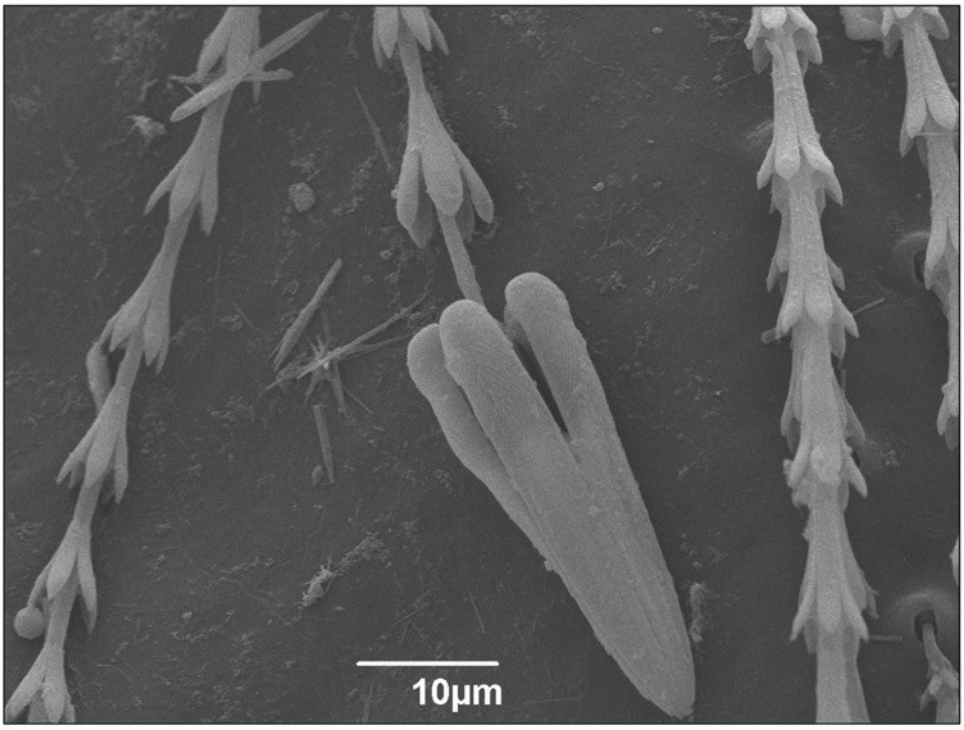

Fig. G. 1900x. Head of the hastiseta and rosettes of hastisetae on the first abdominal tergites.

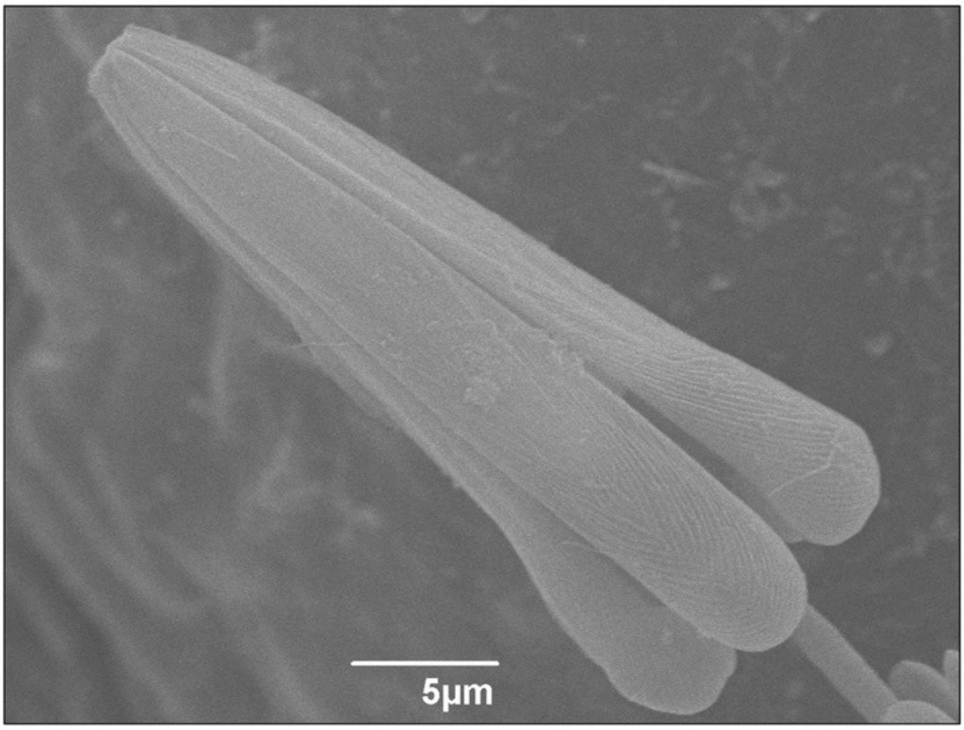

Fig. H. 4000x. Lateral view of the head of the hastiseta (first abdominal segments) illustrating the general profile and septation.

Anthrenus (*Florilinus*) *olgae* Kalík, 1946

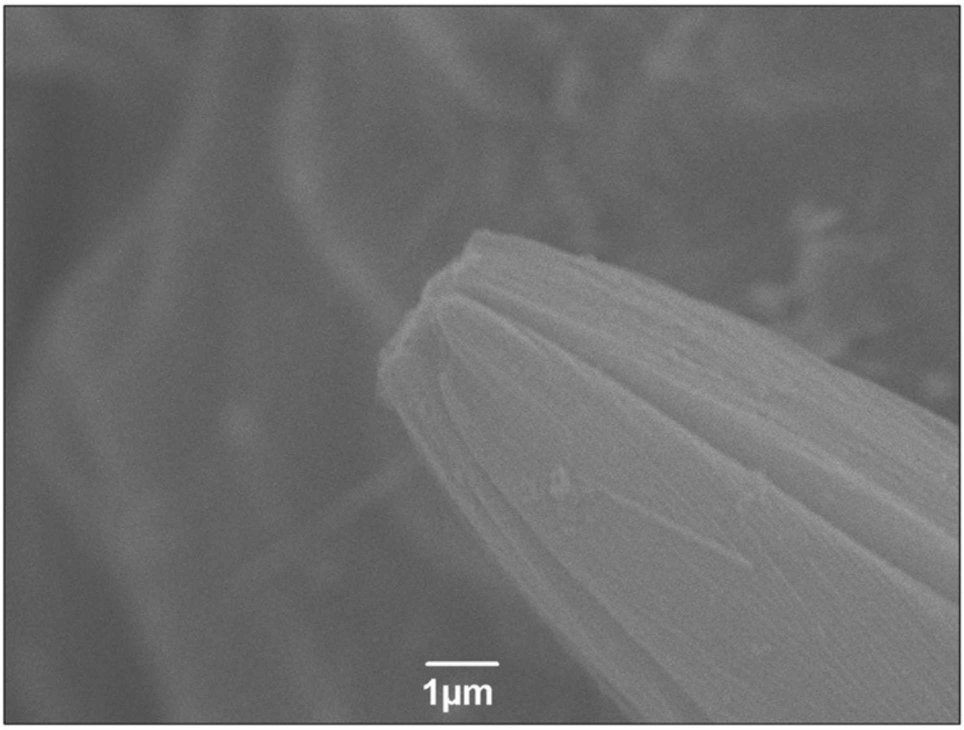

Fig. I. 10000x. Blunt apex of the hastiseta (first abdominal segments); lateral view.

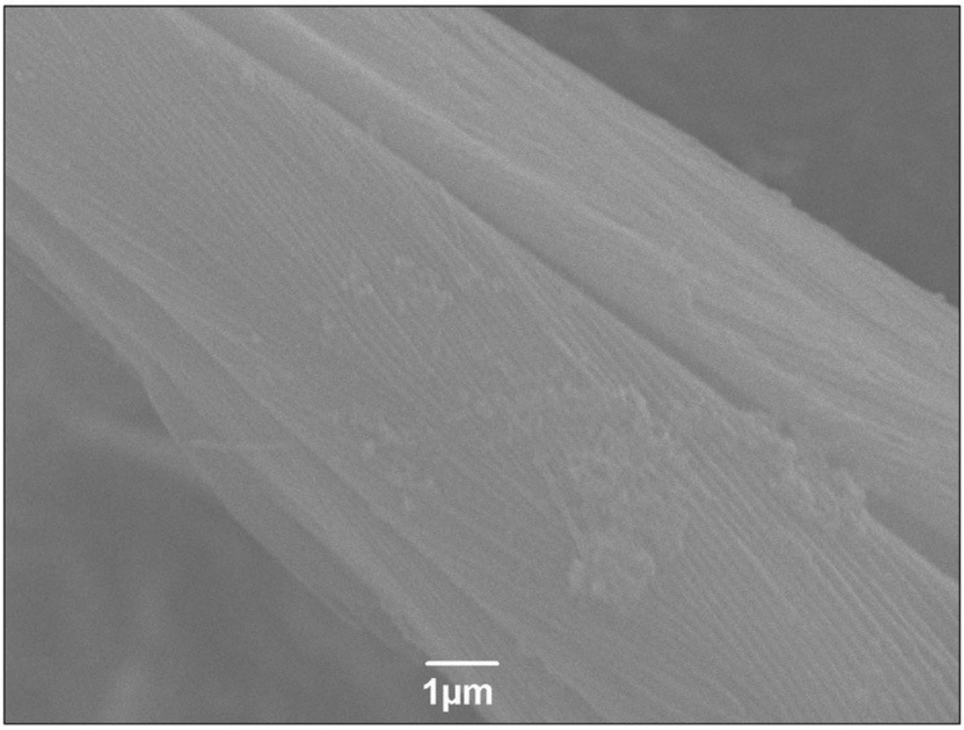

Fig. J. 10000x. Detail of the knurls overing the longitudinal processes of the head of the hastiseta.

Anthrenus (*Florilinus*) *olgae* Kalík, 1946

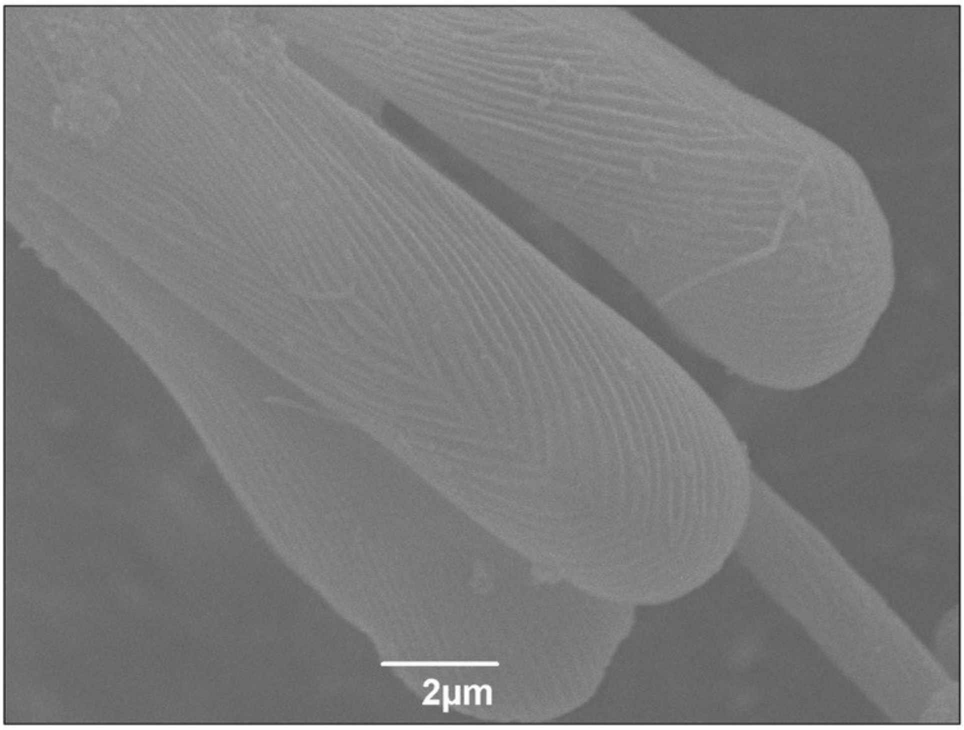

Fig. K. 8000x. Detail of the apex of the longitudinal processes of the head of the hastiseta.

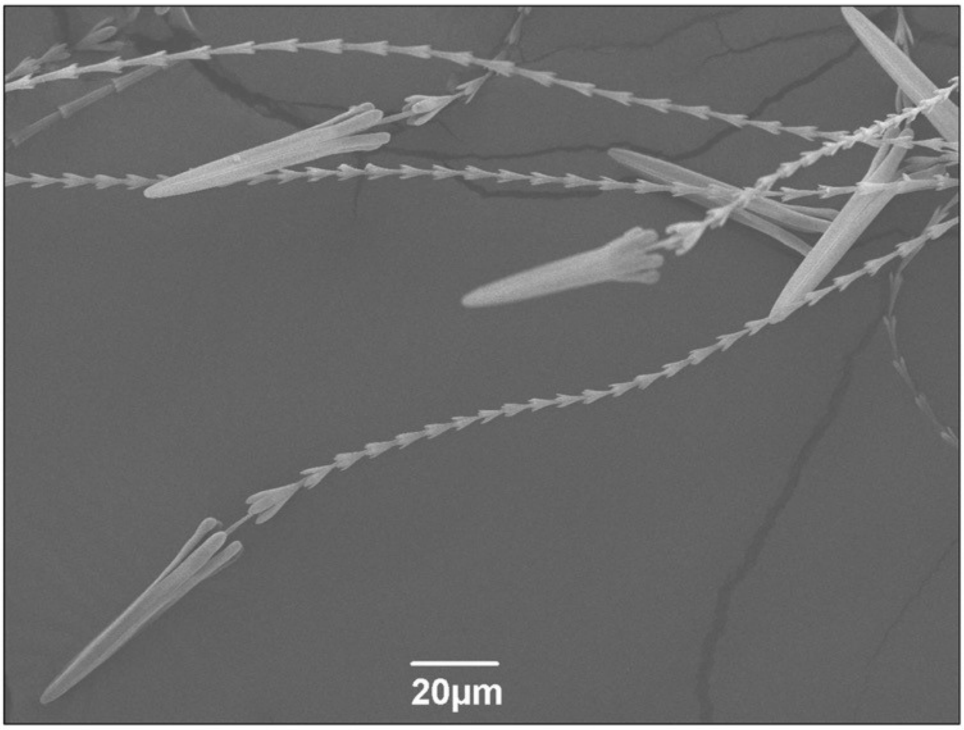

Fig. L. 600x. Hastisetae originating from the sixth abdominal segment; general view.

Anthrenus (*Florilinus*) *olgae* Kalík, 1946

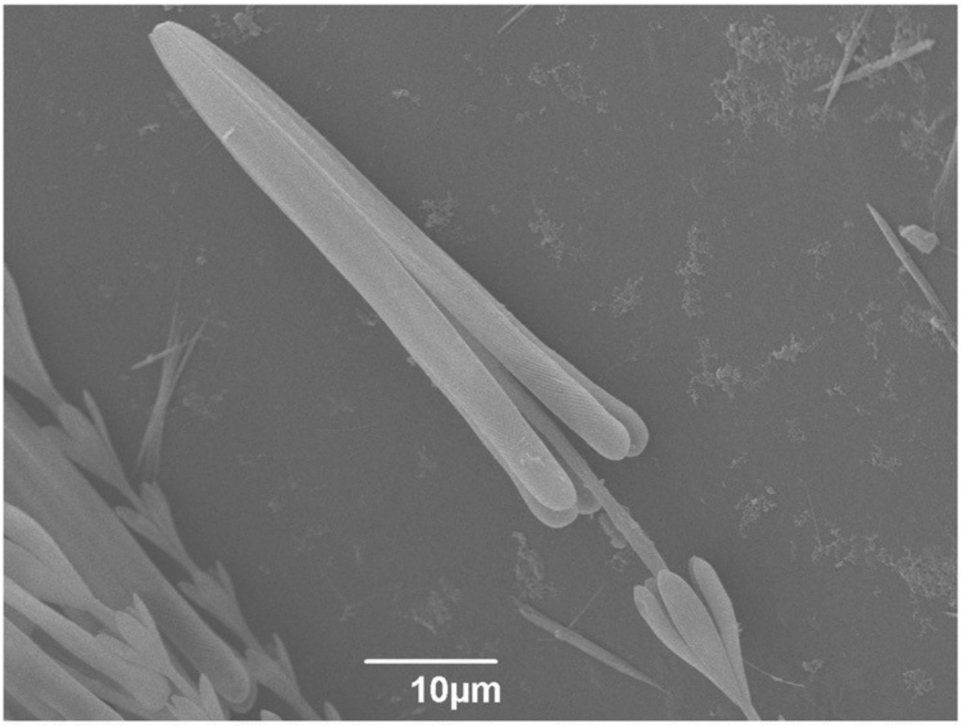

Fig. M. 1800x. Head of the hastiseta and ultimate rosette (hastiseta belonging to hastisetal tufts present of the fifth/sixth abdominal tergite).

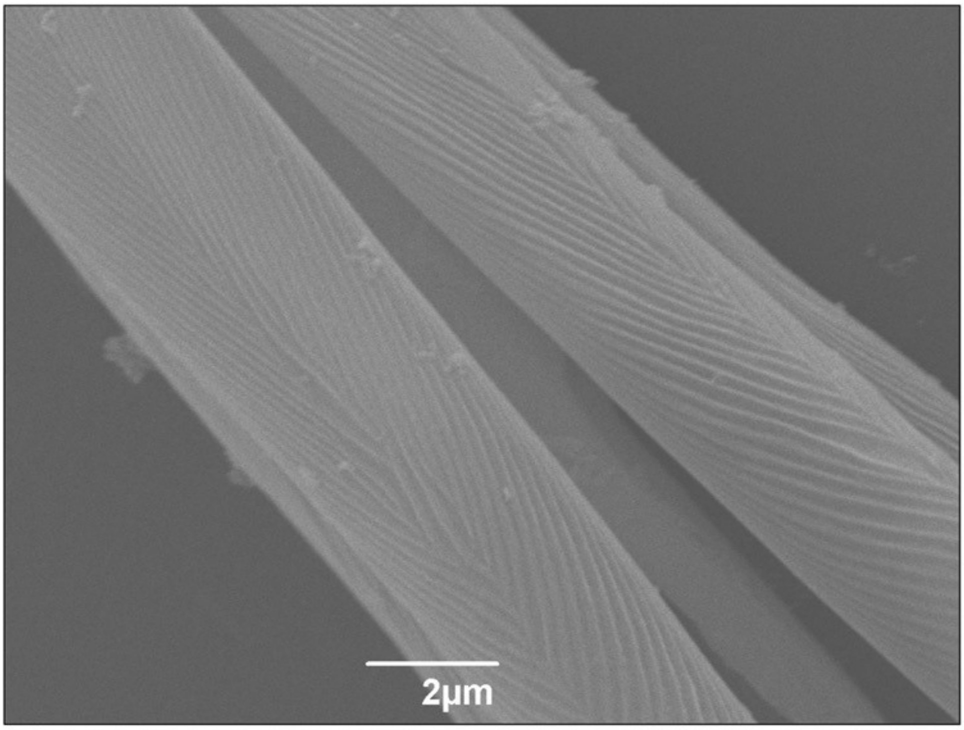

Fig. N. 9900x. Detail of the hastiseta highlighting the peculiar knurls present on the longitudinal processes.

Anthrenus (*Florilinus*) *olgae* Kalík, 1946

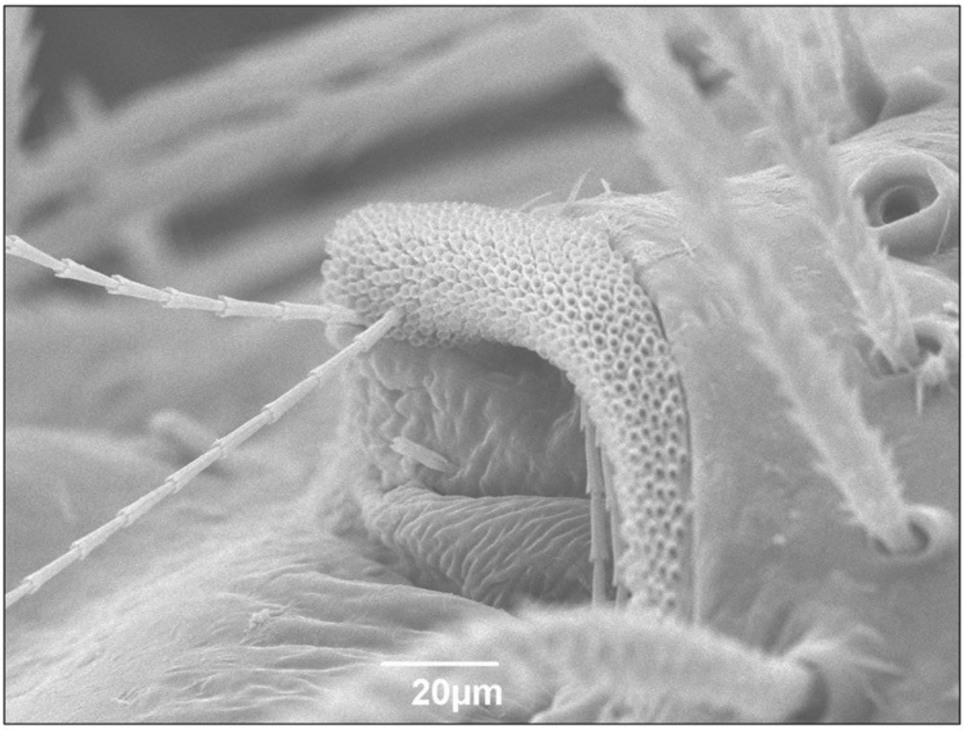

Fig. O. 800x. Insertion of the hastisetae on the membranaceous part of the last abdominal tergites

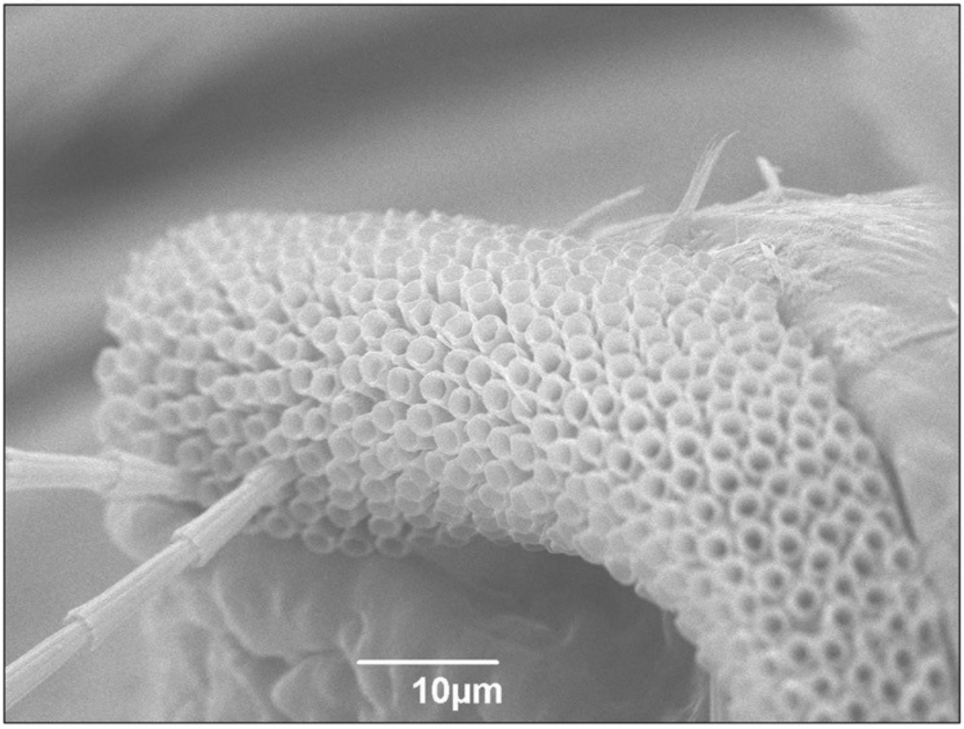

Fig. P. 1900x. Insertion of the hastisetae on the membranaceous part of the last abdominal tergites, detail showing the developed collars enveloping the first rosette and the pedicel.

Supplementary material 6

Anthrenus (*Helocerus*) *fuscus* Olivier, 1789

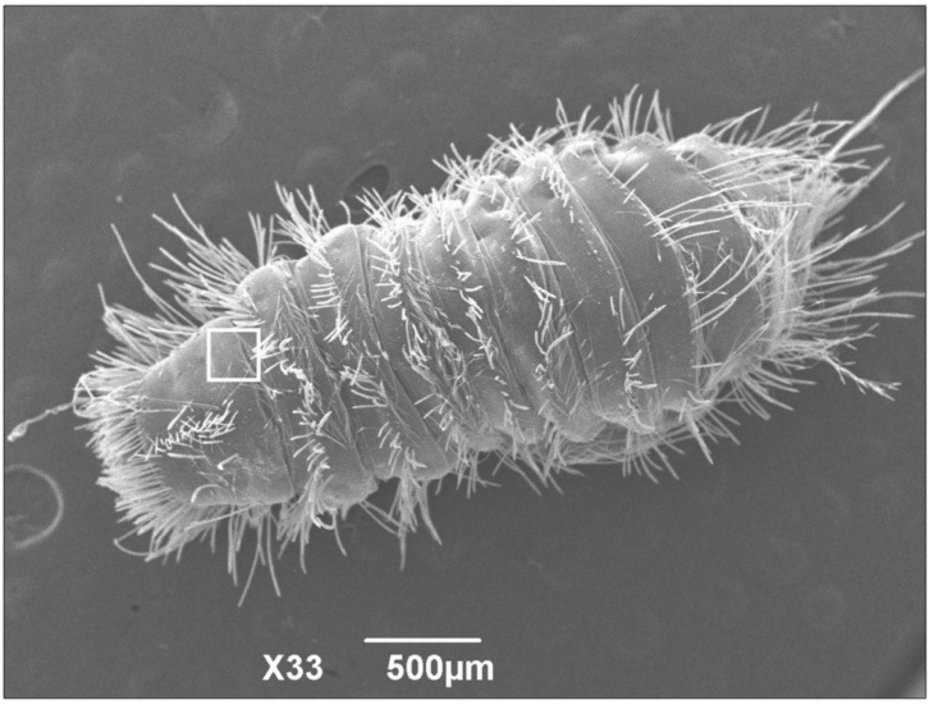

Fig. A. 33x. Mature larva, dorsal view. Insert figure B.

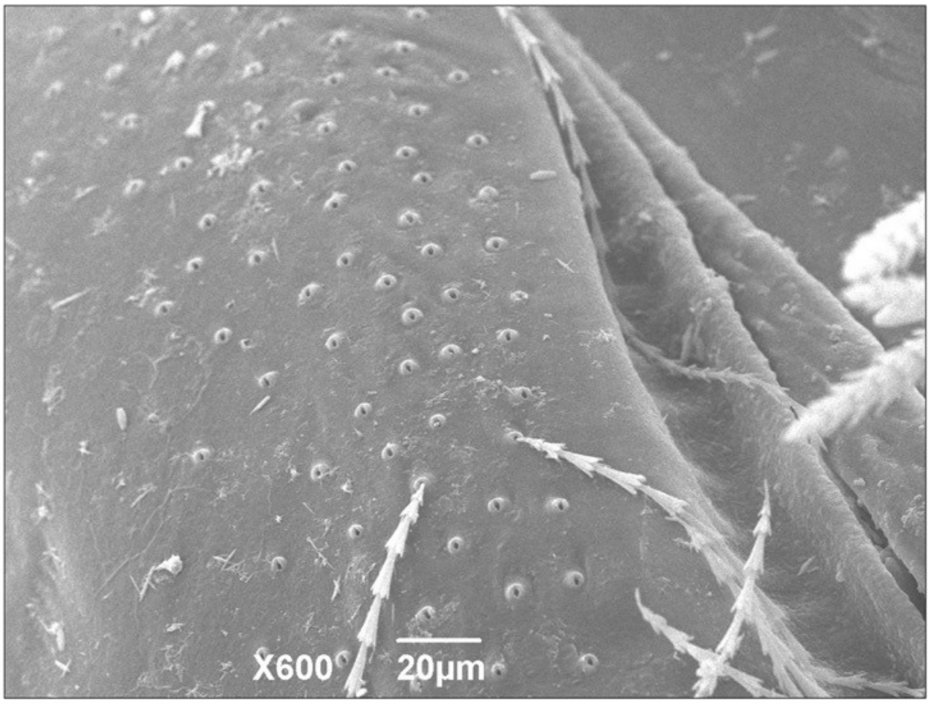

Fig. B. 600x. Detail of the first thoracic tergite showing the insertions of the hastisetae (almost completely detached).

Anthrenus (*Helocerus*) *fuscus* Olivier, 1789

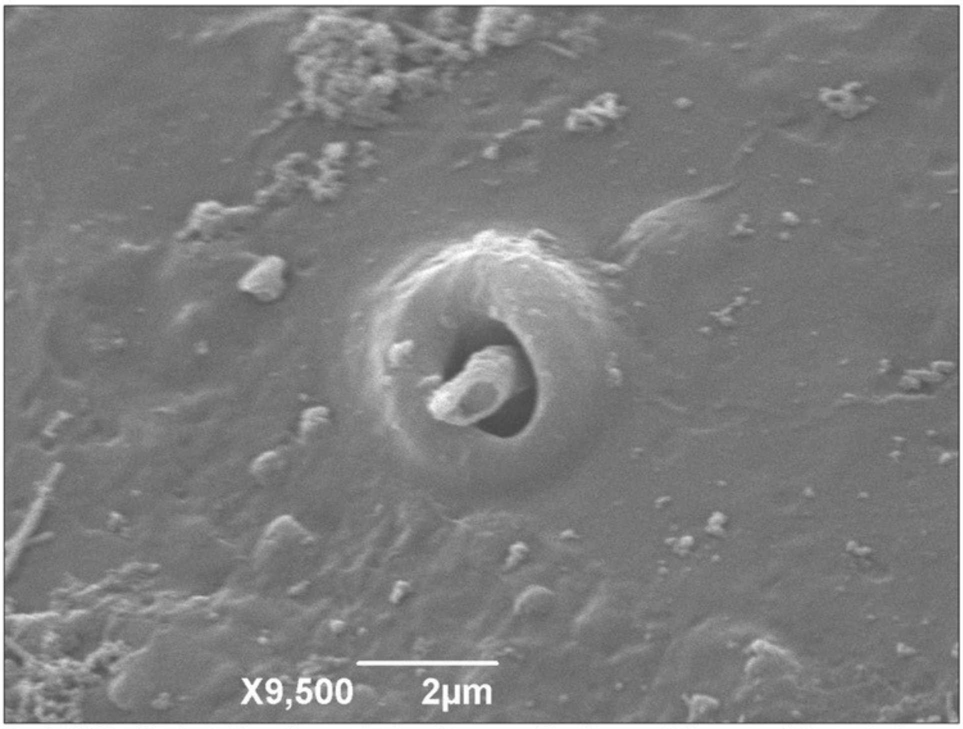

Fig. C. 9500x. Hastiseta insertion on the tergite presenting the remaining part of the pedicel after the seta detachment.

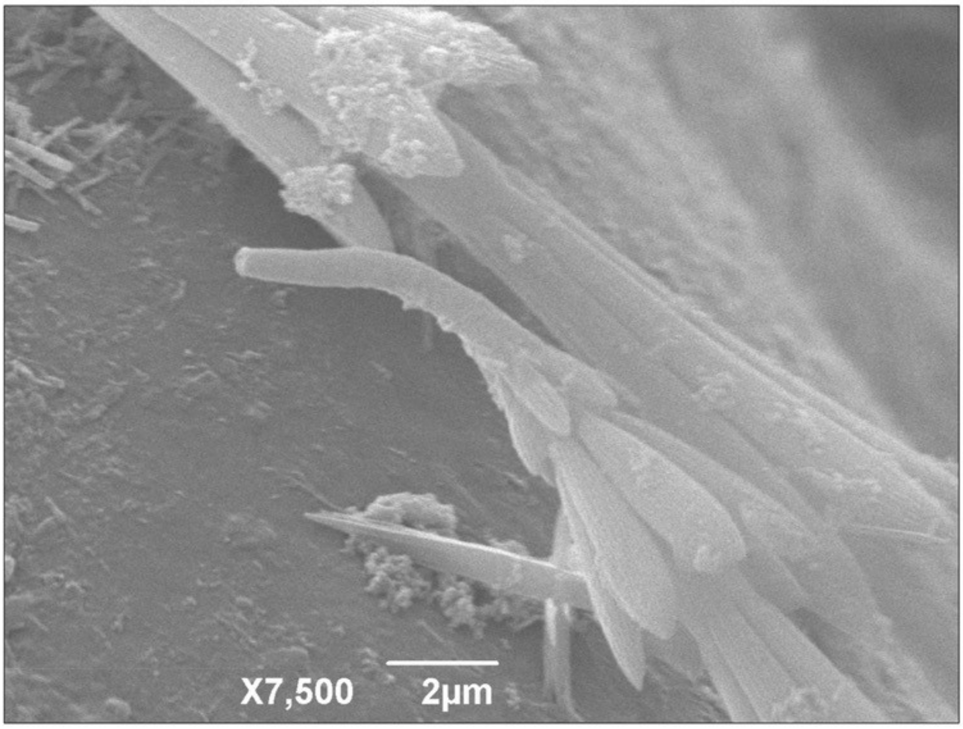

Fig. D. 7500x. Detail of the pedicel and first rosette of a detached hastiseta of the thorax.

Anthrenus (*Helocerus*) *fuscus* Olivier, 1789

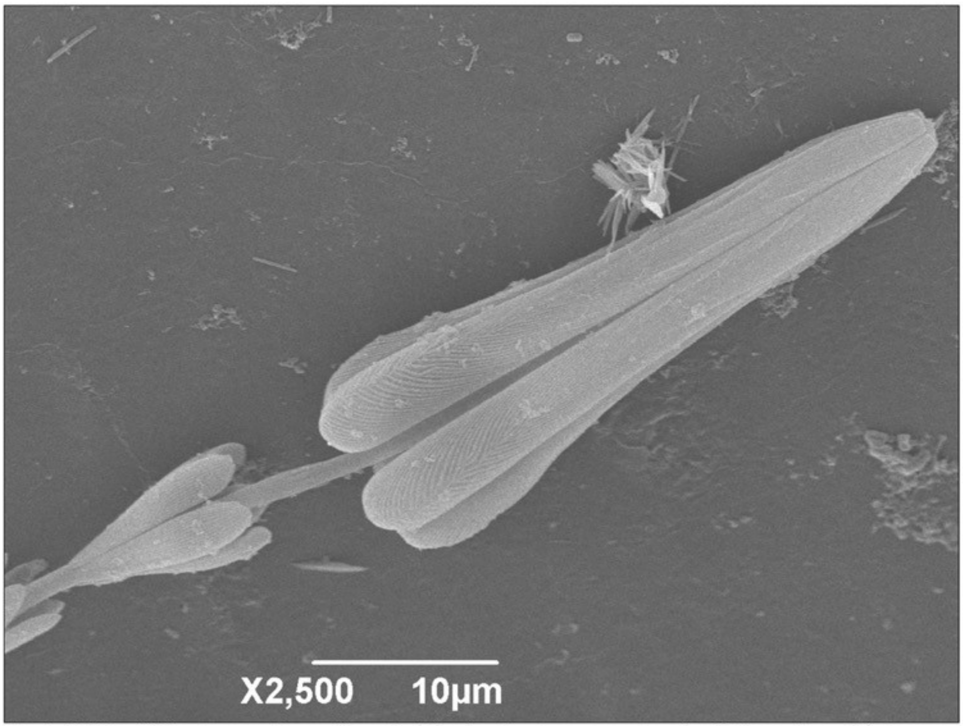

Fig. E. 2500x. Ultimate rosette and head of the hastisetae present on both thoracic and fist abdominal tergites.

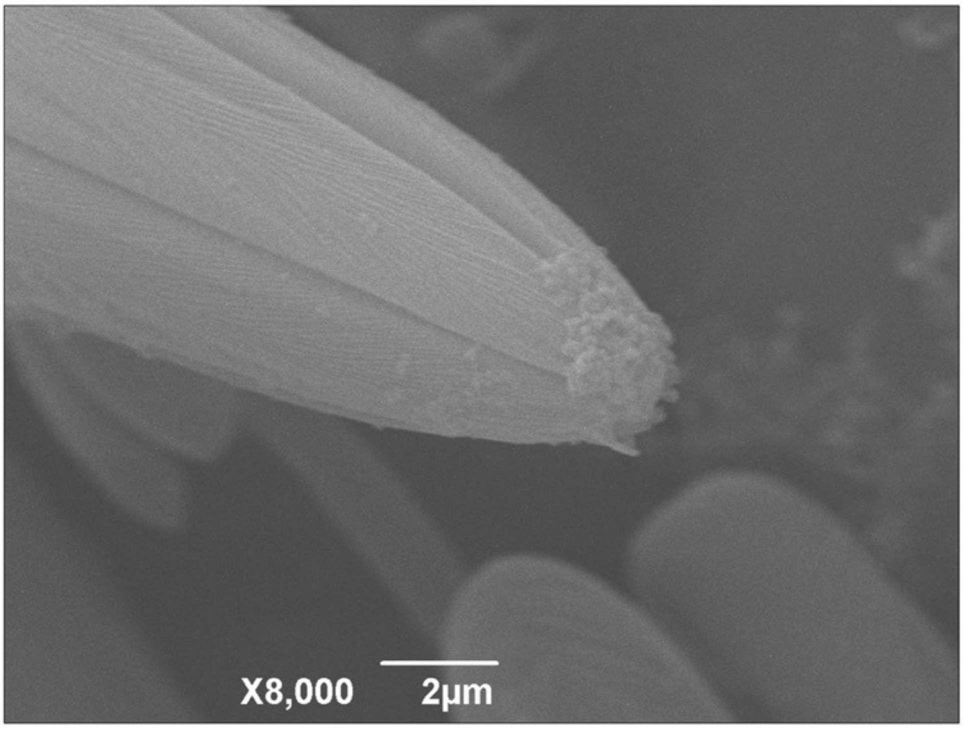

Fig. F. 7500x. Apex of the head of the hastiseta encrusted with an unidentified susbtance.

Anthrenus (*Helocerus*) *fuscus* Olivier, 1789

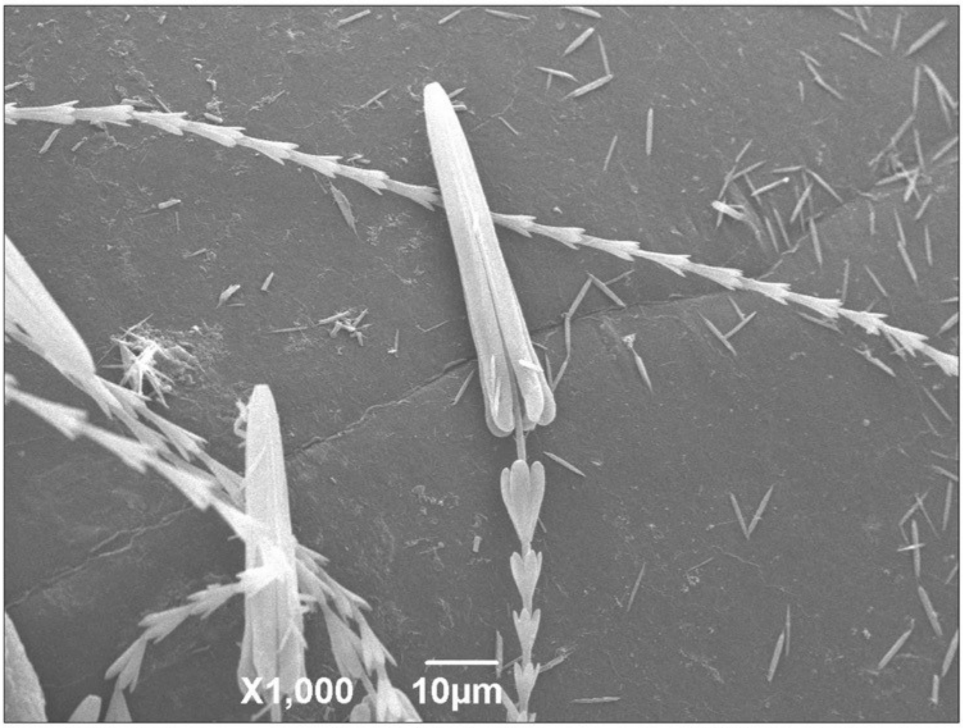

Fig. E. 1000x. Ultimate rosette and head of the hastisetae present on 5th-7th abdominal tergites.

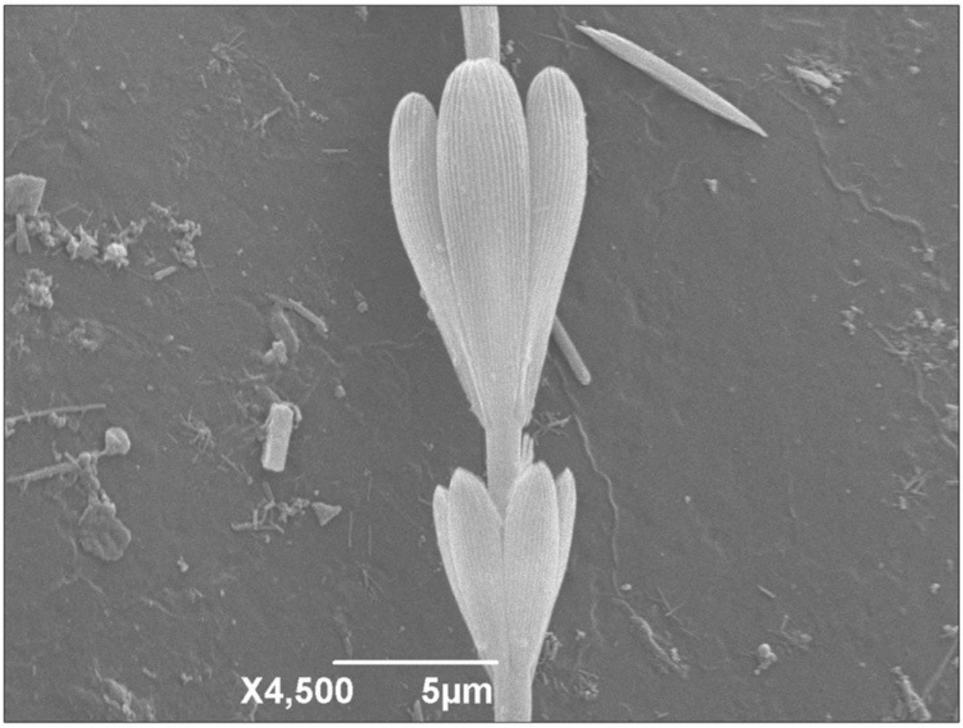

Fig. F. 4500x. Ultimate rosette of the hastisetae present on 5th-7th abdominal tergites

Supplementary material 7

Anthrenus (*Helocerus*) *polonicus* Mroczkowski, 1950

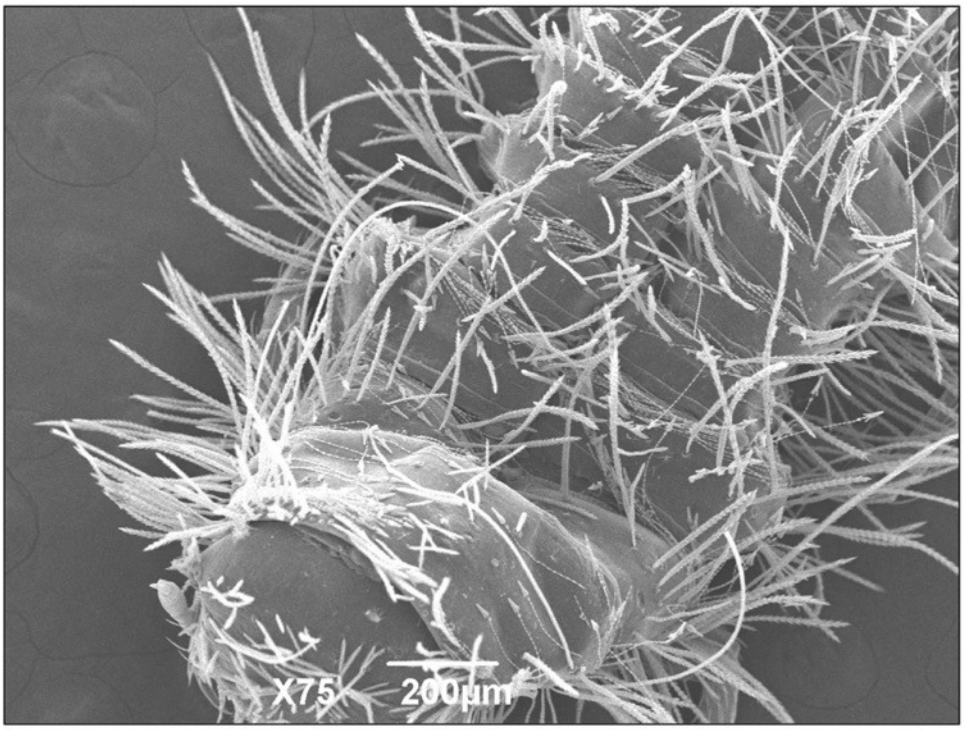

Fig. A. 75x. Mature larva, dorsal view: recumbent tufts of hastisetae on the sclerotized part of the thoracic and fist abdominal tergites.

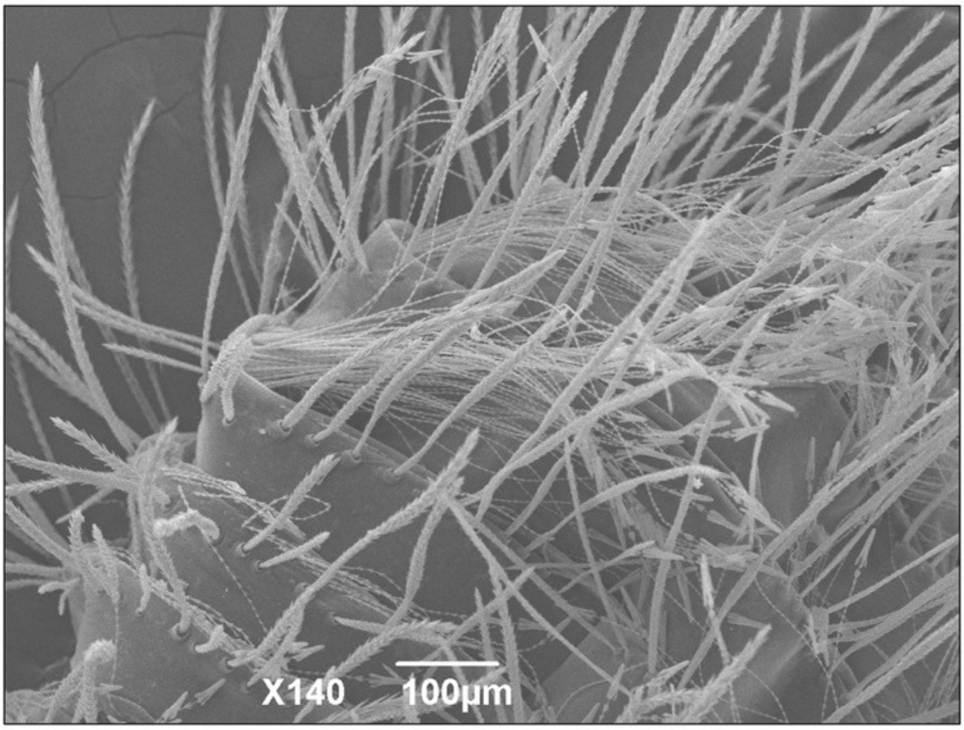

Fig. B. 140x. Mature larva, dorsal view: tufts of hastisetae on the membranous part of 5th-7th abdominal tergites.

Anthrenus (*Helocerus*) *polonicus* Mroczkowski, 1950

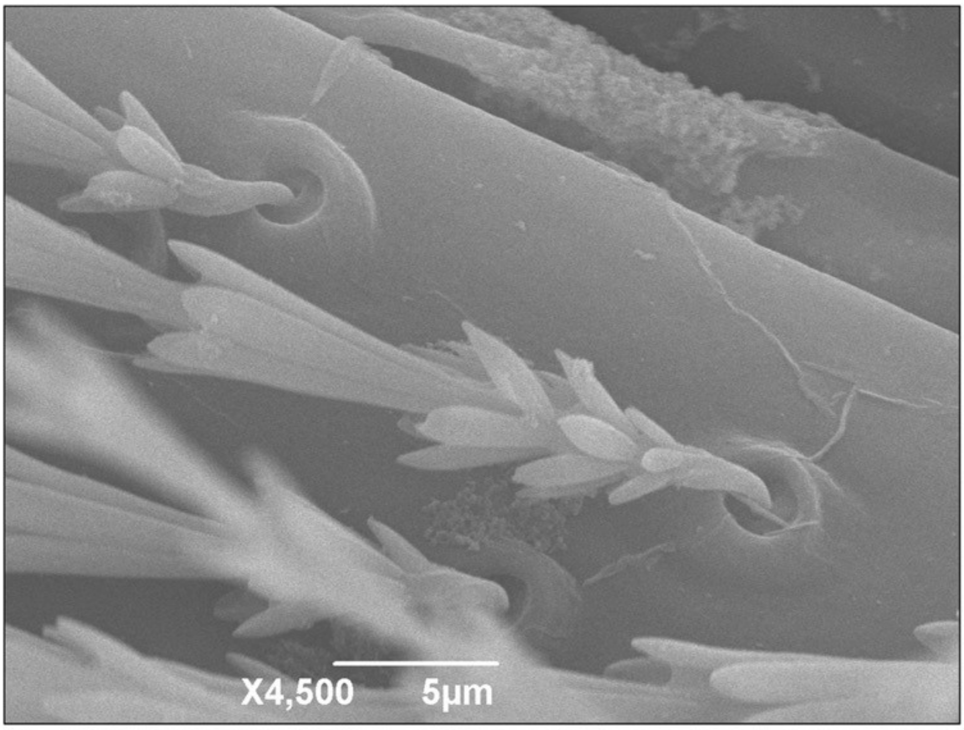

Fig. C. 4500x. Detail of the insertion of the hastisetae on the sclerotized part of the tergite.

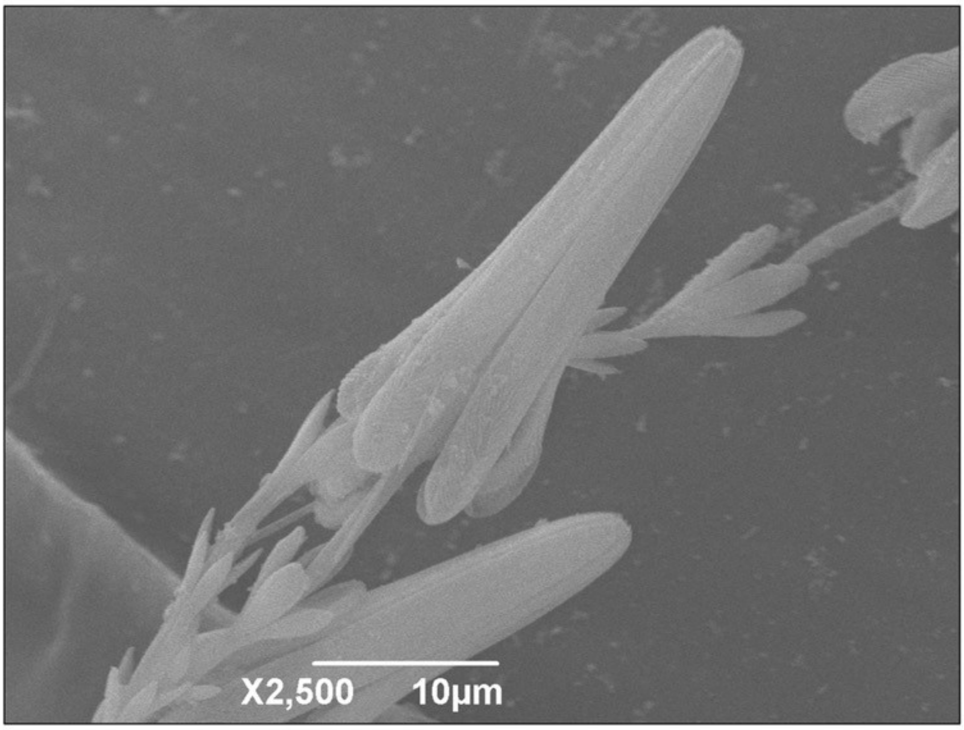

Fig. D. 2500x. Head of the hastiseta belonging to the the hastisetae on the sclerotized part of the tergite.

Anthrenus (*Helocerus*) *polonicus* Mroczkowski, 1950

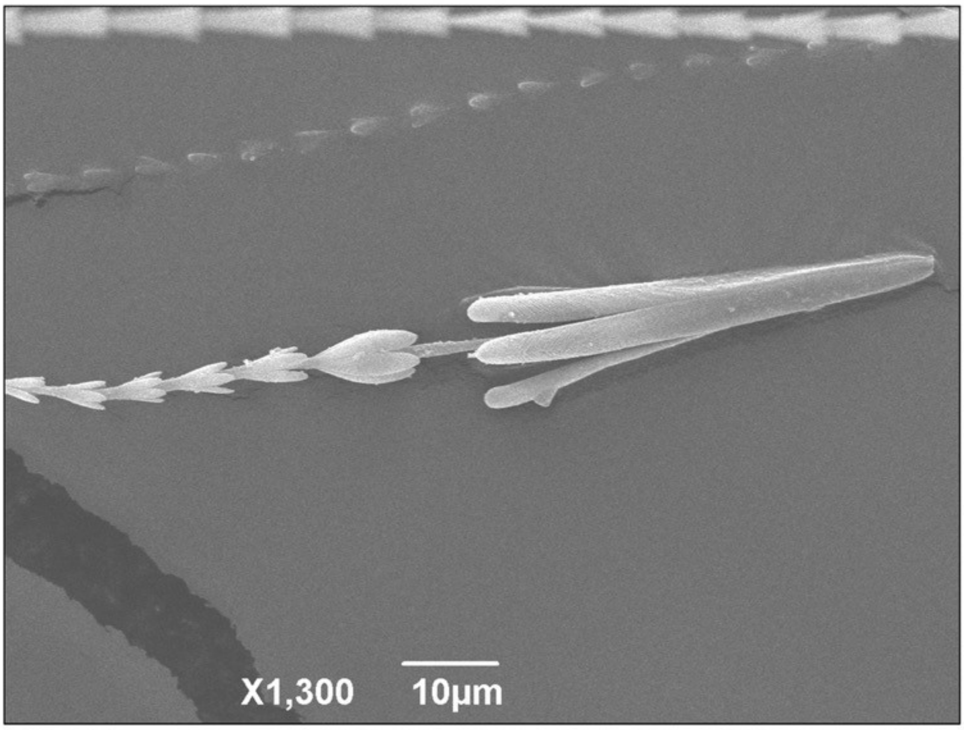

Fig. E. 1300x. Detail of the apical part of the hastisetae belonging to the 5th-7th abdominal tergite.

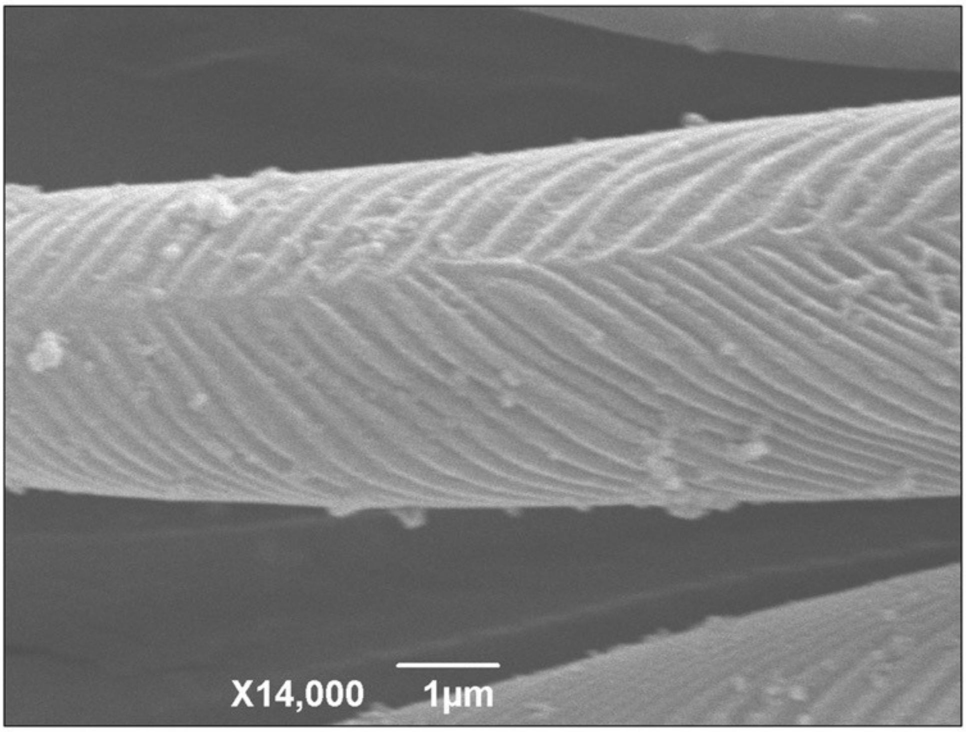

Fig. F. 2500x. Detail of the knurls covering the longitudinal processes of the head of the hastiseta.

Anthrenus (*Helocerus*) *polonicus* Mroczkowski, 1950

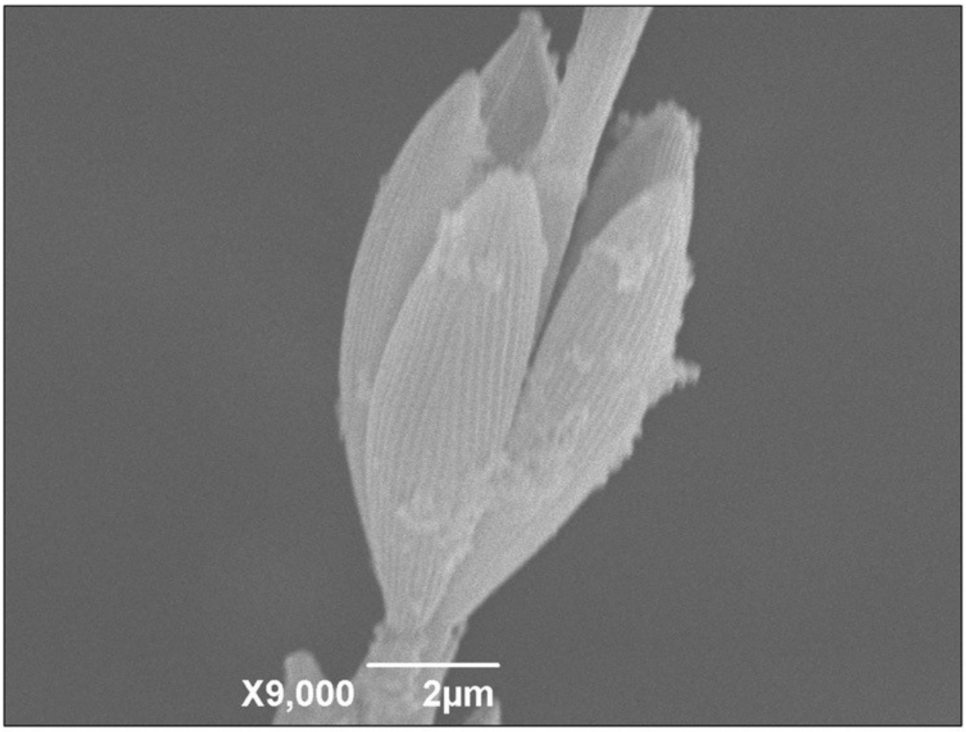

Fig. G. 9000x. Rosette of the hastisetae belonging to the 5th-7th abdominal tergite.

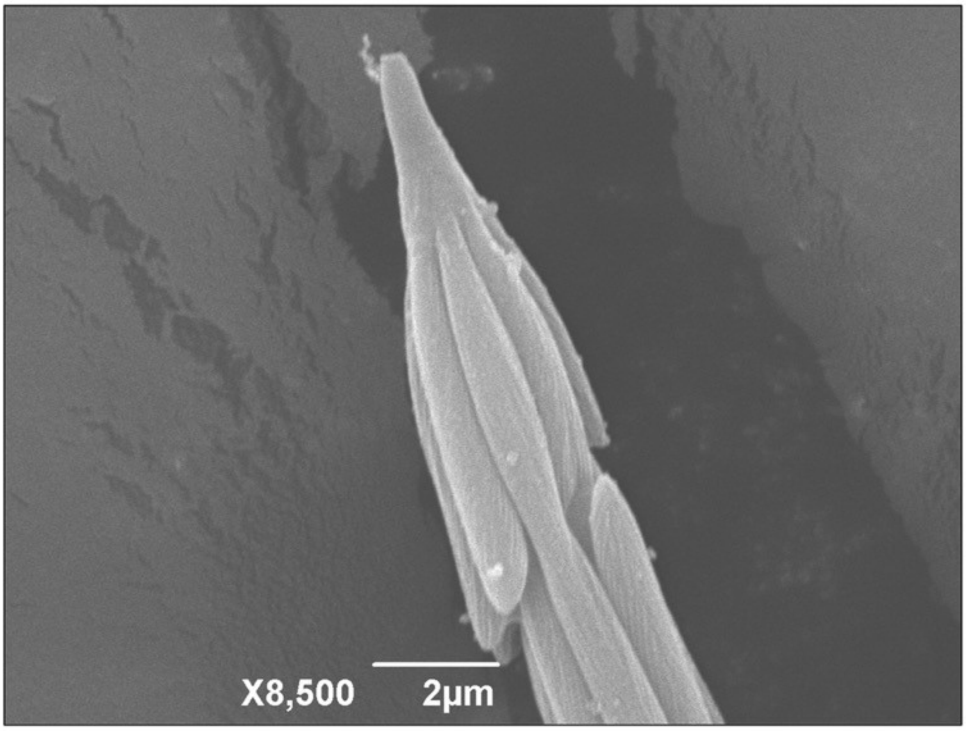

Fig. H. 8500x. Detail of the pedicel and first rosette of one hastiseta belonging to the 5th-7th abdominal tergite.

Supplementary material 8

Anthrenus spp. 1 (USA)

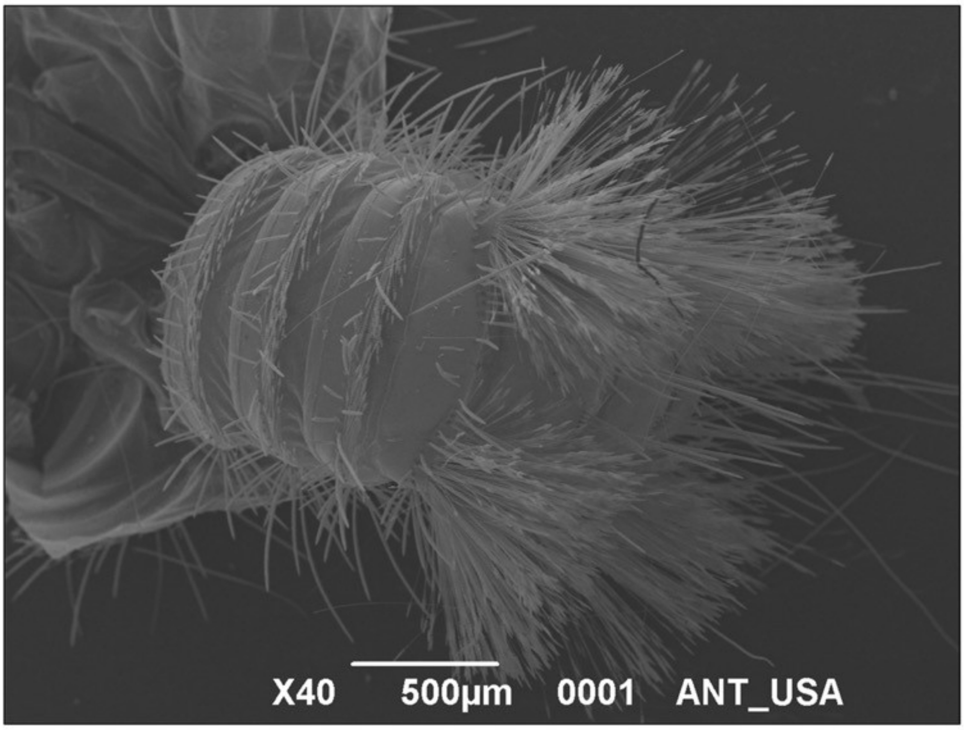

Fig. A. 40x. Exuvia, dorsal view. Detail of the last seven abdominal tergites.

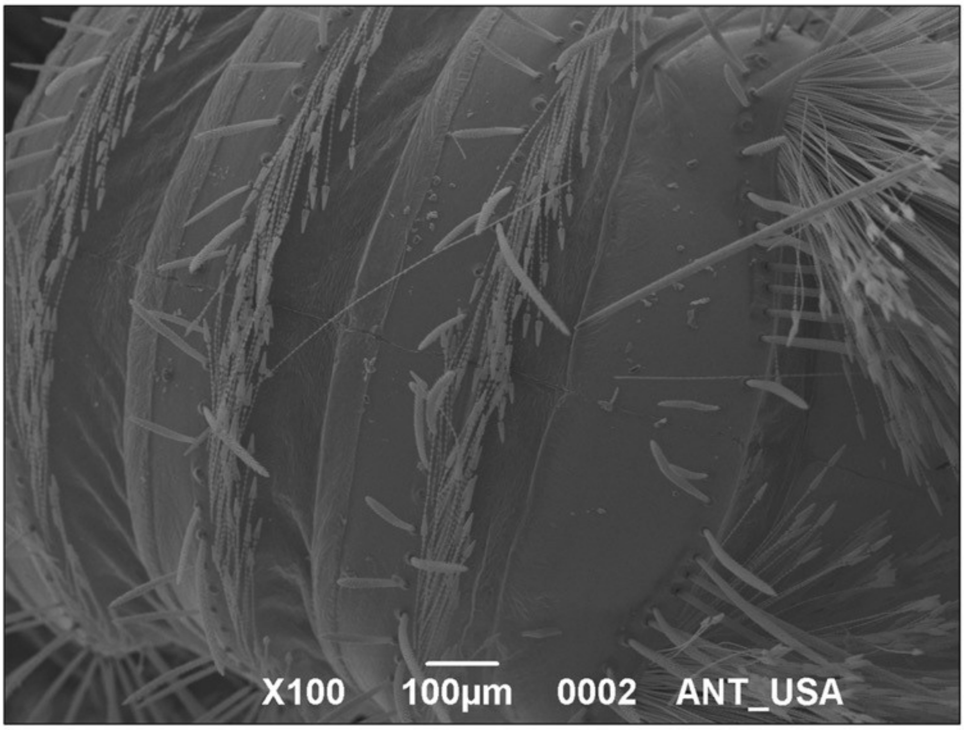

Fig. B. 100x. Detail of the second to fourth abdominal tergite, dorsal view.

Anthrenus spp. 1 (USA)

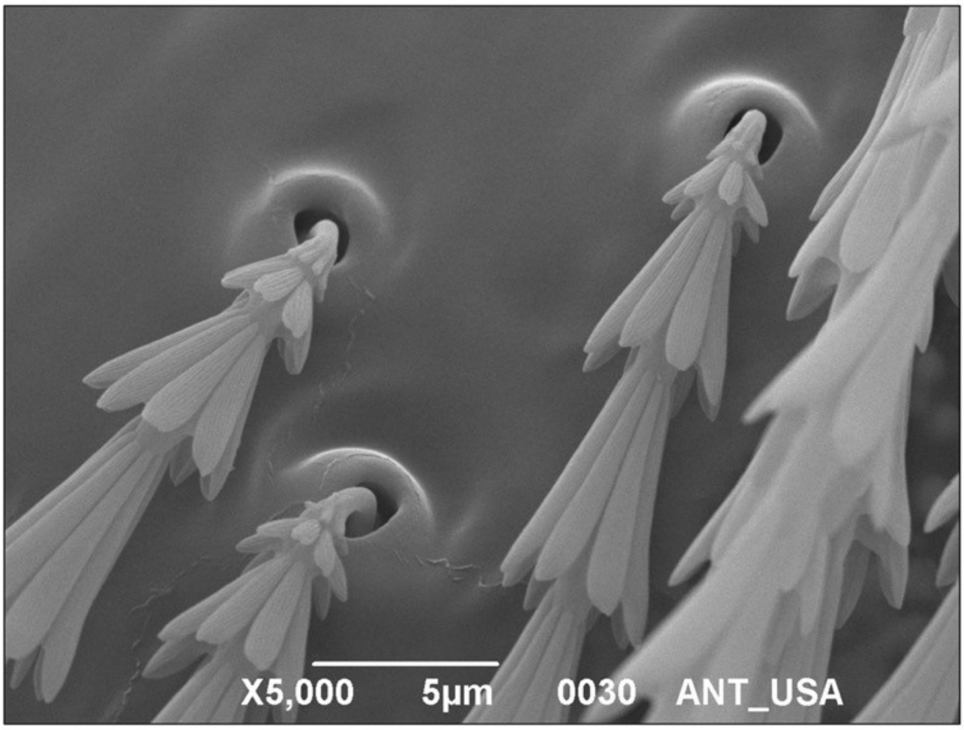

Fig. C. 5000x. Insertion of hastisetae on the first abdominal tergites.

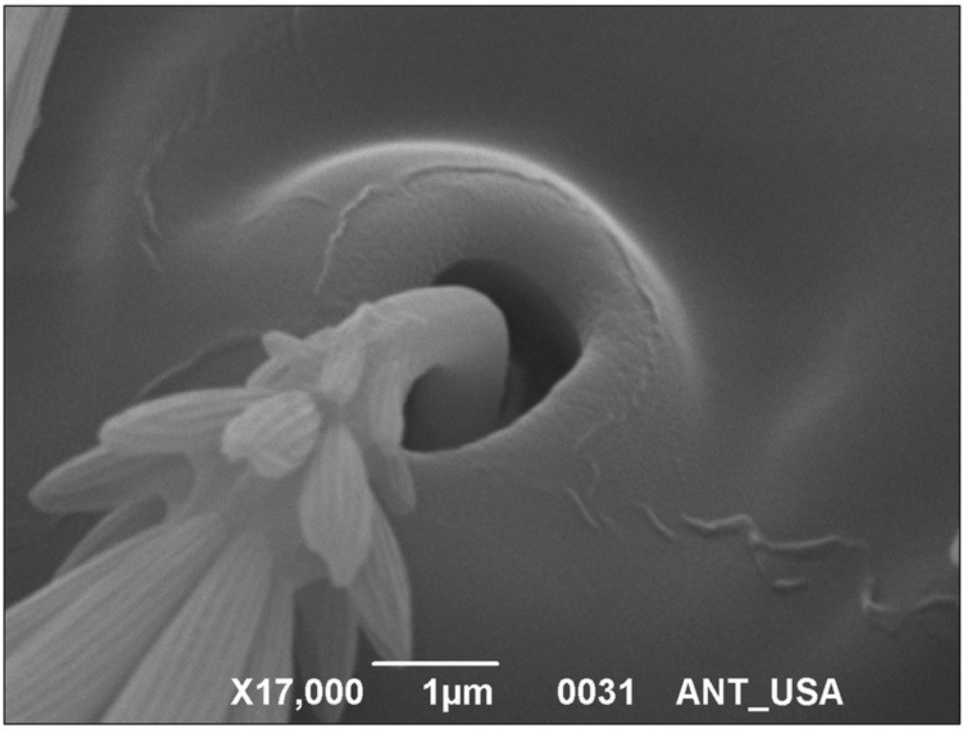

Fig. D. 17000x. Detail of the insertion of the hastiseta showing the curved pedicel.

Anthrenus spp. 1 (USA)

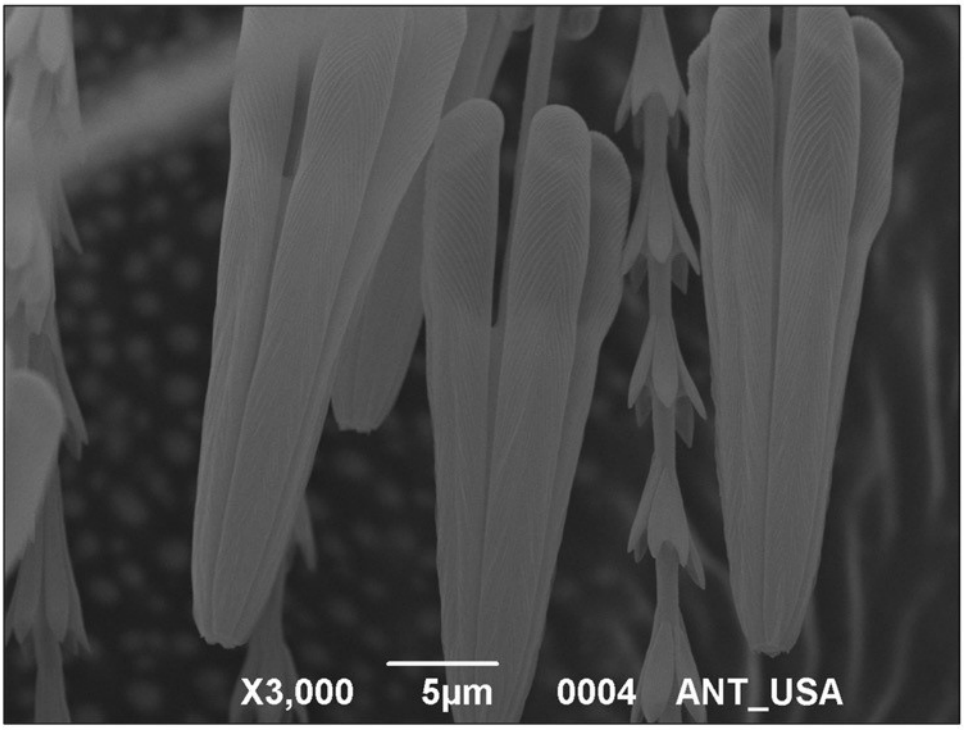

Fig. E. 3000x. Head of the hastiseta on the first abdominal tergites.

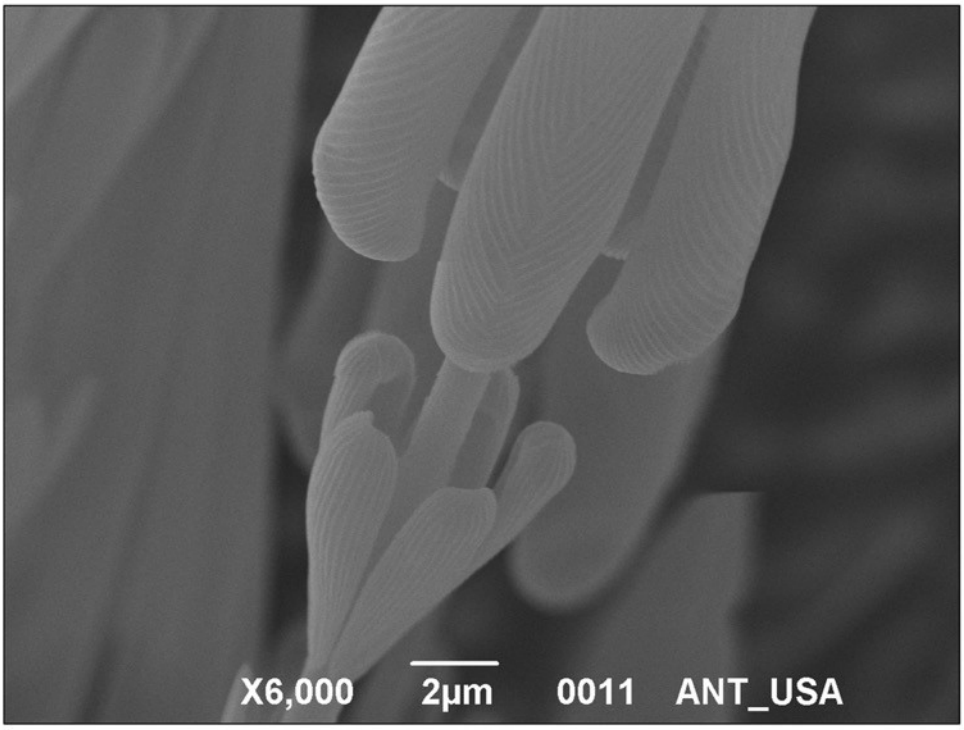

Fig. F. 6000x. Detail of the last rosette and the stalk.

Anthrenus spp. 1 (USA)

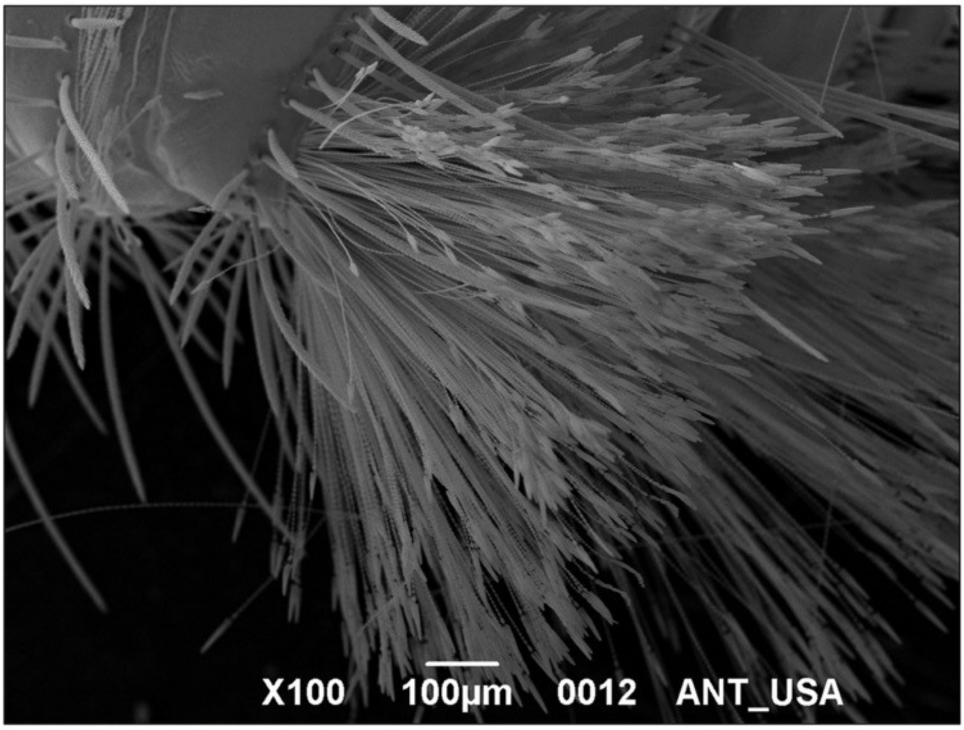

Fig. H. 100x. Dorsal view of one tuft of hastisetae on the posterior corner of the 5th abdominal tergite

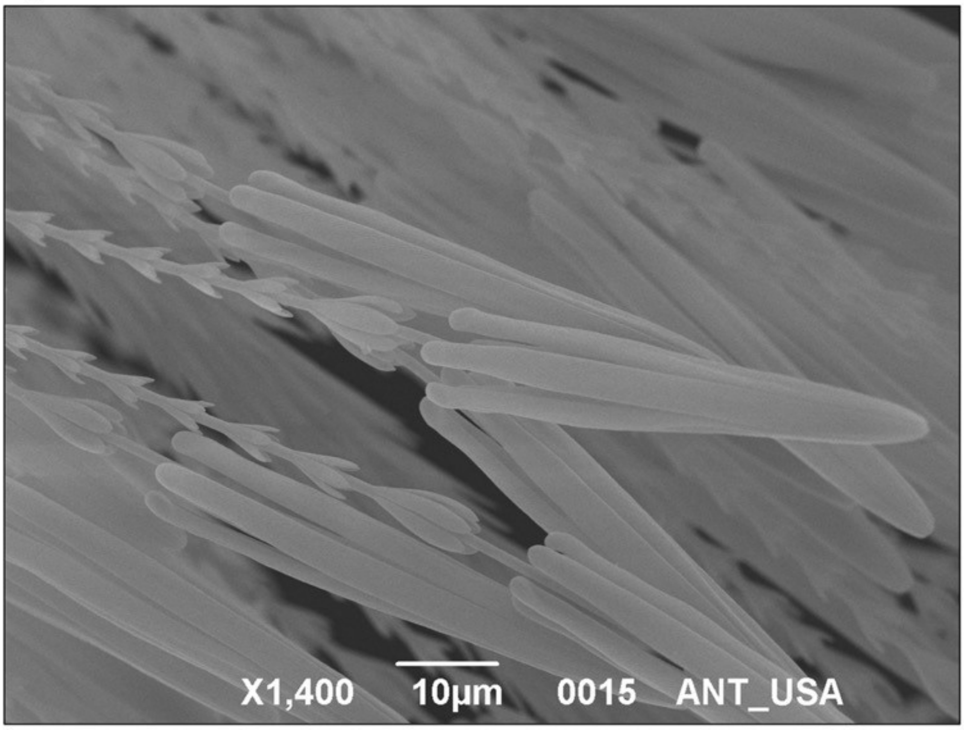

Fig. I. 1400x. Detail of the head of the hastisetae on the last abdominal tegites..

Anthrenus spp. 1 (USA)

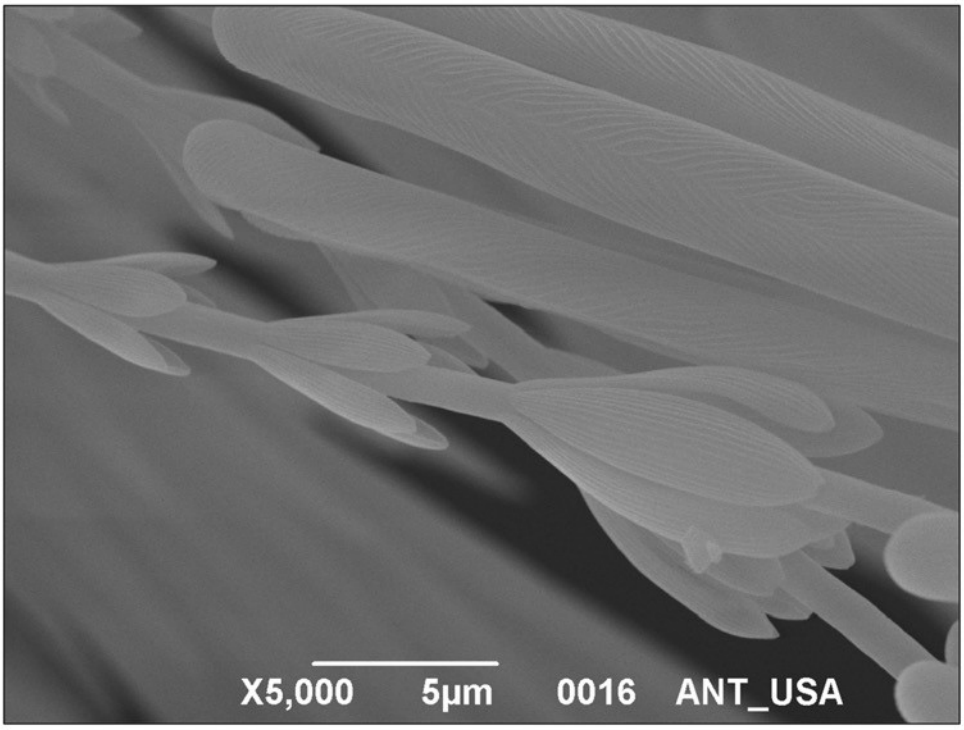

Fig. J. 5000x. Size comparison between the last rosette and the previous two.

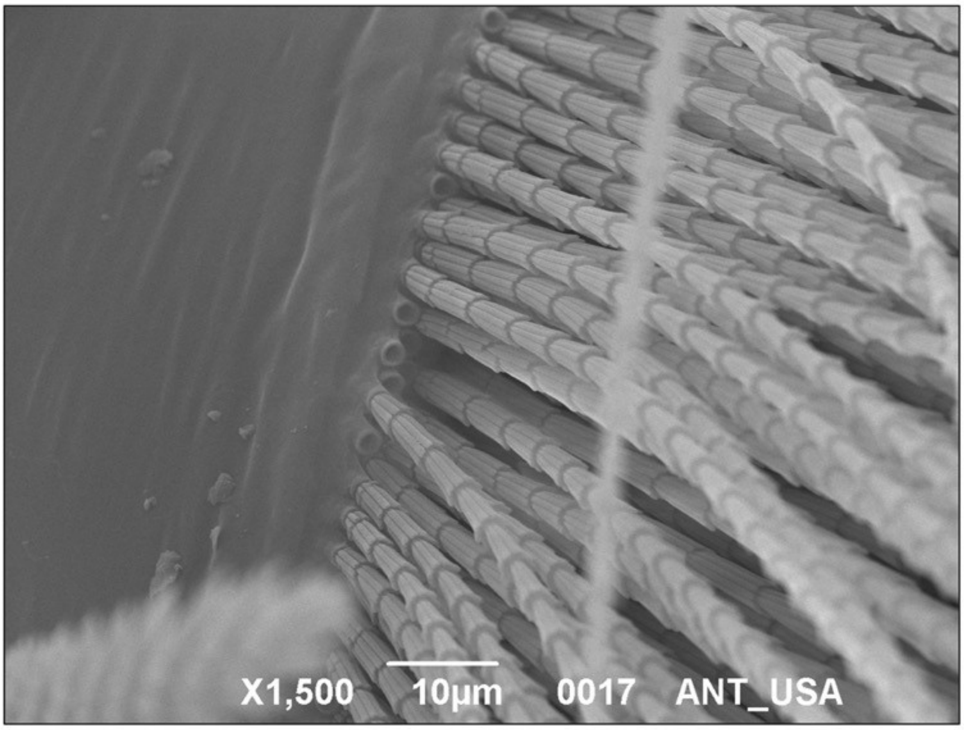

Fig. K. 1500x. Detail of the membranous part of the firth abdominal tergite with the hastisetae inserted.

Anthrenus spp. 1 (USA)

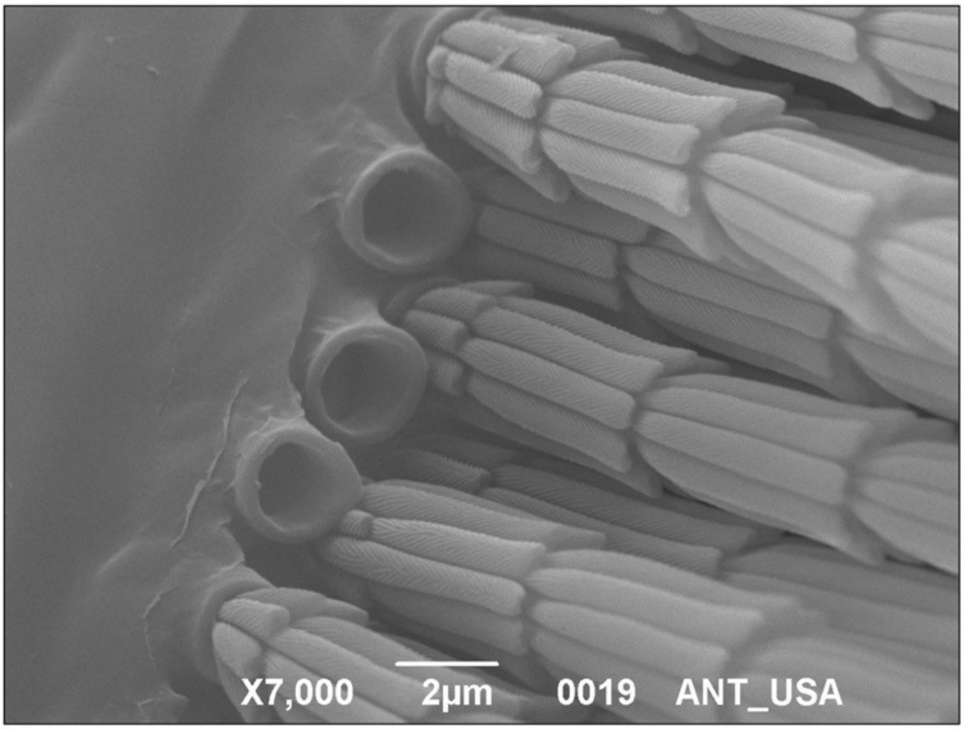

Fig. L. 7000x. Detail of the insertion of the hastisetae on the last abdominal tergites.

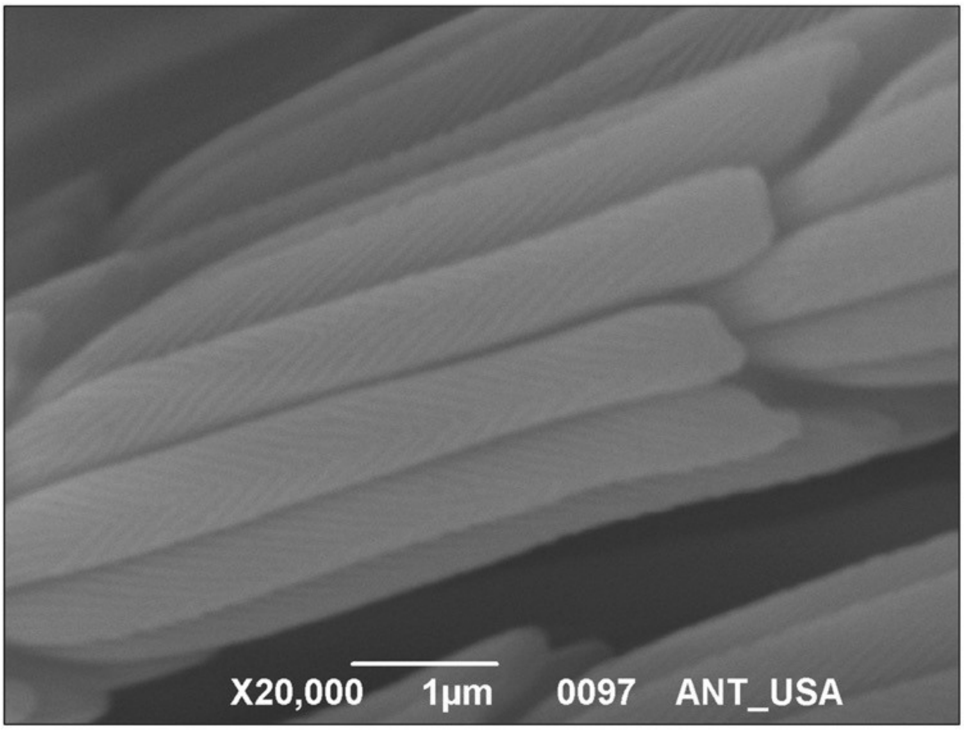

Fig. M. 130x. Detail one basal rosette, showing the peculiar columnar profile.

Supplementary material 9

Anthrenus spp. 2 (Portugal)

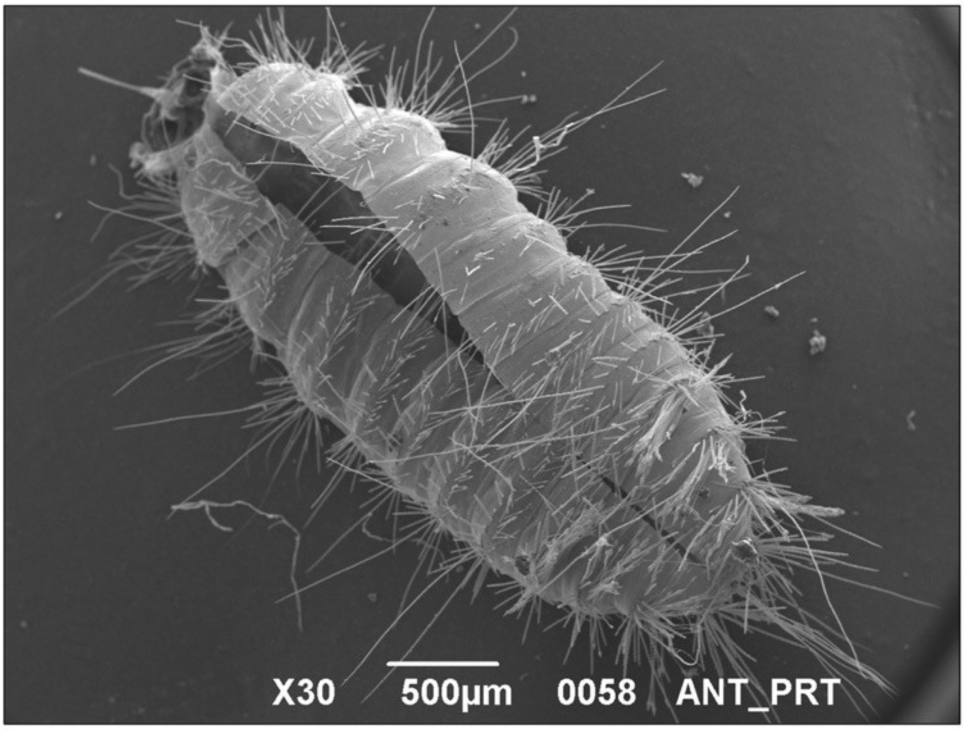

Fig. A. 30x. Exuvia, dorsal view.

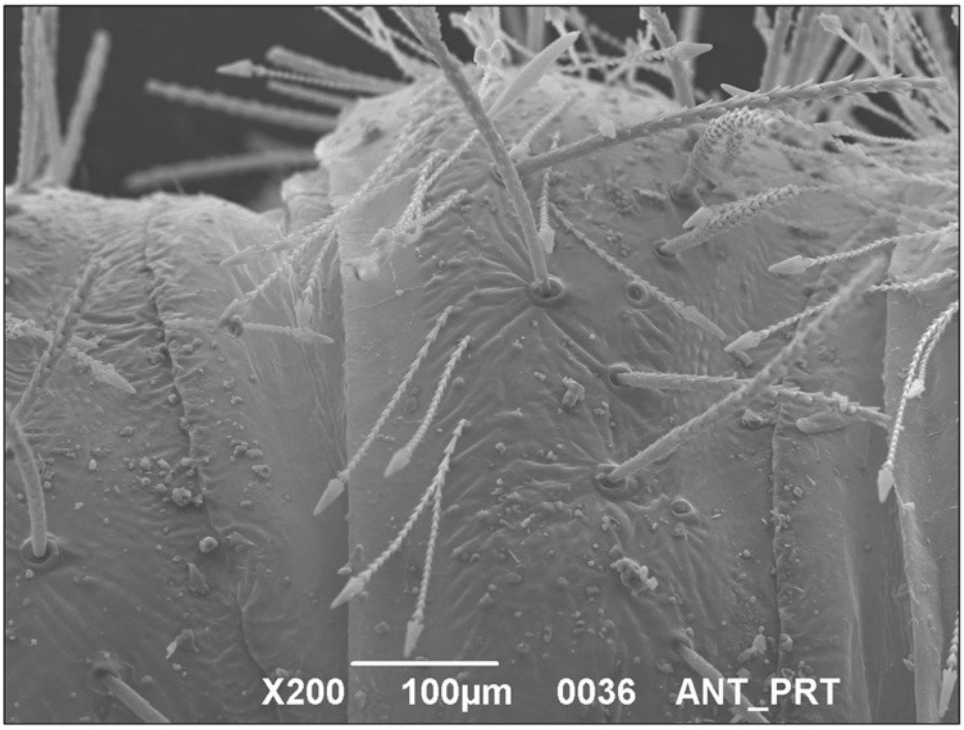

Fig. B. 200x. Detail of the first and second thoracic tergite, dorsal view.

Anthrenus spp. 2 (Portugal)

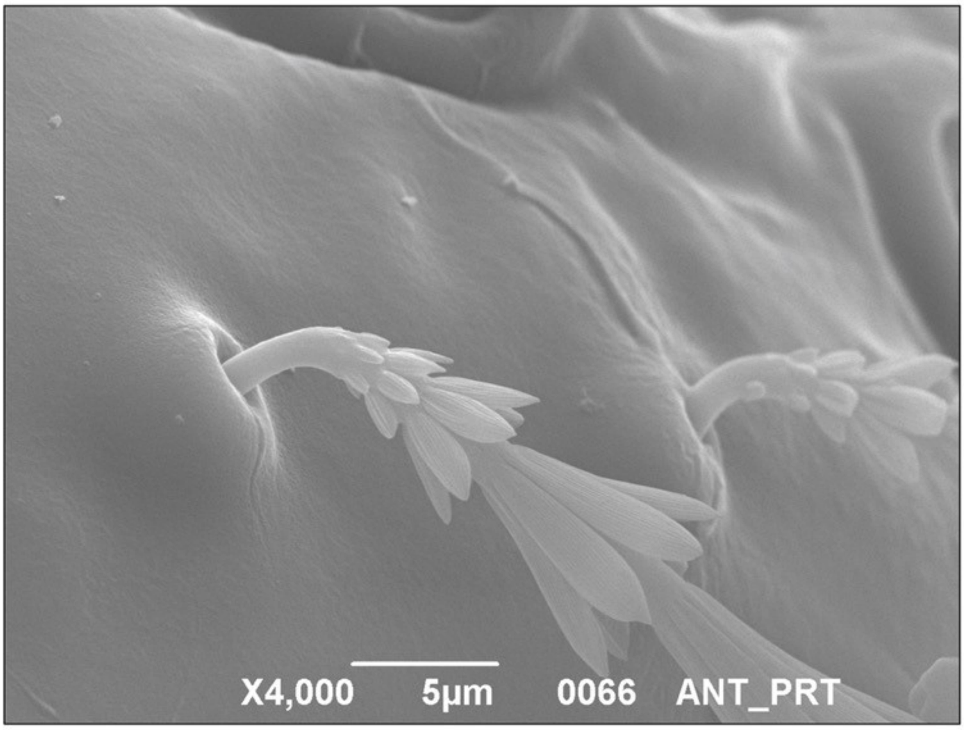

Fig. C. 4000x. Detail of the insertion of the hastisetae on thoracic and first abdominal tergites.

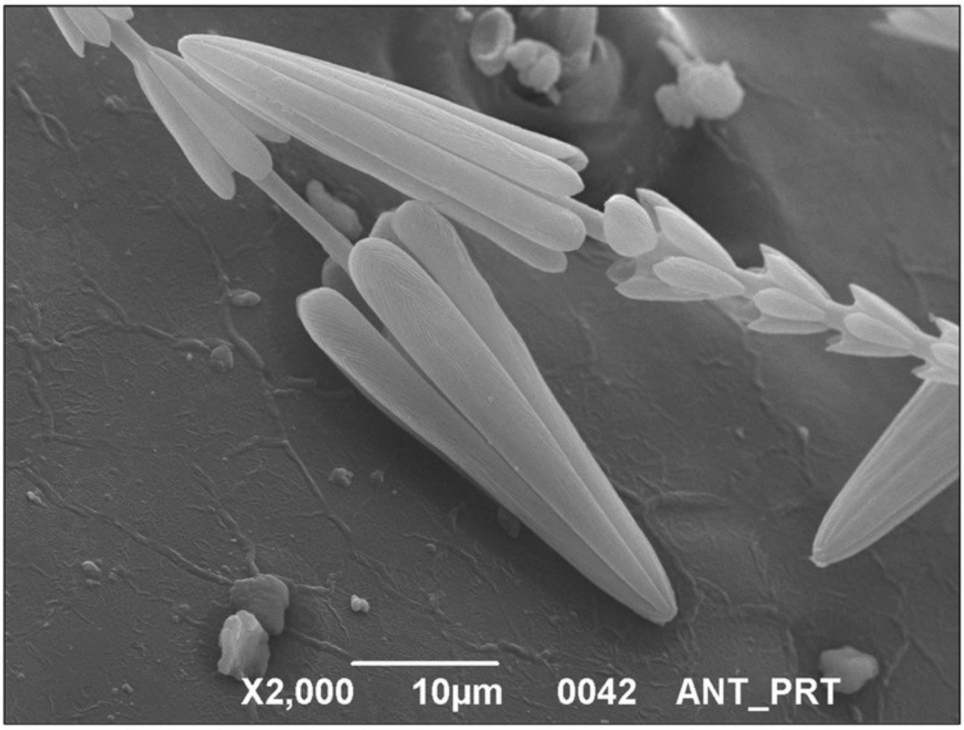

Fig. D. 2000x. Head of the hastisetae on thoracic and first abdominal tergites.

Anthrenus spp. 2 (Portugal)

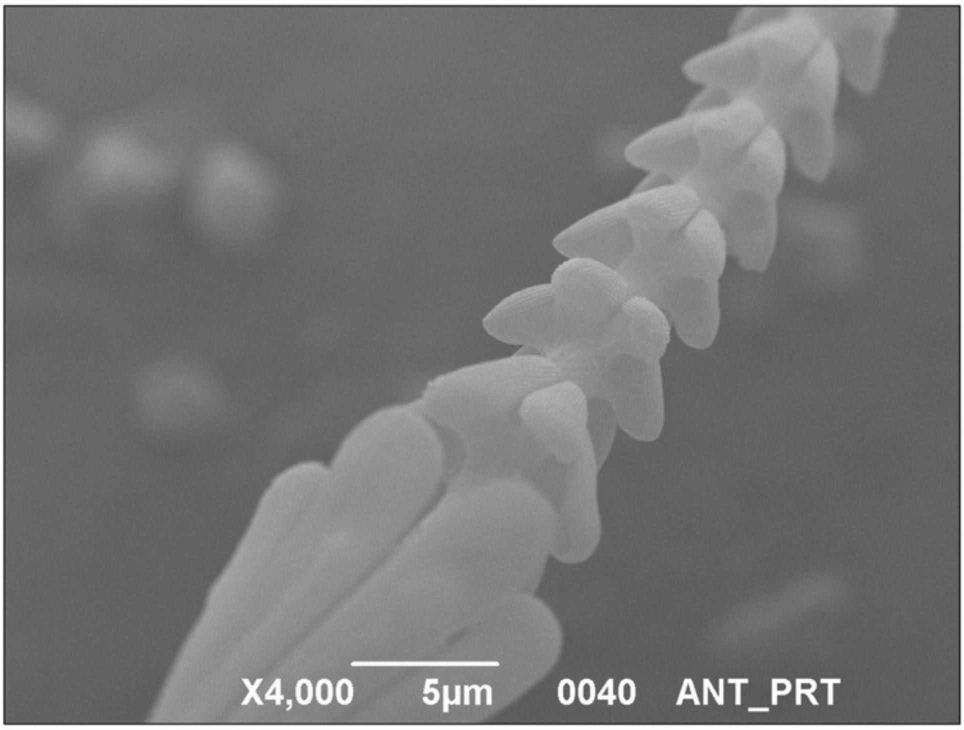

Fig. E. 4000x. Antero-lateral view of the last part of the hastiseta, highlighting rosettes structure.

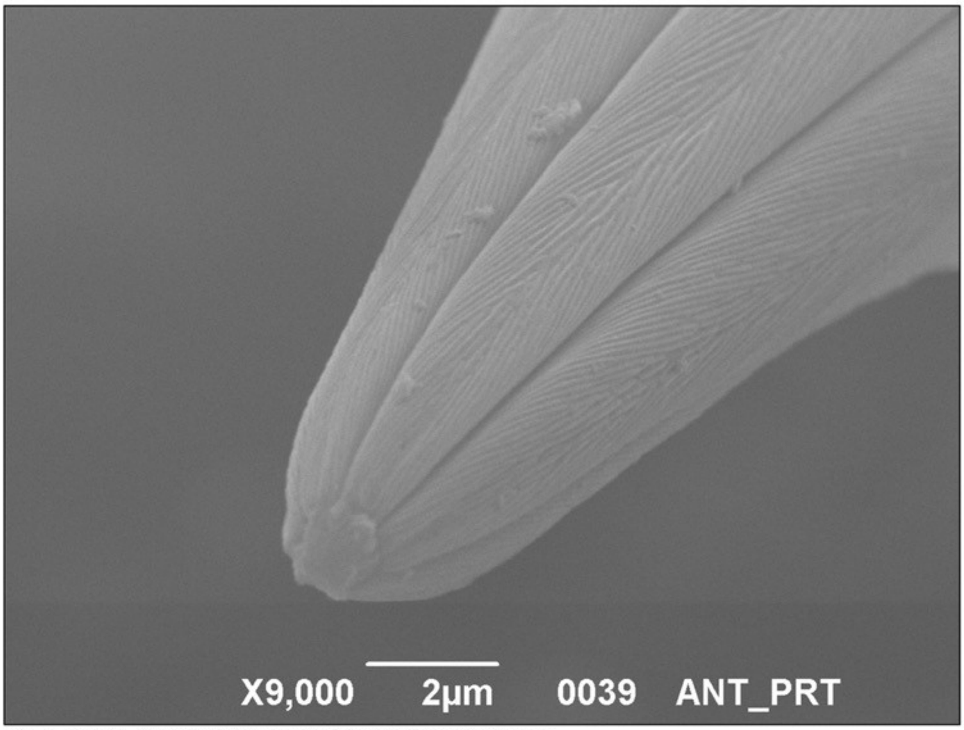

Fig. F. 9000x. Detail of the apex of head of the hastiseta.

Anthrenus spp. 2 (Portugal)

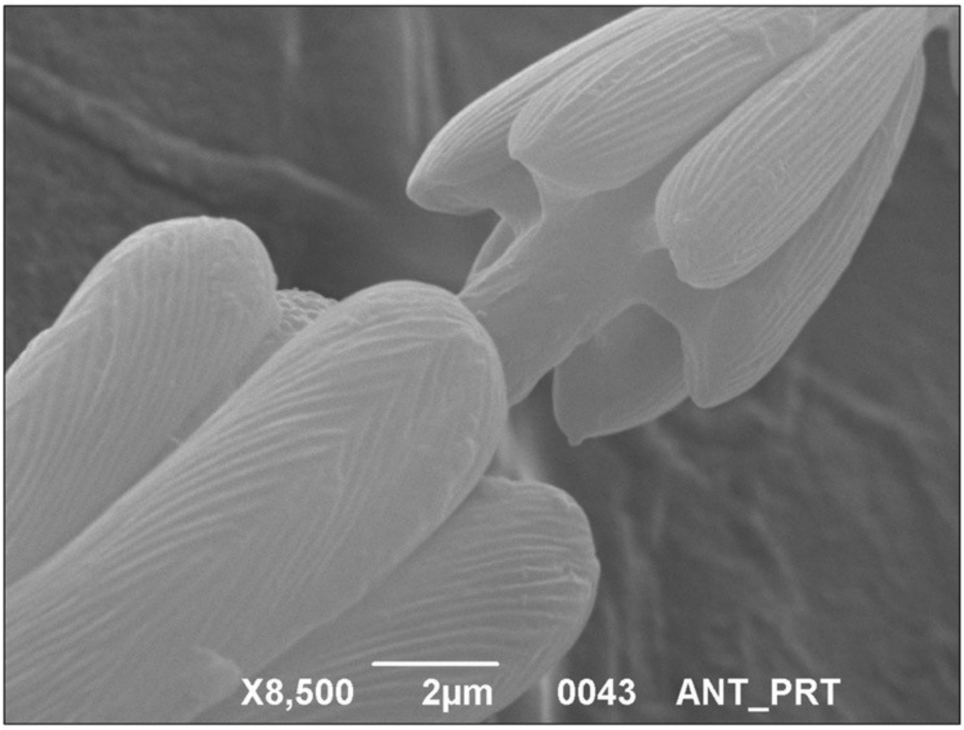

Fig. G. 8500x. Detail of the last rosette and the apical parts of the longitudinal processes of the head.

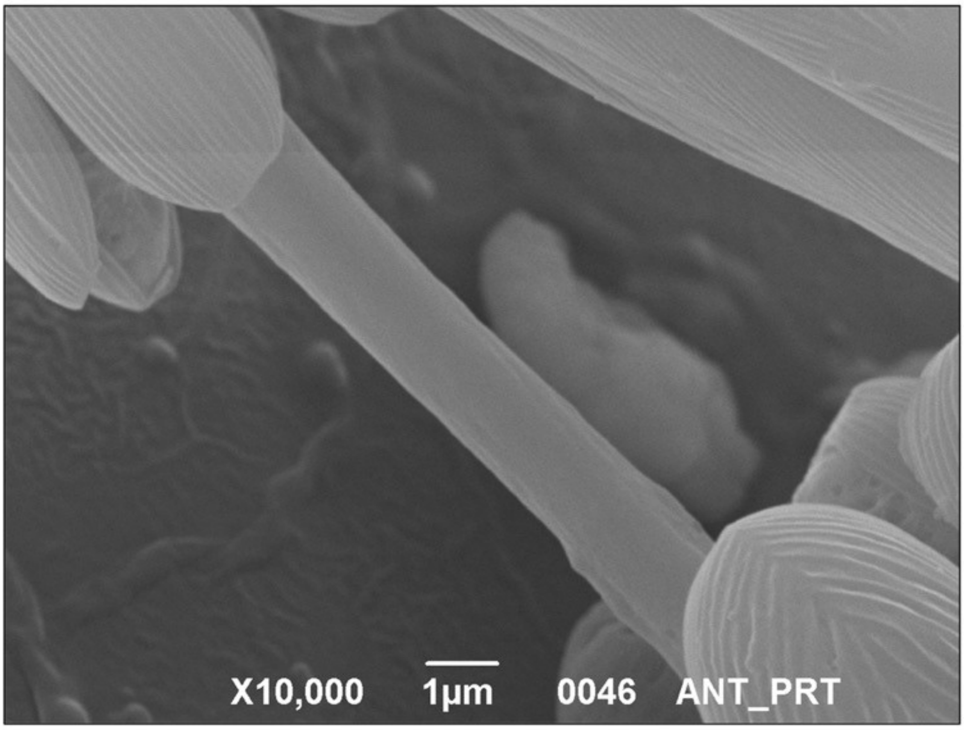

Fig. H. 1000x. Detail of the stalk between the last rosette and the head of the hastiseta.

Anthrenus spp. 2 (Portugal)

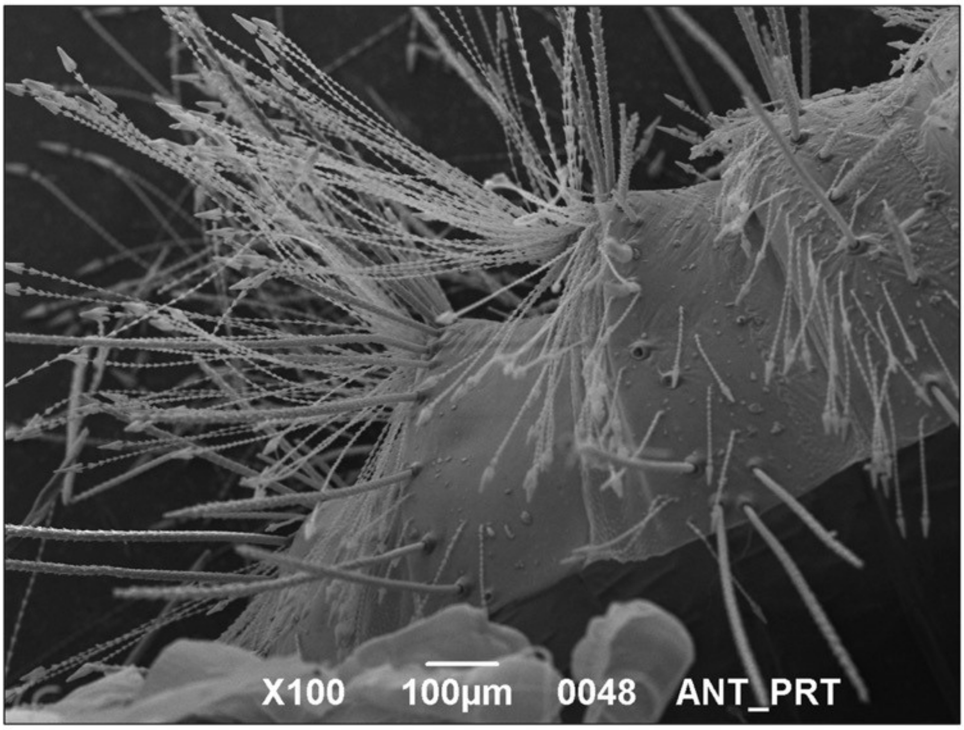

Fig. I. 100x. Tufts of erected hastisetae on the last abdominal tergites, lateral view.

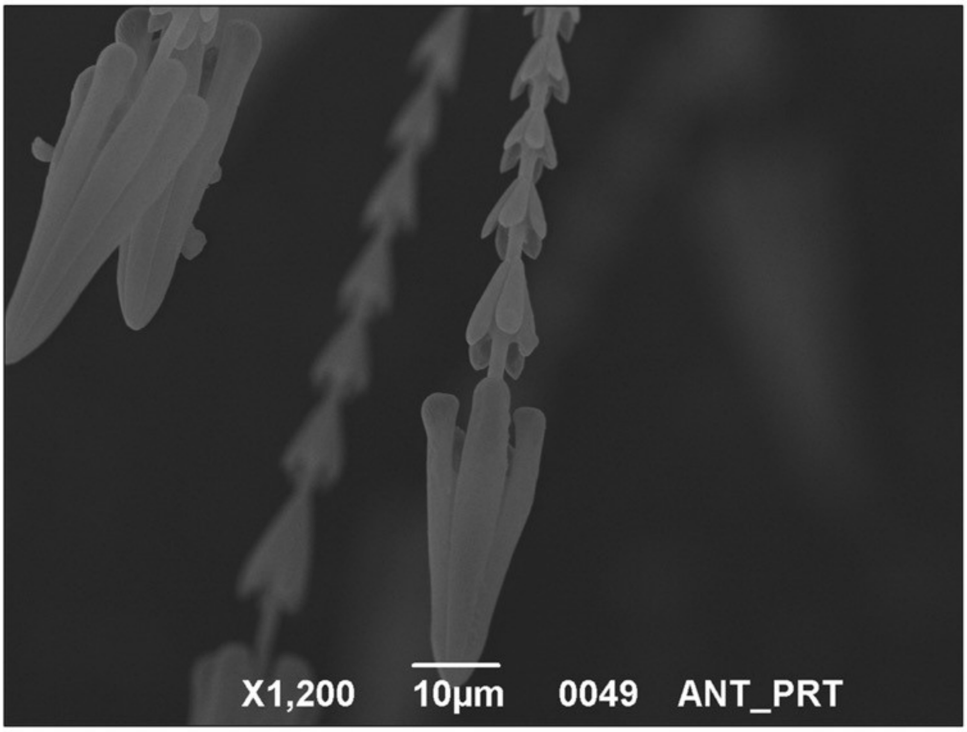

Fig. J. 1200x. Apex of the hastiseta of the last abdominal tergites.

Anthrenus spp. 2 (Portugal)

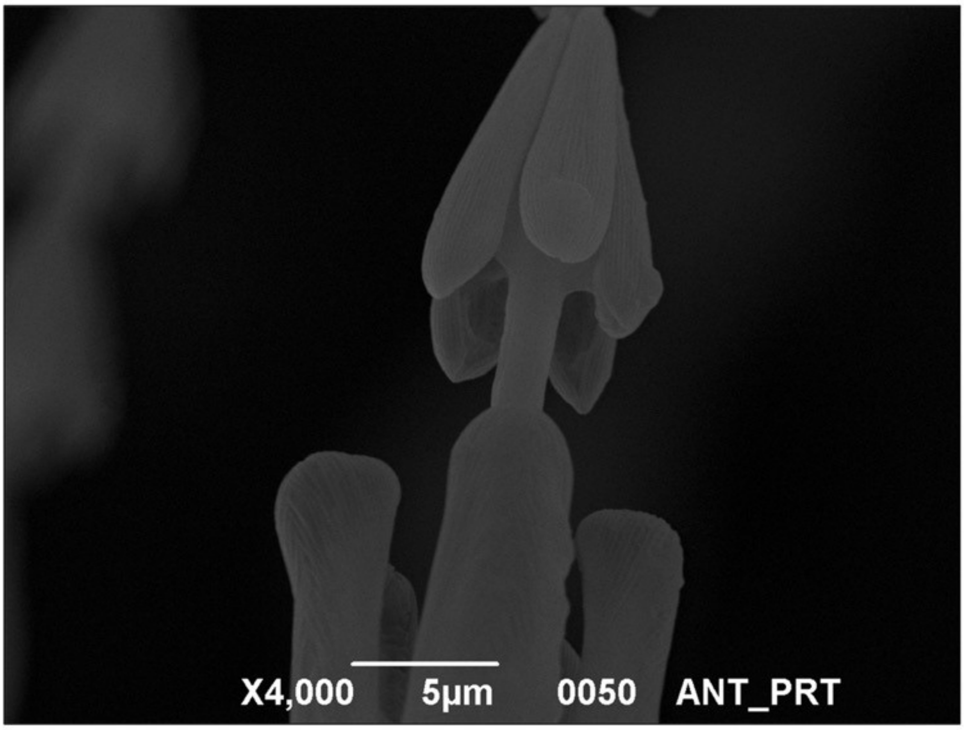

Fig. K. 4000x. Detail of the last rosette in antero-lateral view.

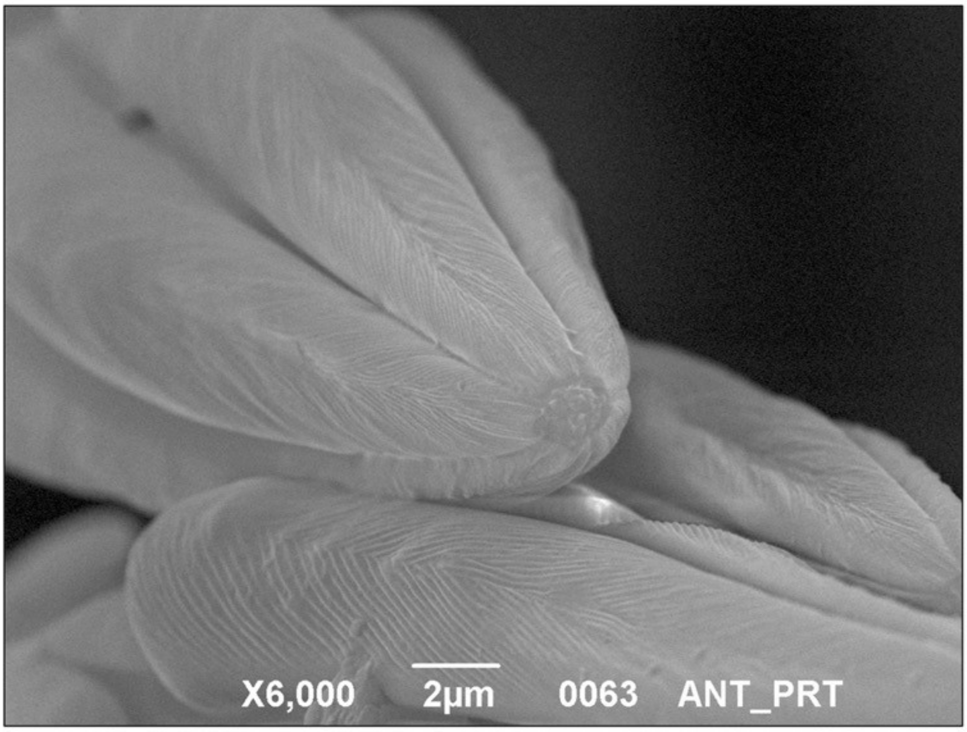

Fig. L. 6000x. Detail of the apex of the head of the hastiseta showing the circular depression.

Anthrenus spp. 2 (Portugal)

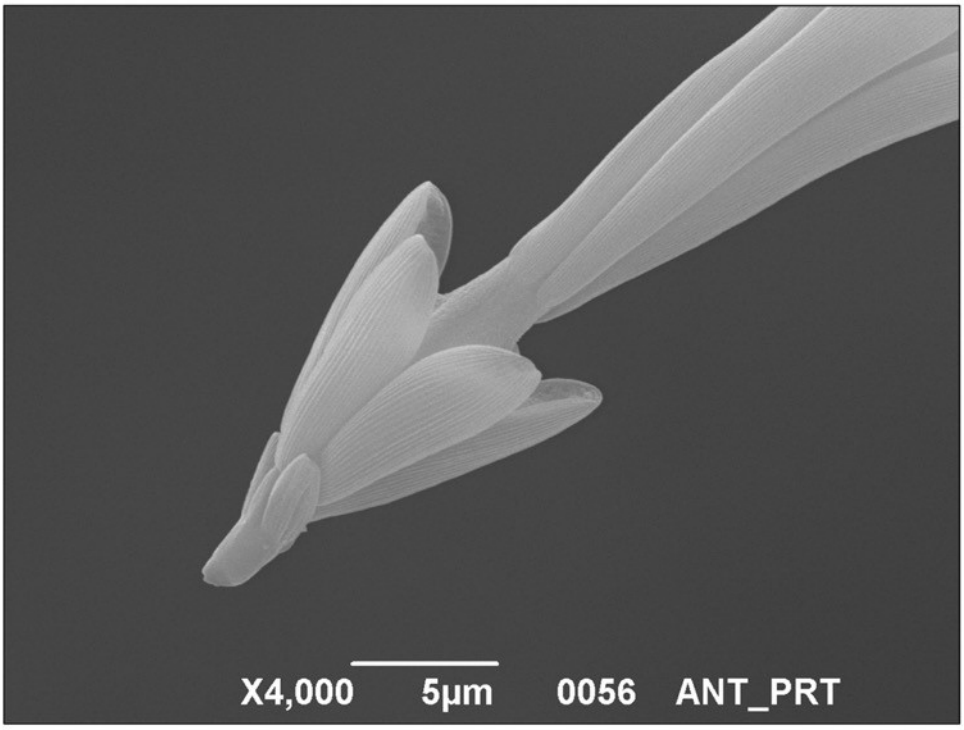

Fig. M. 4000x. Detail of the pedicel of one hastiseta belonging to the tuft on the last tergites.

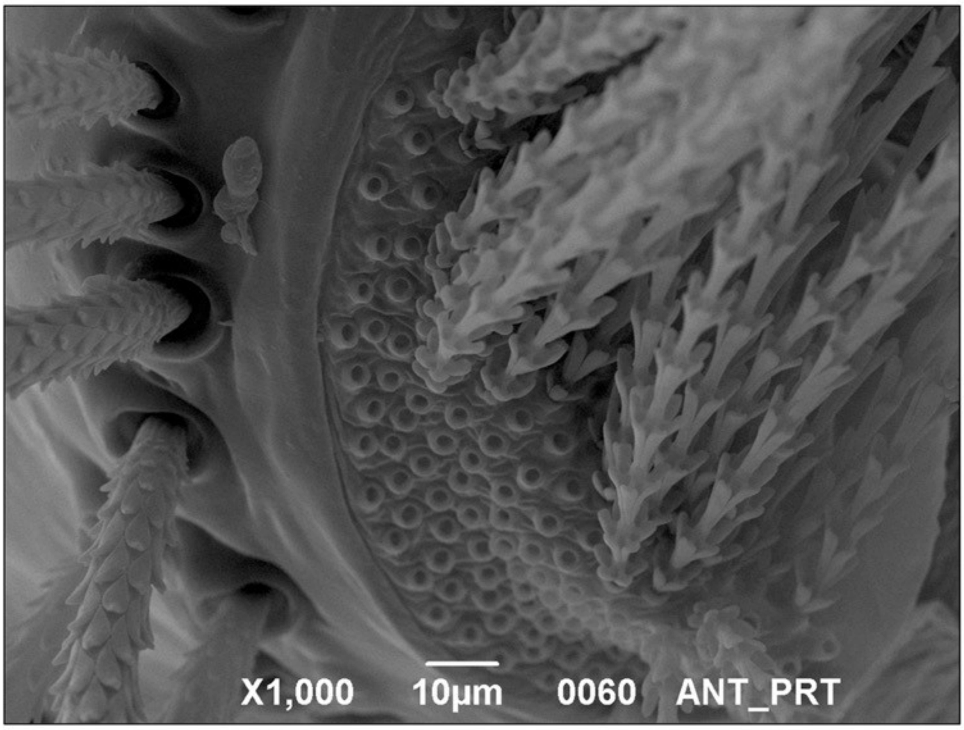

Fig. N. 1000x. Detail the membranous part of the 6th abdominal tergite showing the insertion of the tuft of hastisetae.

Supplementary material 10

Ctesias serra (Fabricius, 1792)

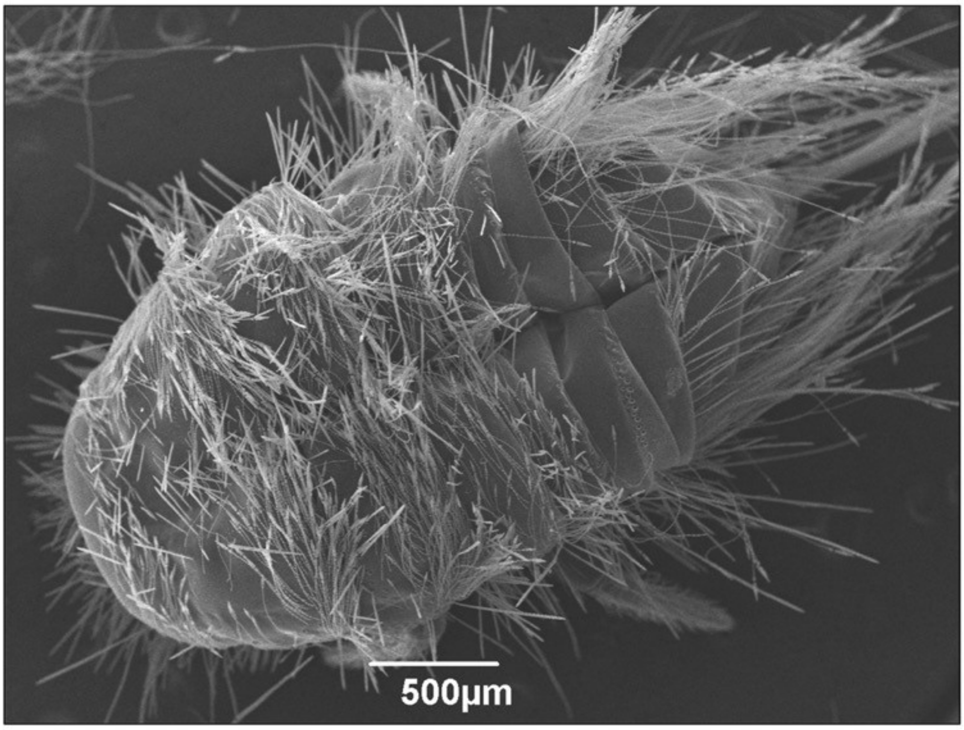

Fig. A. 35x. Mature larva, dorsal view. Insert figure B.

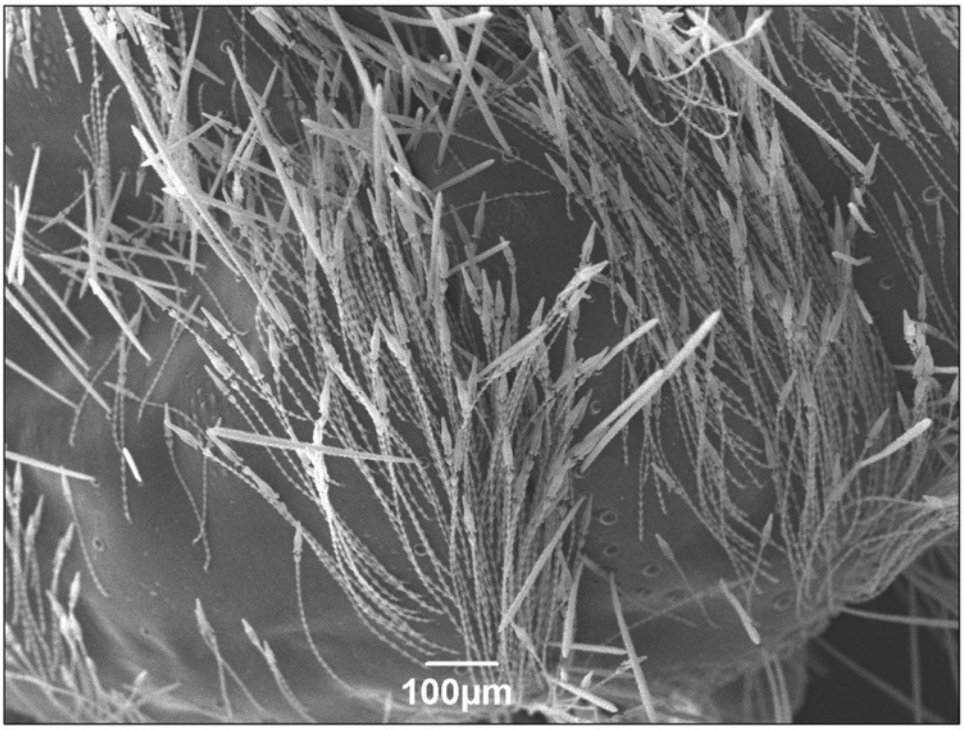

Fig. B. 100x. Detail of the first and second thoracic tergite, dorsal view.

Ctesias serra (Fabricius, 1792)

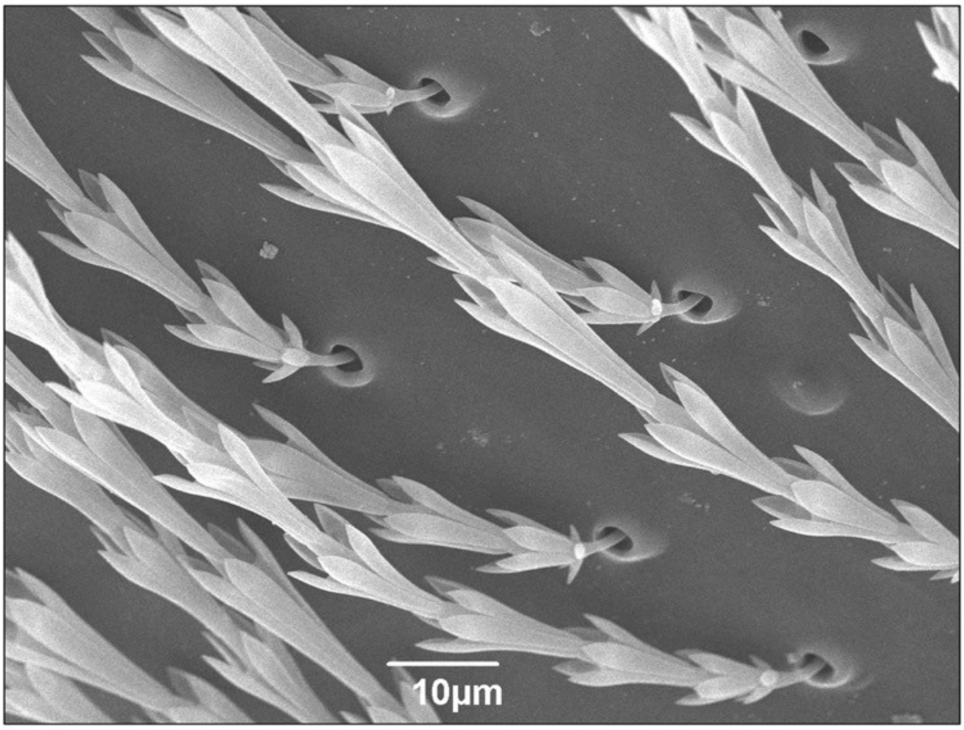

Fig. C. 1500x. Insertion of the hastisetae on thoracic and first abdominal tergites.

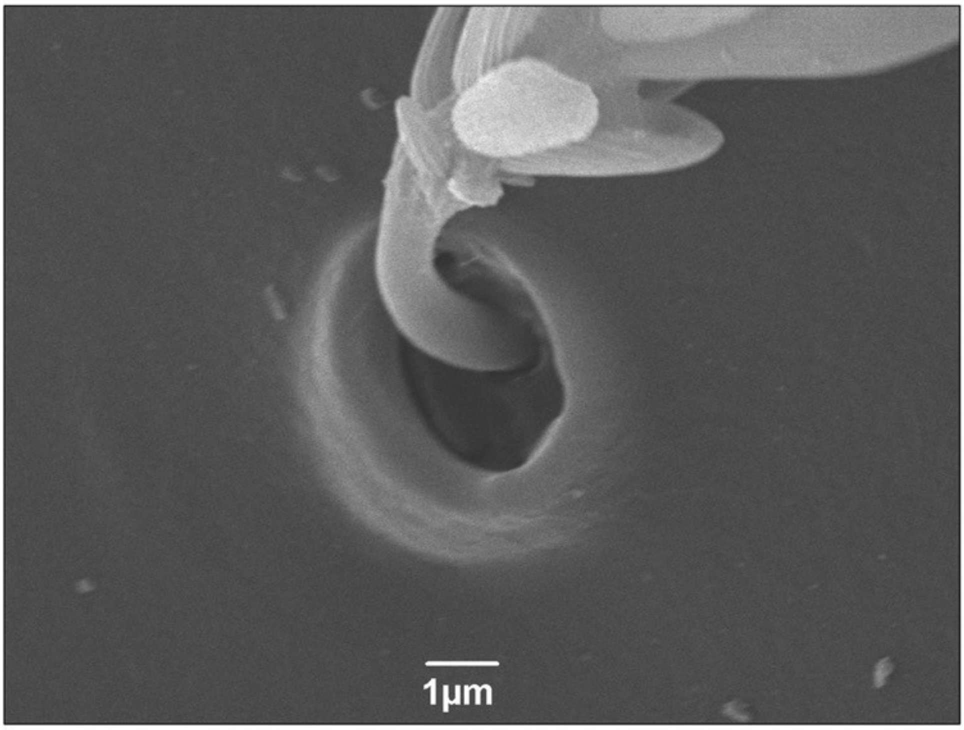

Fig. D. 10000x. Detail of one insertion showing the curved pedicel and the chitin sheath.

Ctesias serra (Fabricius, 1792)

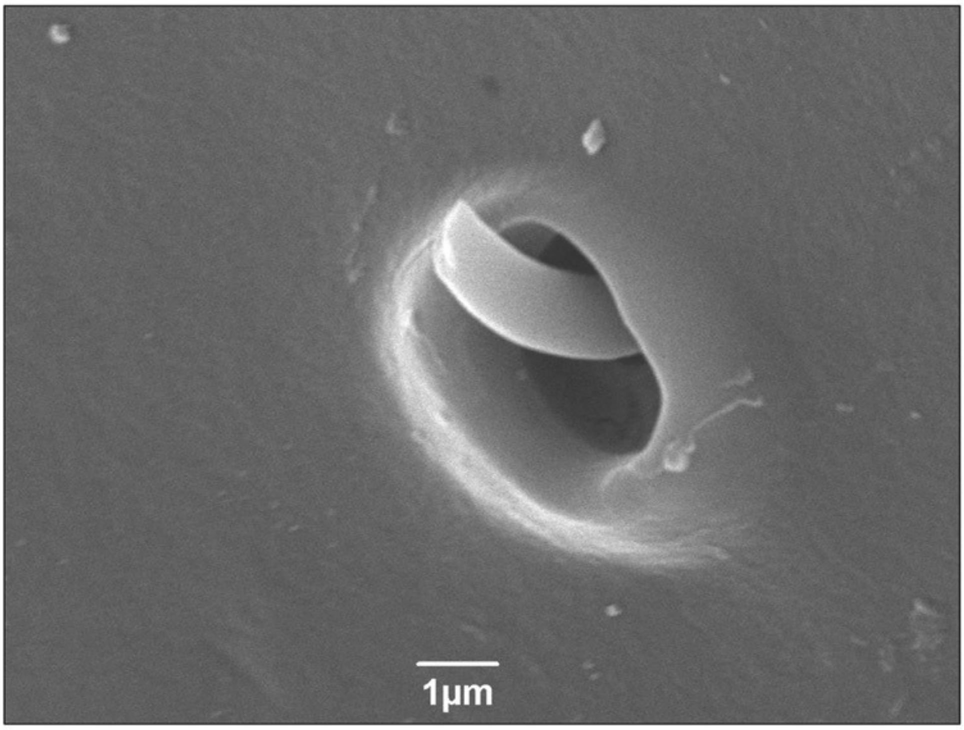

Fig. E. 11000x. Detail of a broken pedicel showing the clear breaking point.

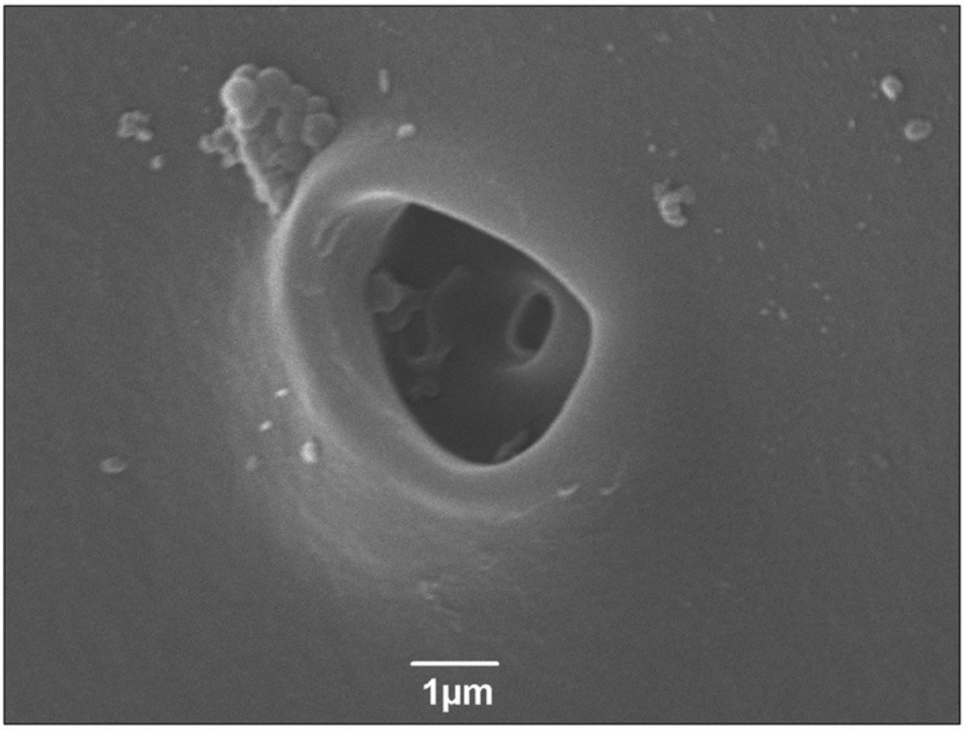

Fig. F. 12000x. Detail of the papilla and of the chitin sheath surrounding the insertion of the pedicel.

Ctesias serra (Fabricius, 1792)

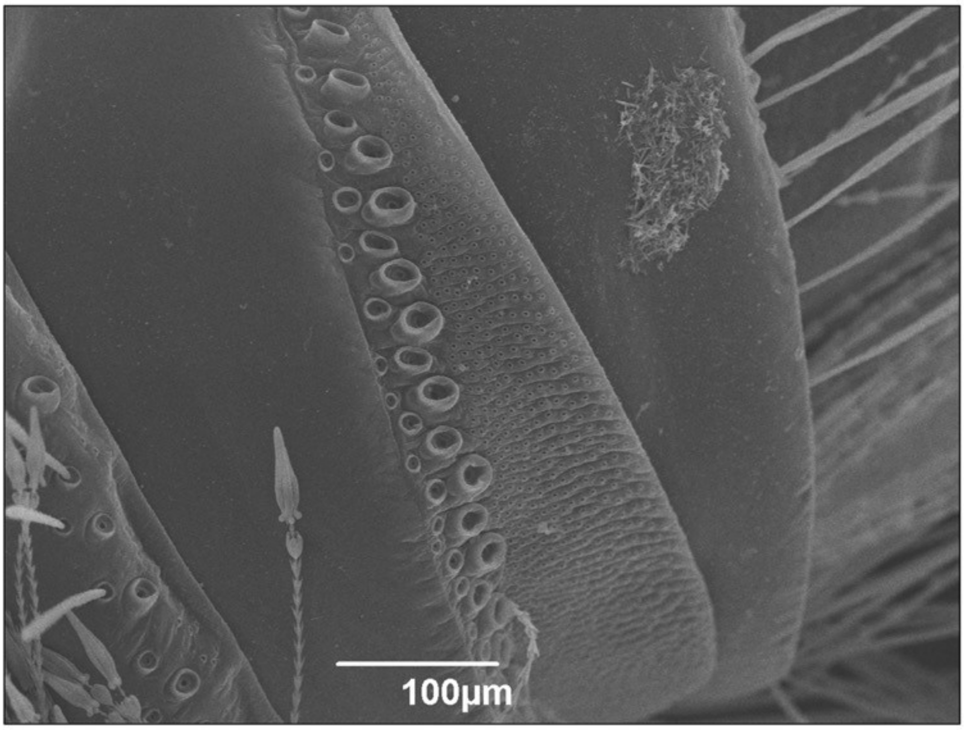

Fig. G. 220x. Detail of the membranous part of the 4th abdominal tergite (hastisetae removed)

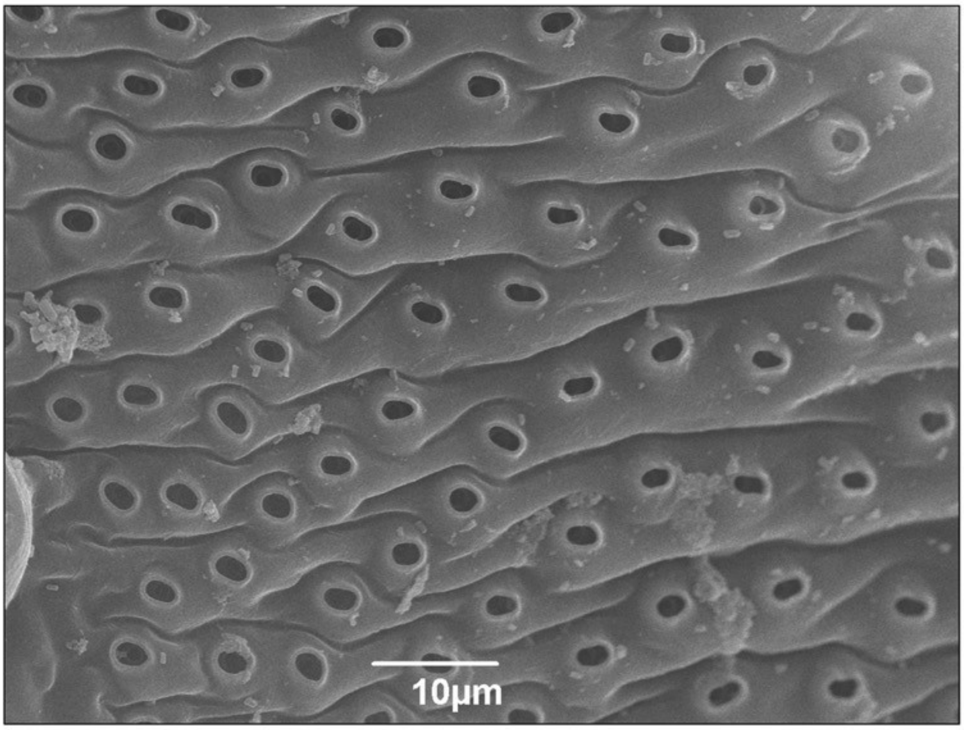

Fig. H. 1700x. Detail showing the peculiar shape and organization of the insertion of the hastisetae

Ctesias serra (Fabricius, 1792)

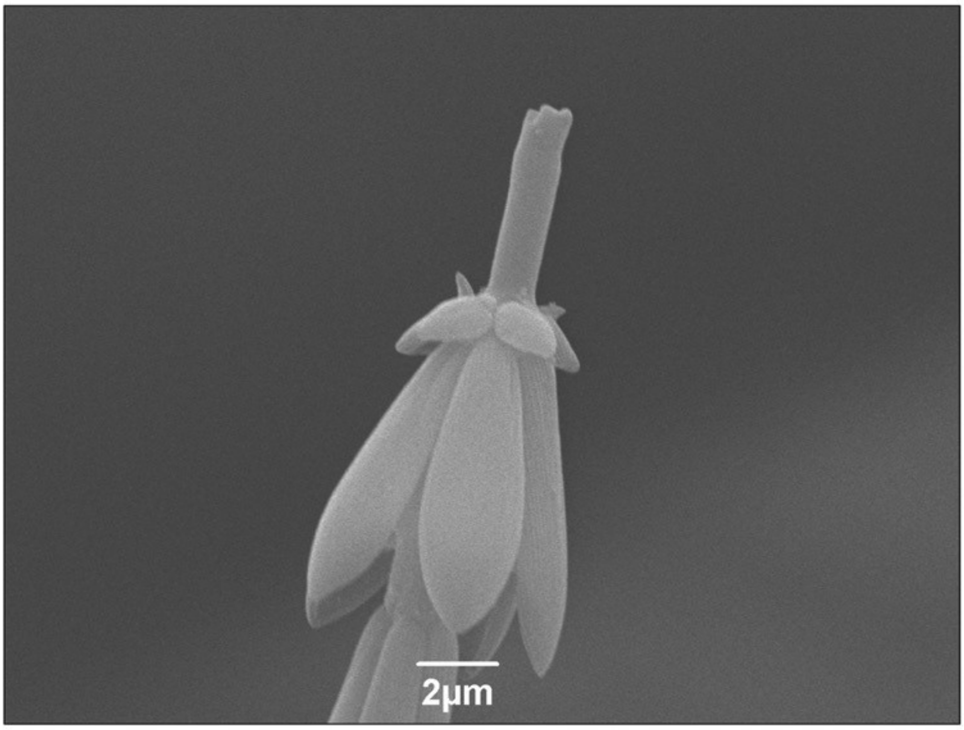

Fig. I. 5500x. Detail of the pedicel of one hastisetae belonging to the tufts on the last abdominal tergites.

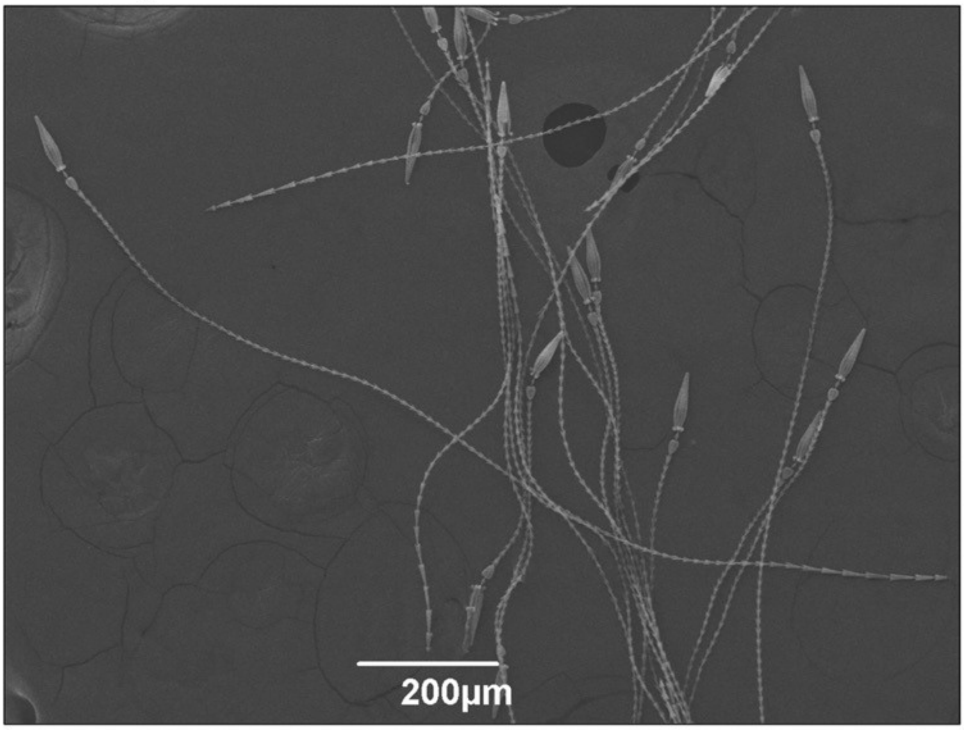

Fig. J. 1700x. Hastisetae of the 4th-7th abdominal tergites.

Ctesias serra (Fabricius, 1792)

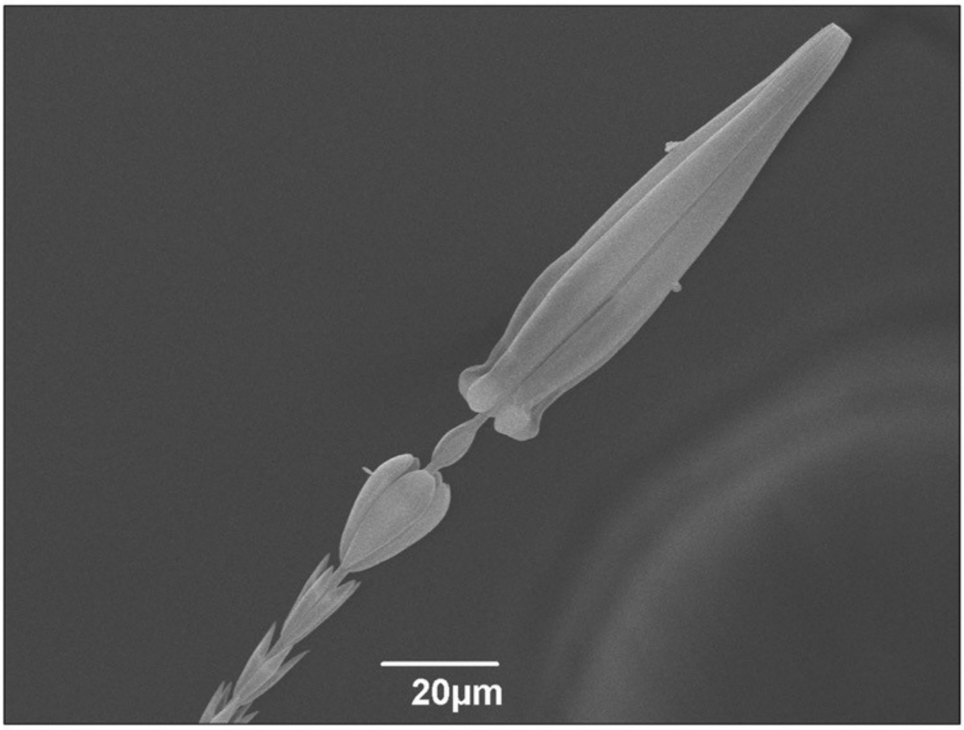

Fig. K. 800x. Detail of apex of the hastiseta showing the peculiar shape of the last rosette, stalk and head of the hastiseta.

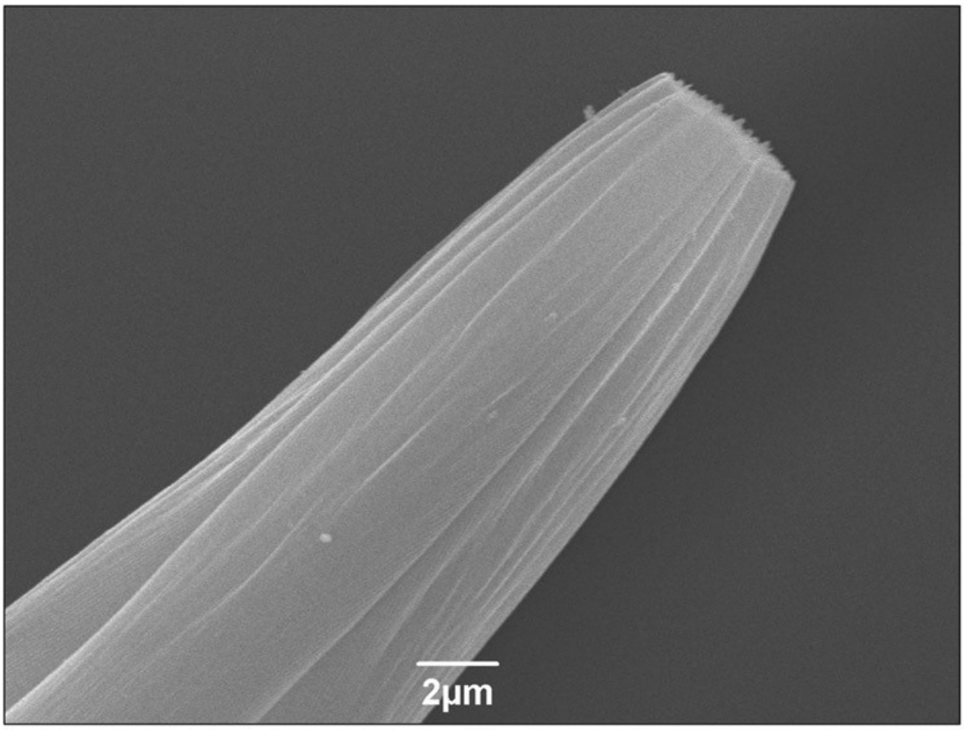

Fig. L. 5500x. Apex of the head of the hastiseta, lateral view.

Ctesias serra (Fabricius, 1792)

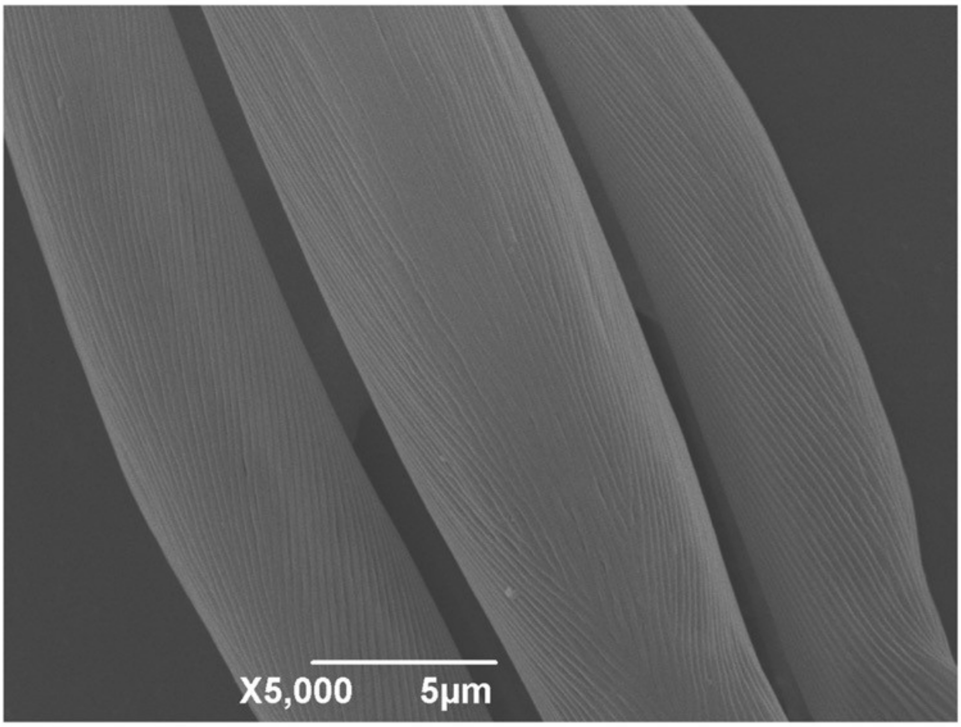

Fig. M. 800x. Detail of the knurls covering the longitudinal processed of the head of the hastiseta.

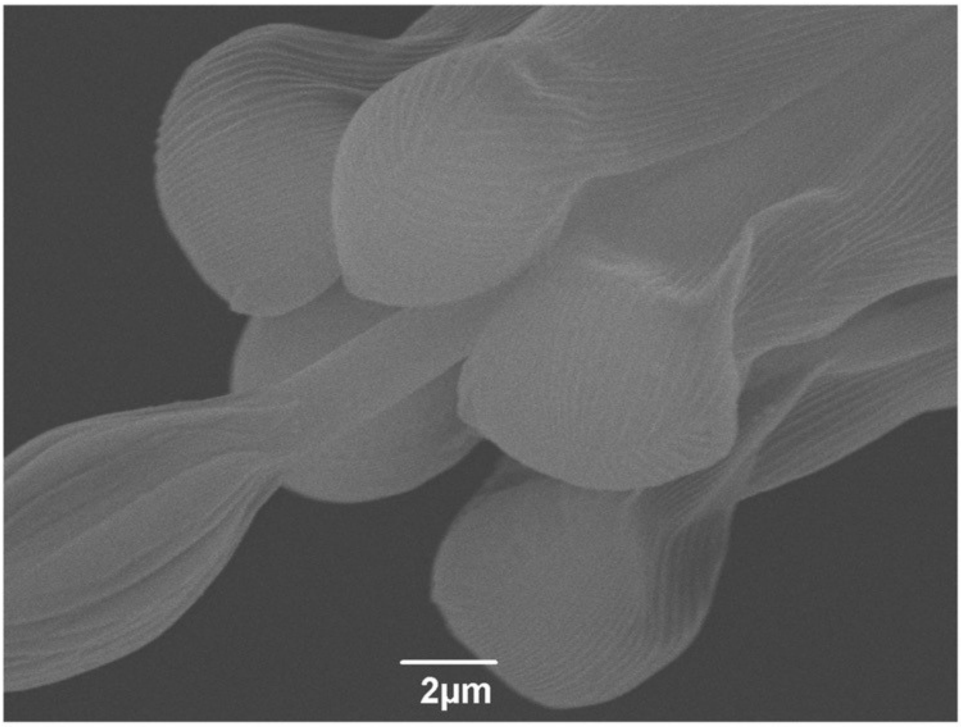

Fig. N. 6500x. Detail of the apical knob of the longitudinal processes of the hastiseta.

Ctesias serra (Fabricius, 1792)

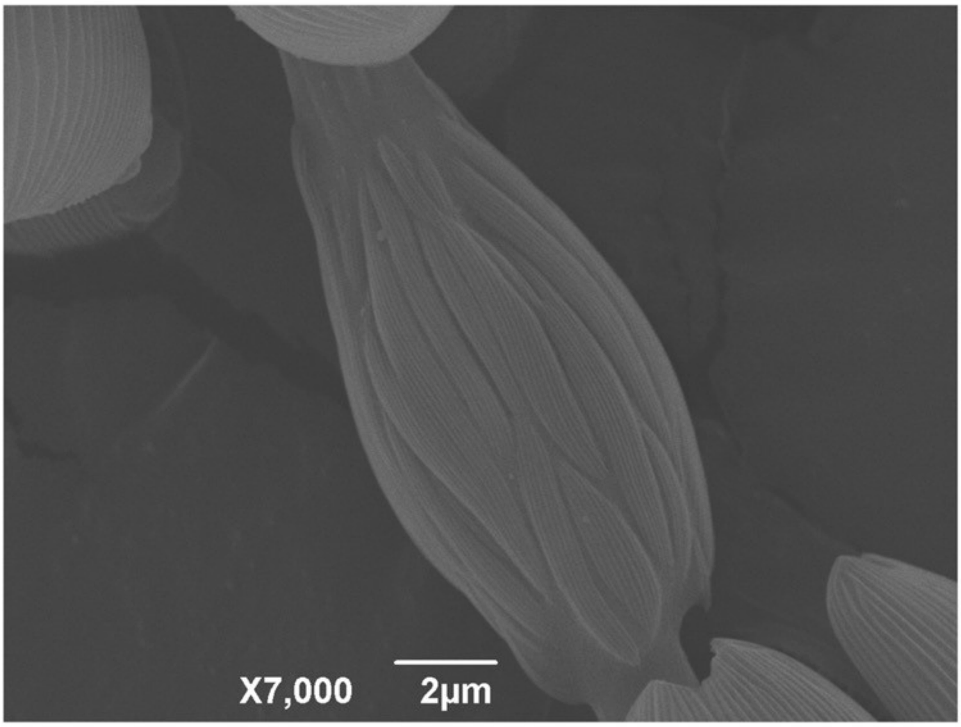

Fig. O. 700x. Detail of the bud-like shaft before the apical head of the hastiseta.

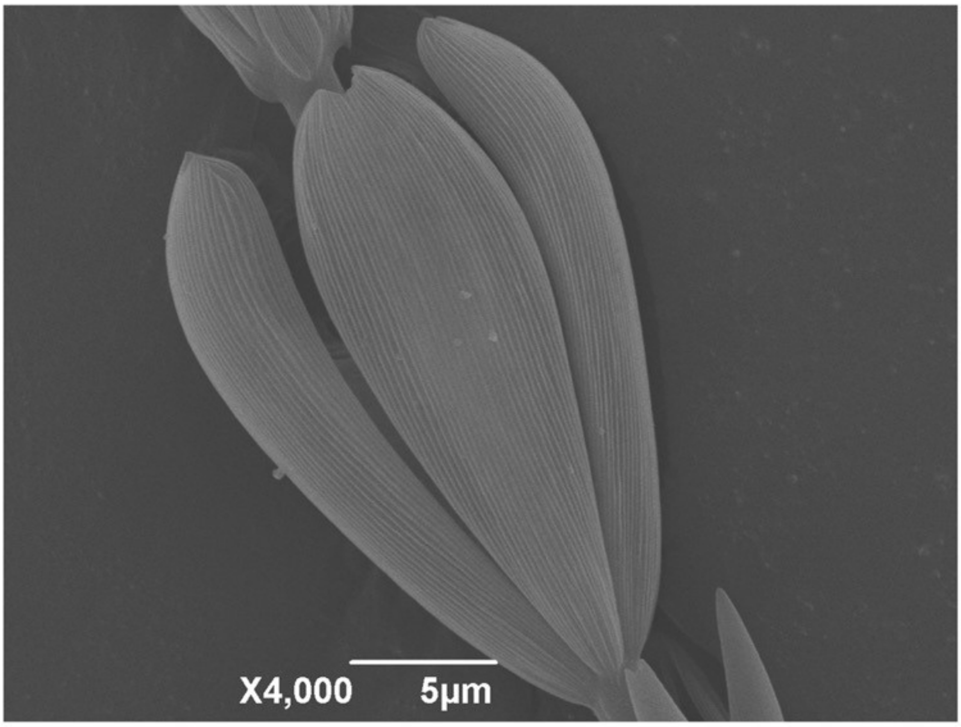

Fig. P. 4000x. Detail of the ultimate rosette, lateral view.

Supplementary material 11

Megatoma (*Megatoma*) *undata* (Linnaeus, 1758)

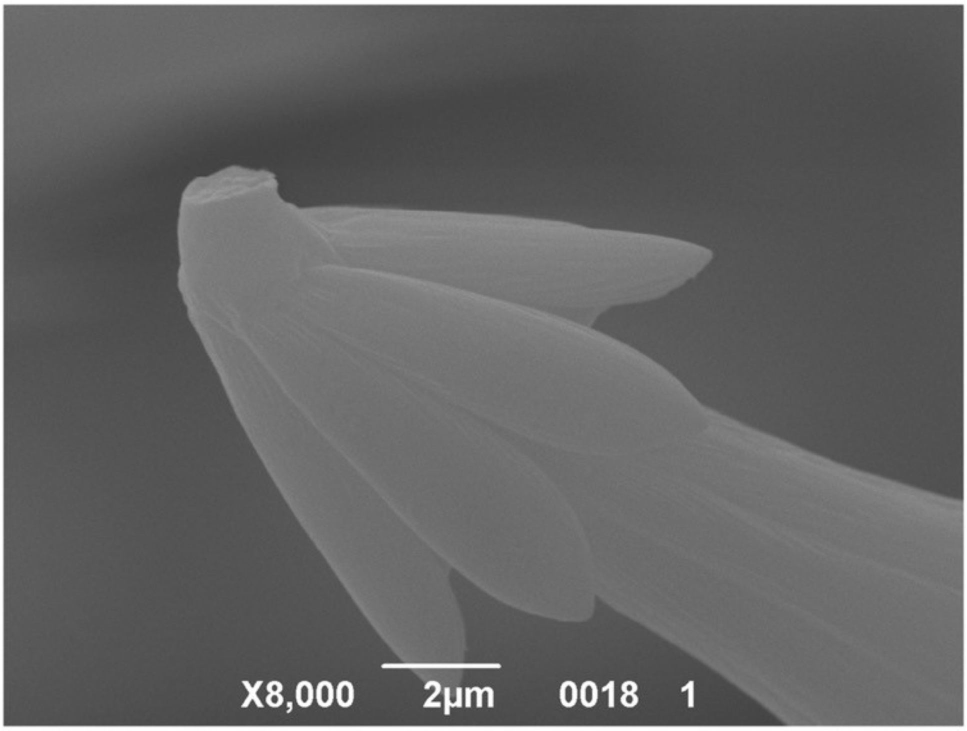

Fig. A. 8000x. Detail of a detached hastiseta showing the broken pedicel.

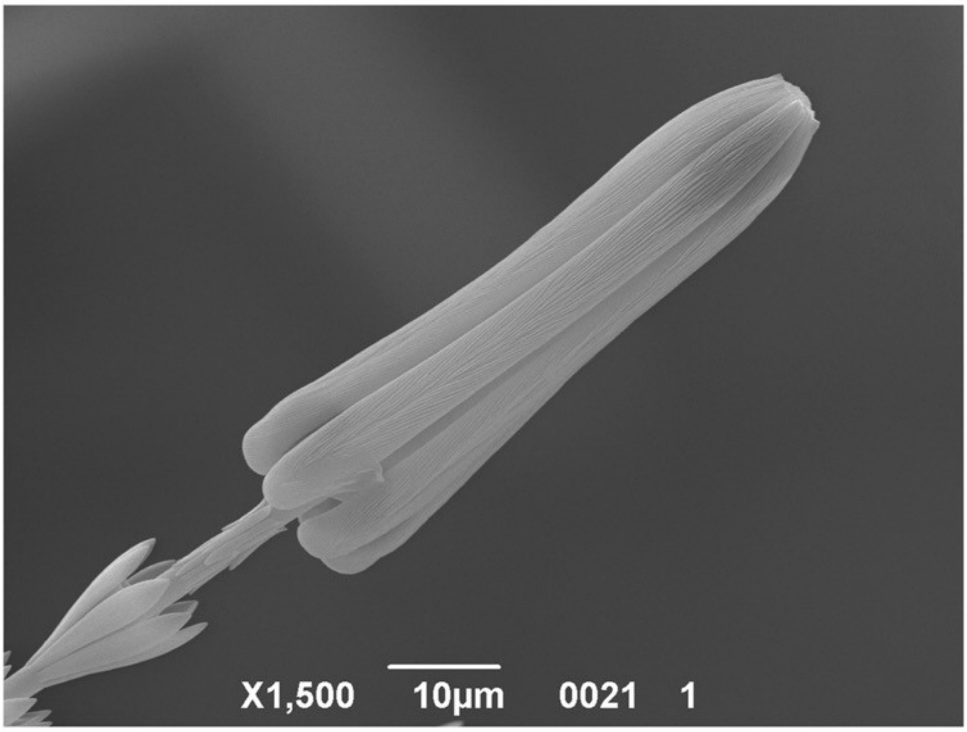

Fig. B. 1500x. Lateral view of the apical part of the hastiseta showing last rosette, stalk and head of the hastiseta.

Megatoma (*Megatoma*) *undata* (Linnaeus, 1758)

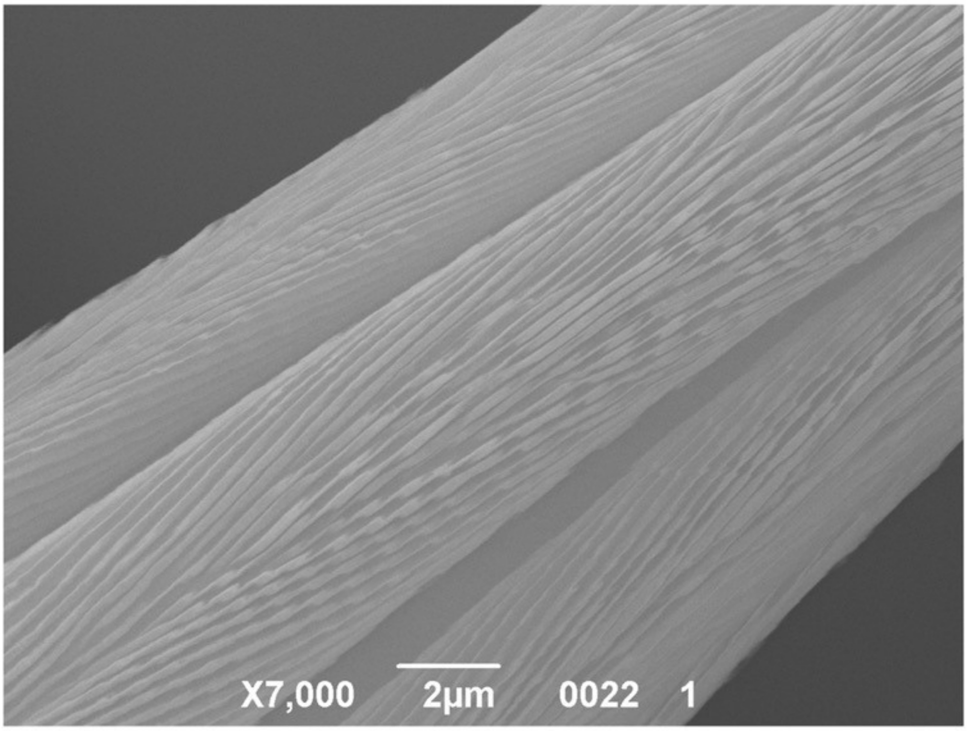

Fig. C. 7000x. Detail of the knurls covering the longitudinal processed of the head of the hastiseta.

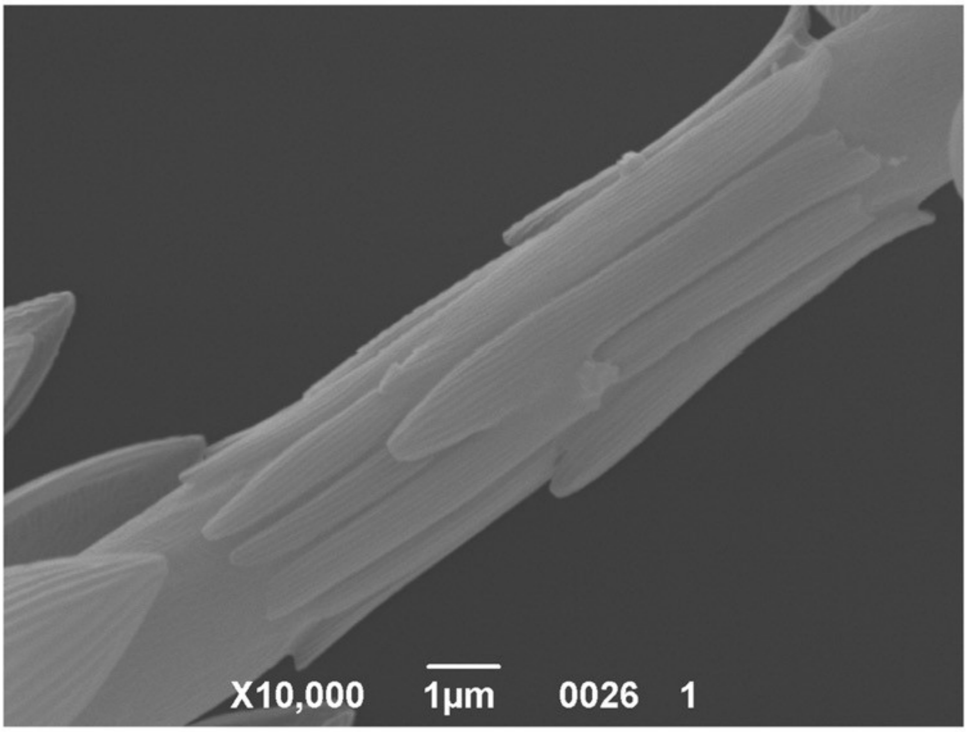

Fig. D. 10000x. Detail of the shaft highlighting the scale-like structures.

Megatoma (*Megatoma*) *undata* (Linnaeus, 1758)

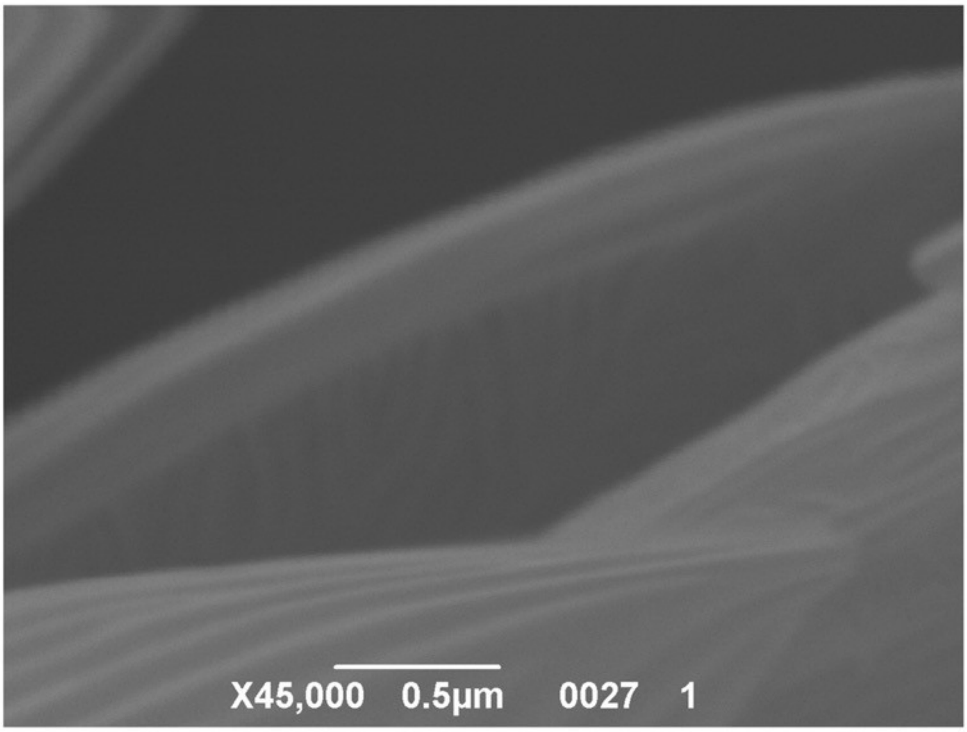

Fig. E. 45000x. Detail of the inner part of one scale of the rosette showing the amorphous chitin matrix.

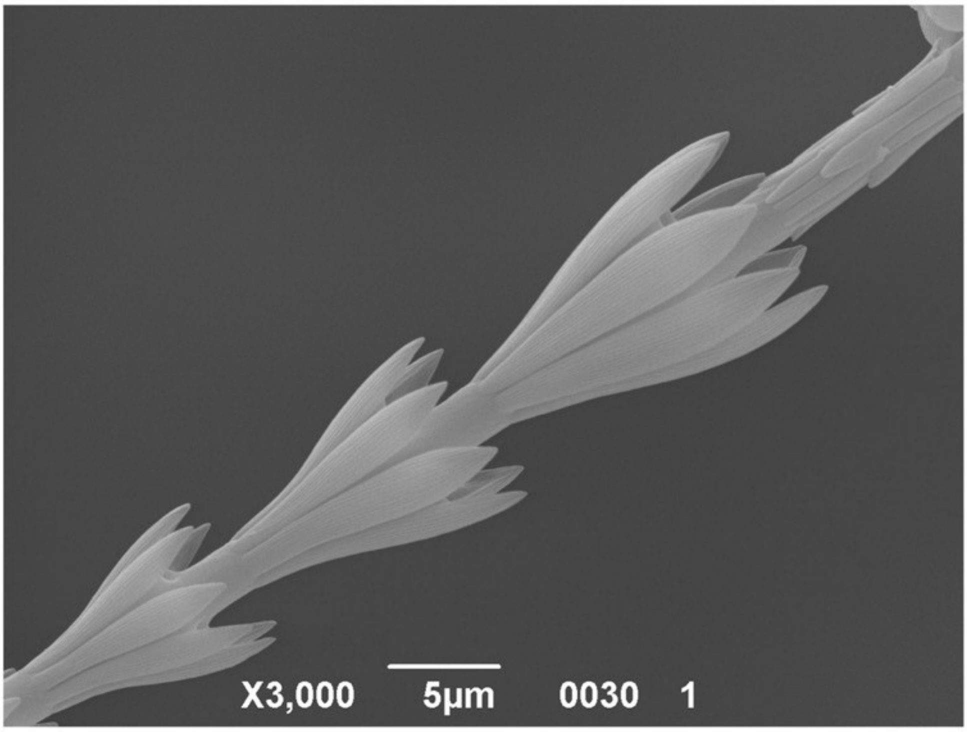

Fig. F. 10000x. Shape and size comparison between the last three rosettes of the hastiseta.

Megatoma (*Megatoma*) *undata* (Linnaeus, 1758)

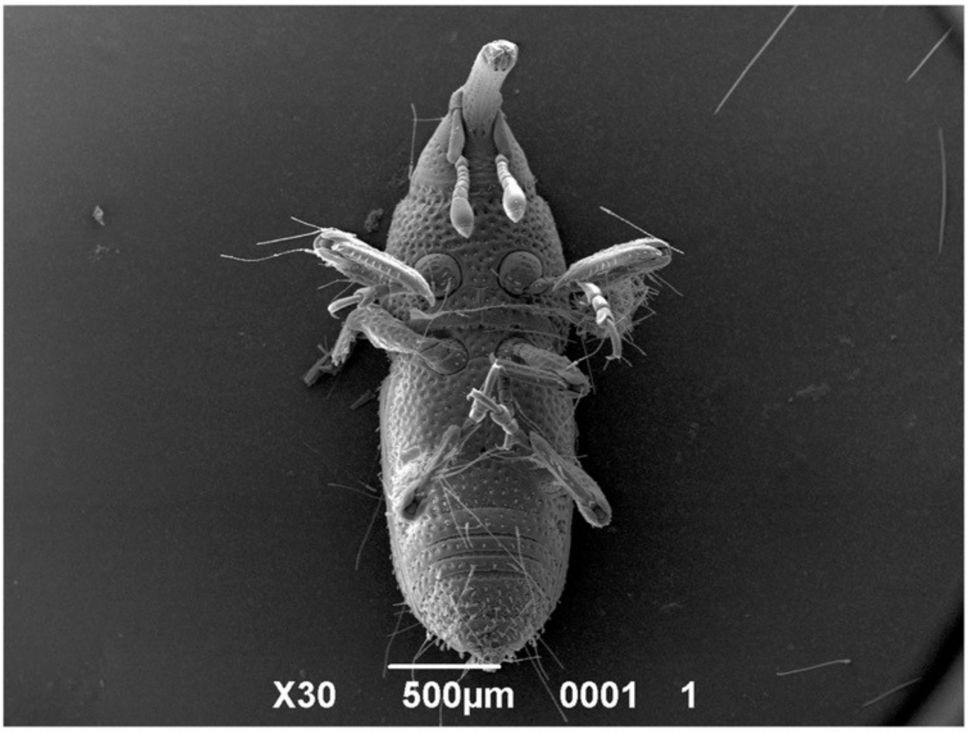

Fig. G. 5000x. *Sitophilus* sp. presenting hastisetae attached on scales.

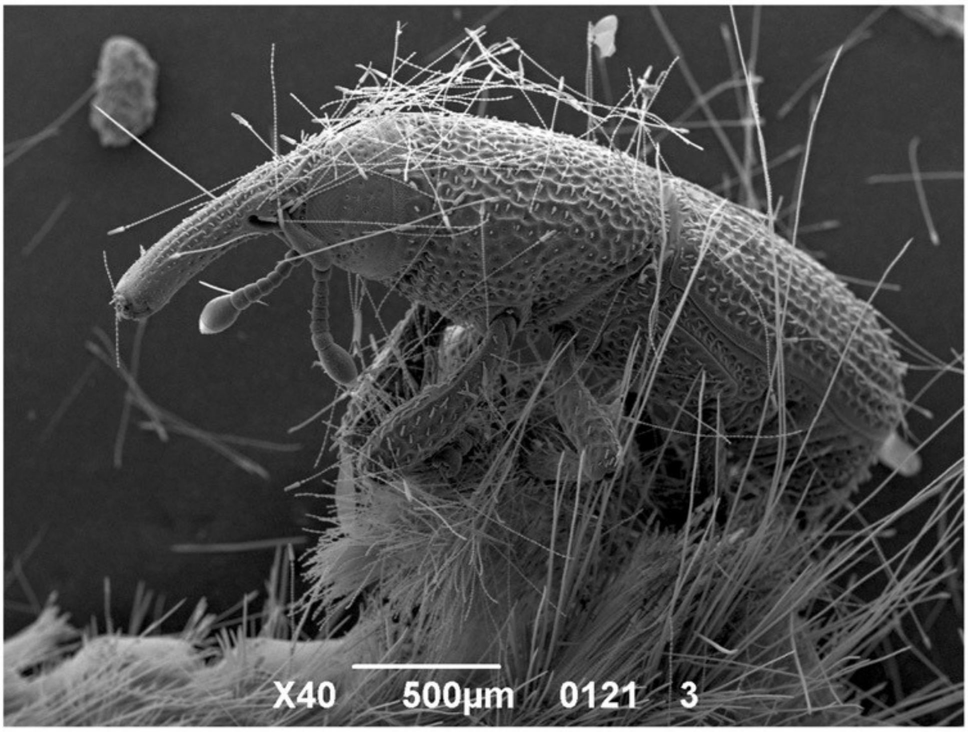

Fig. H. 10000x. *Sitophilus* sp. trapped on a *Megatoma* exuvia.

Megatoma (*Megatoma*) *undata* (Linnaeus, 1758)

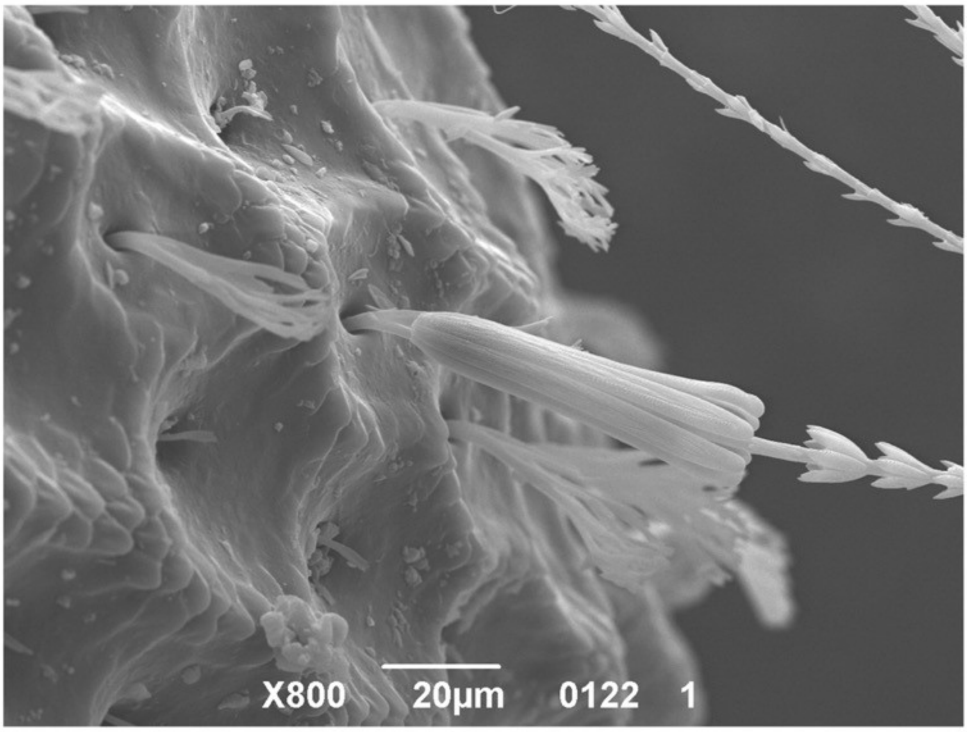

Fig. I. 800x. Head of the hastiseta attached to one *Sitophilus* sp. hair.

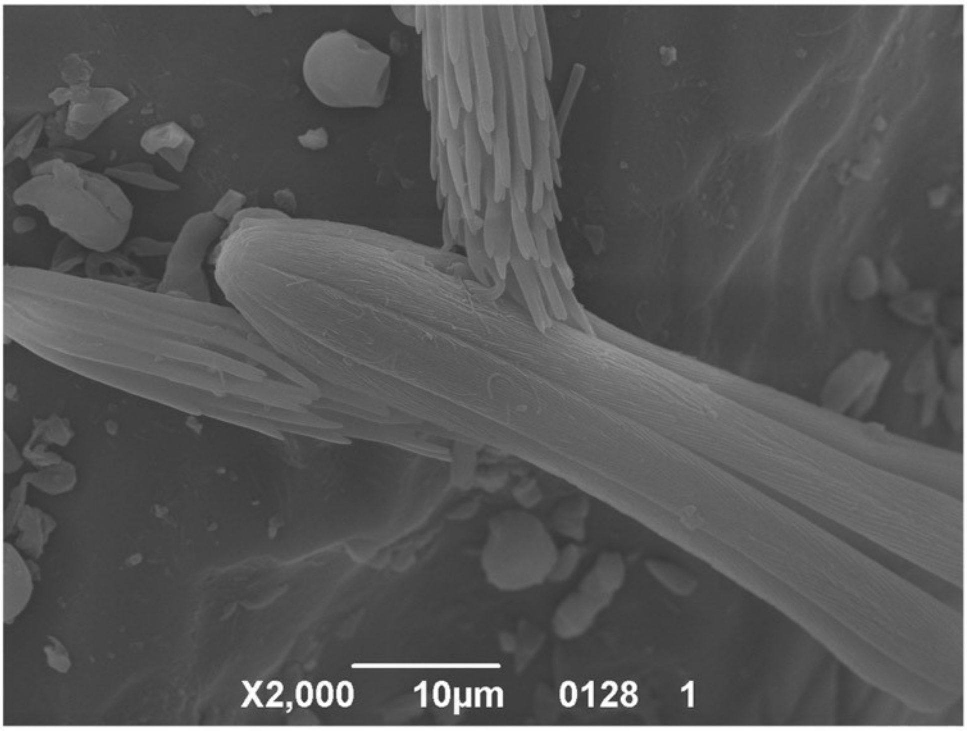

Fig. L. 2000x. Detail of two hairs entangles on the head of the hastiseta.

Supplementary material 12

Thaumaglossa rufocapillata Redtenbacher, 1867

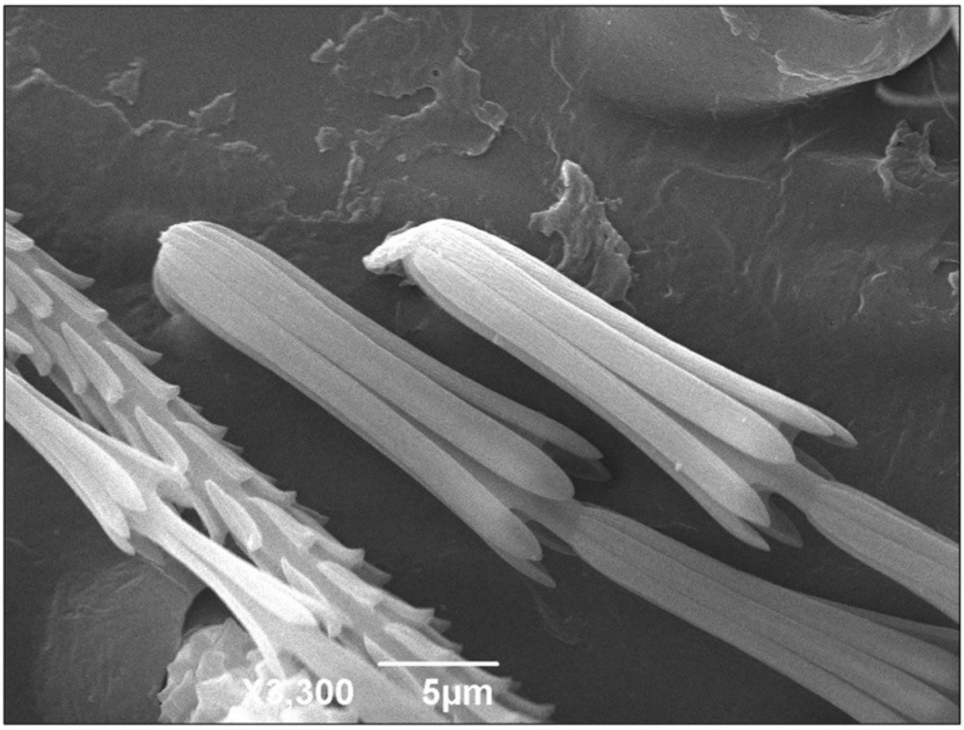

Fig. A. 3300x. Insertion of hastisetae on the thoracic and first abdominal tergites.

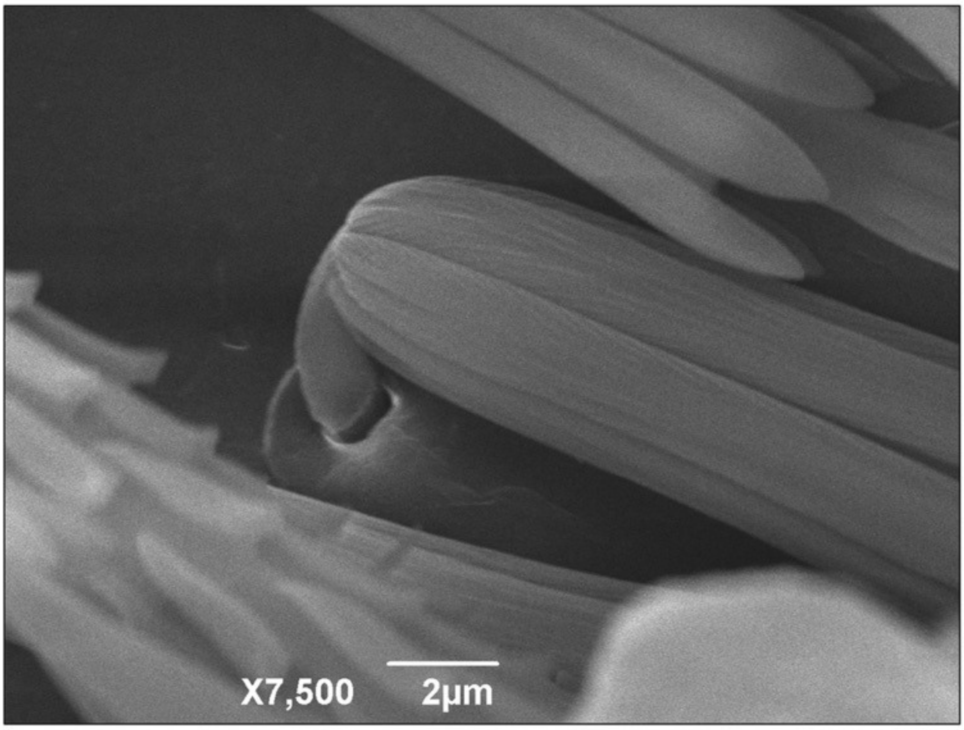

Fig. B. 130x. Detail of insertion of the hastiseta showing the curved pedicel.

Thaumaglossa rufocapillata Redtenbacher, 1867

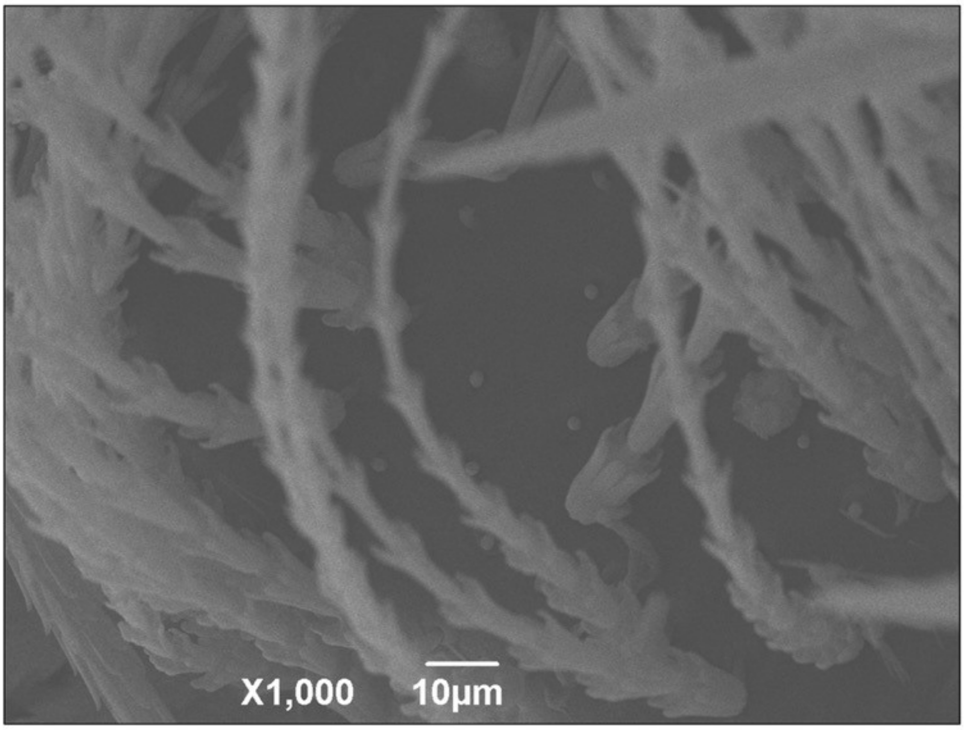

Fig. C. 3300x. Tuft showing detached and still attached hastisetae.

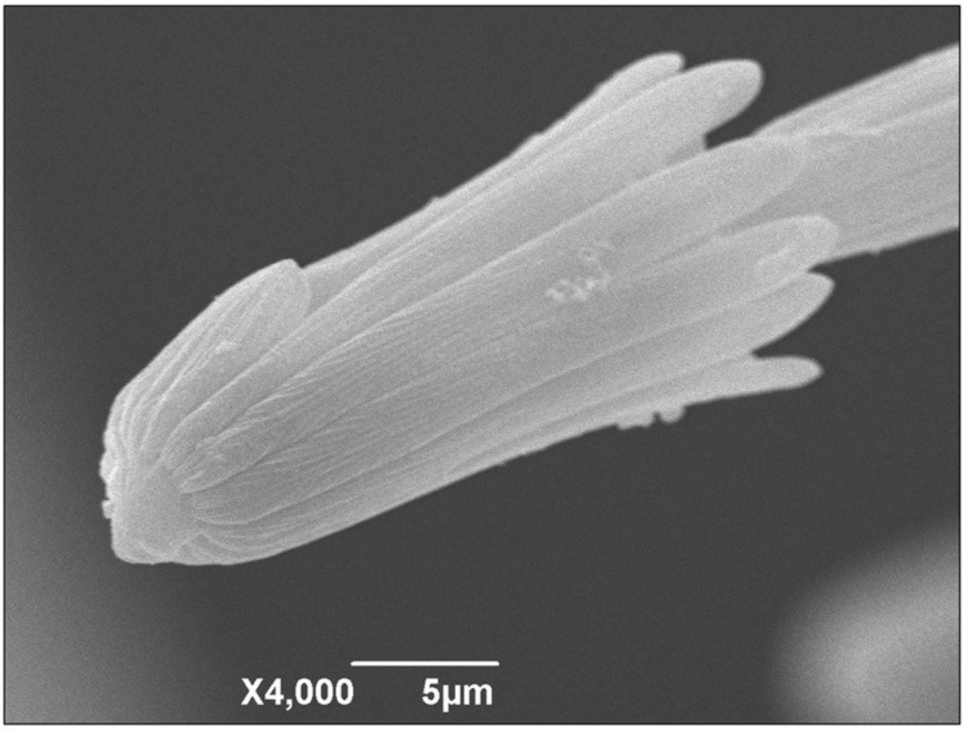

Fig. D. 130x. Detail of the first rosette and the remaining part of the broken pedicel.

Thaumaglossa rufocapillata Redtenbacher, 1867

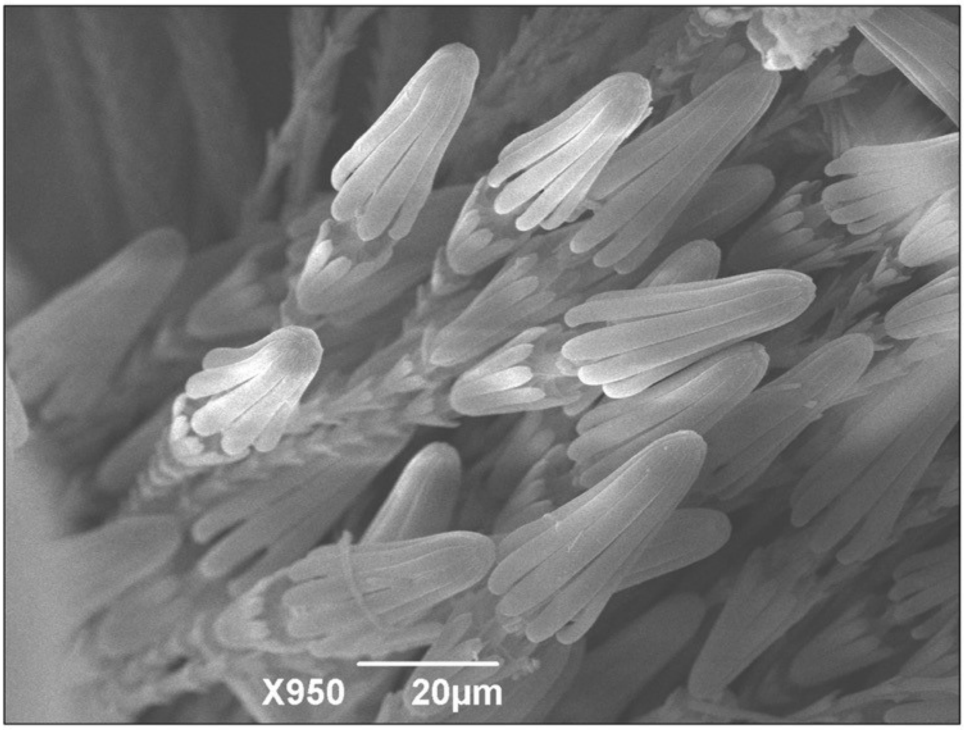

Fig. E. Apical view of one tuft of hastisetae.

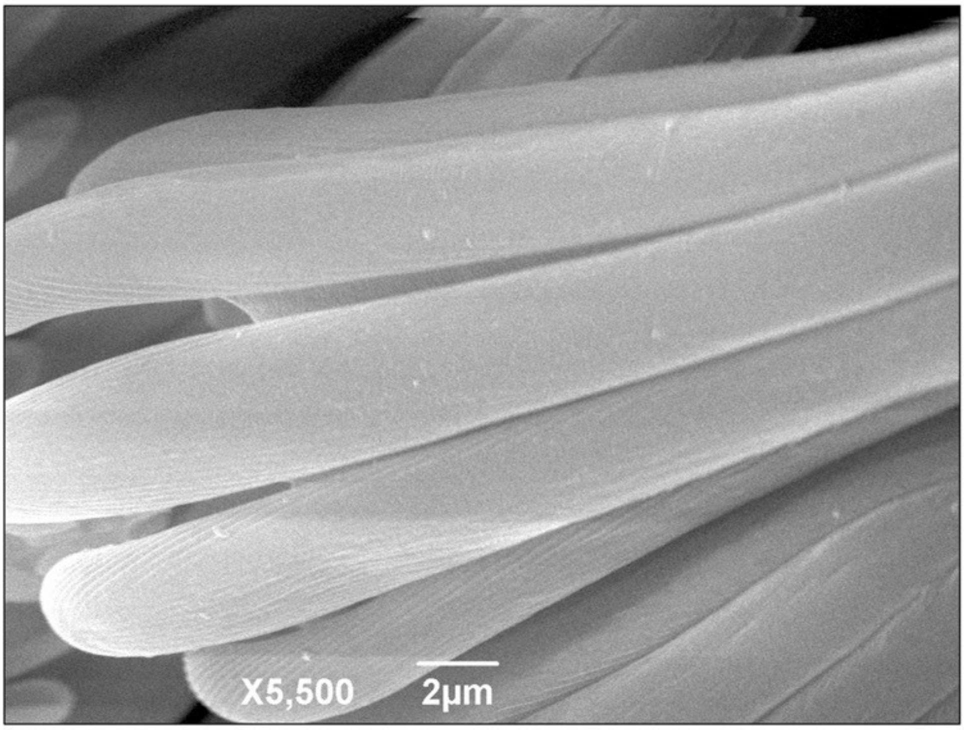

Fig. F. 130x. Detail of the knurls on the longitudinal processes of the head of the hastiseta.

Thaumaglossa rufocapillata Redtenbacher, 1867

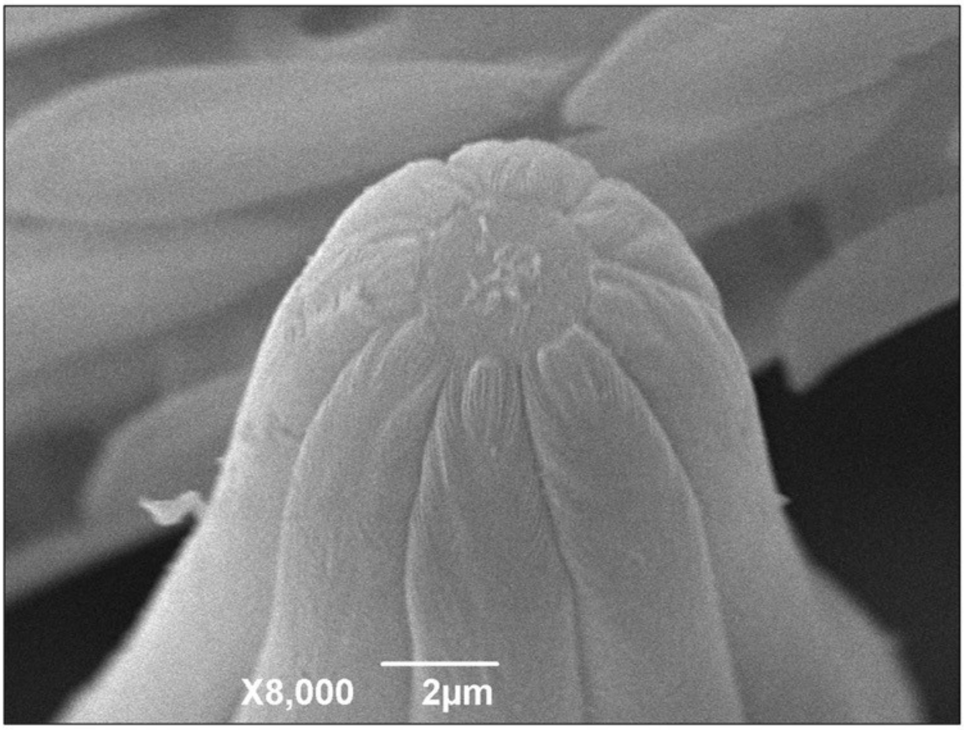

Fig. G. Apex of the head of the hastiseta presenting the circular depression.

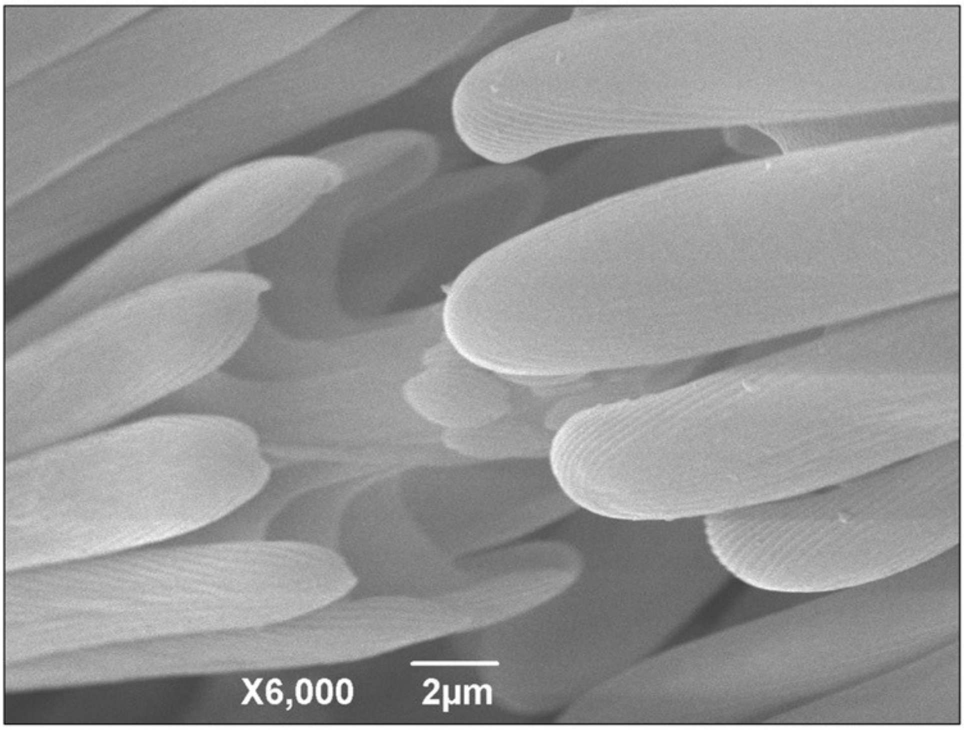

Fig. H. 130x. Antero-lateral view of the ultime rosette showing the cuticle bridges supporting the scales.

Supplementary material 13

Trogoderma granarium Everts, 1898

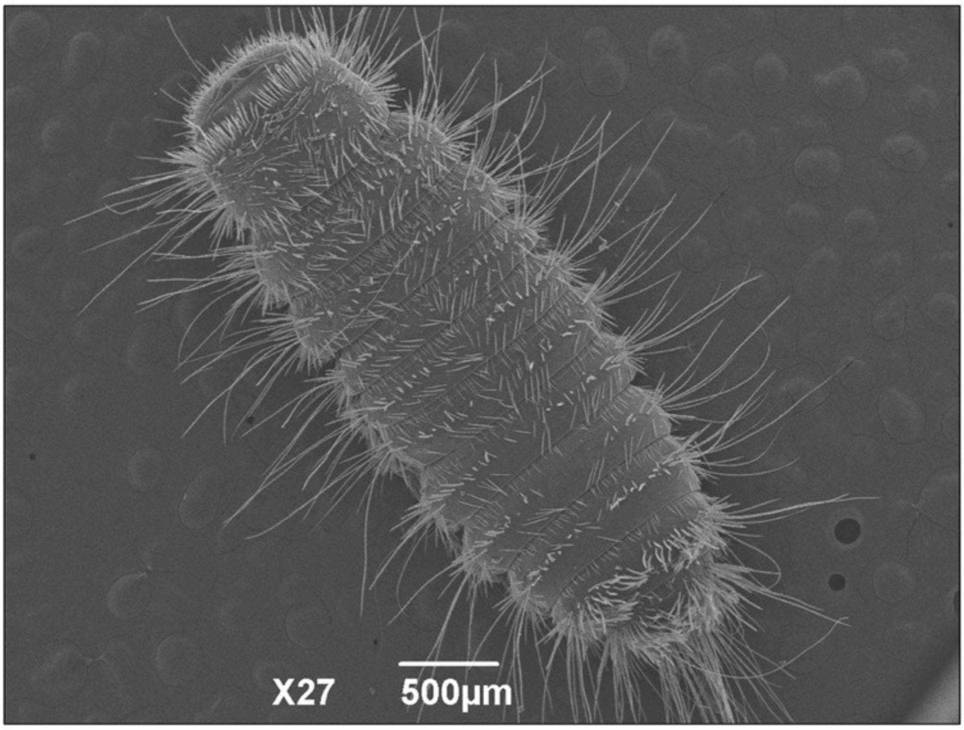

Fig. A. 27x. Mature larva, dorsal view.

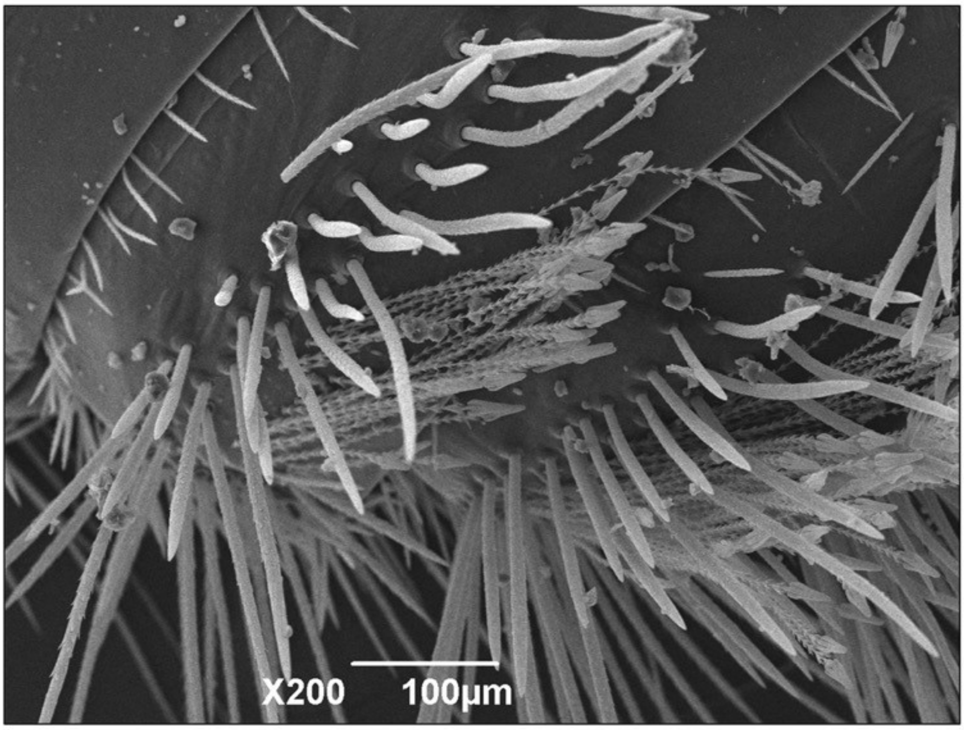

Fig. B. 200x. Detail of the tufts of hastisetae on the posterior corners of the 7th and 8th abdominal tergite.

Trogoderma granarium Everts, 1898

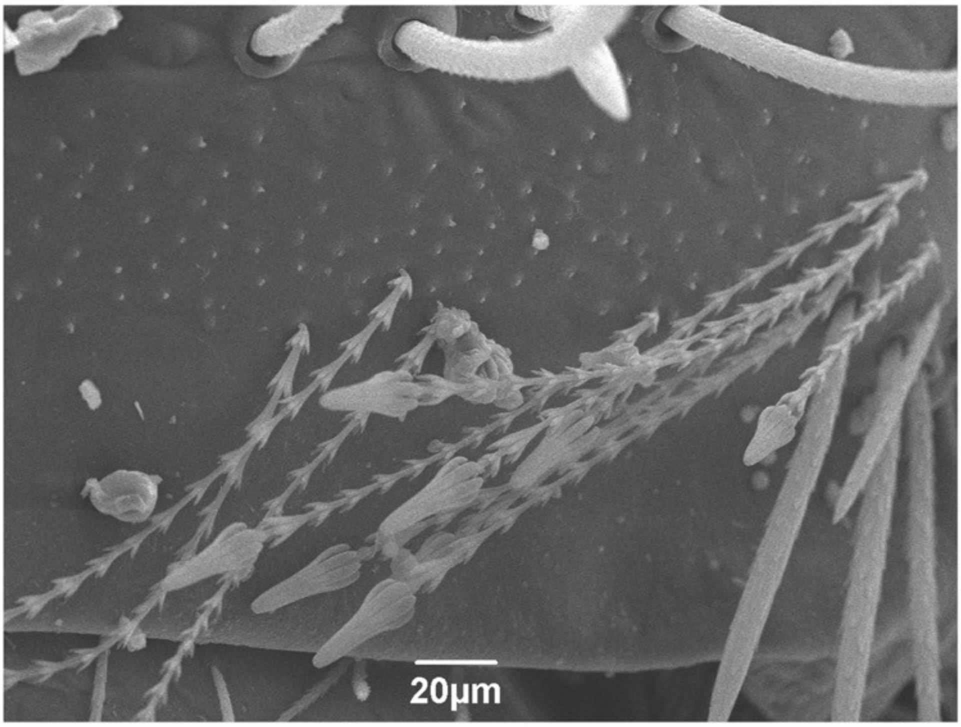

Fig. C. 550x. Detail the 6th abdominal tergite showing attached and the remaining pedicels of the detached hastisetae.

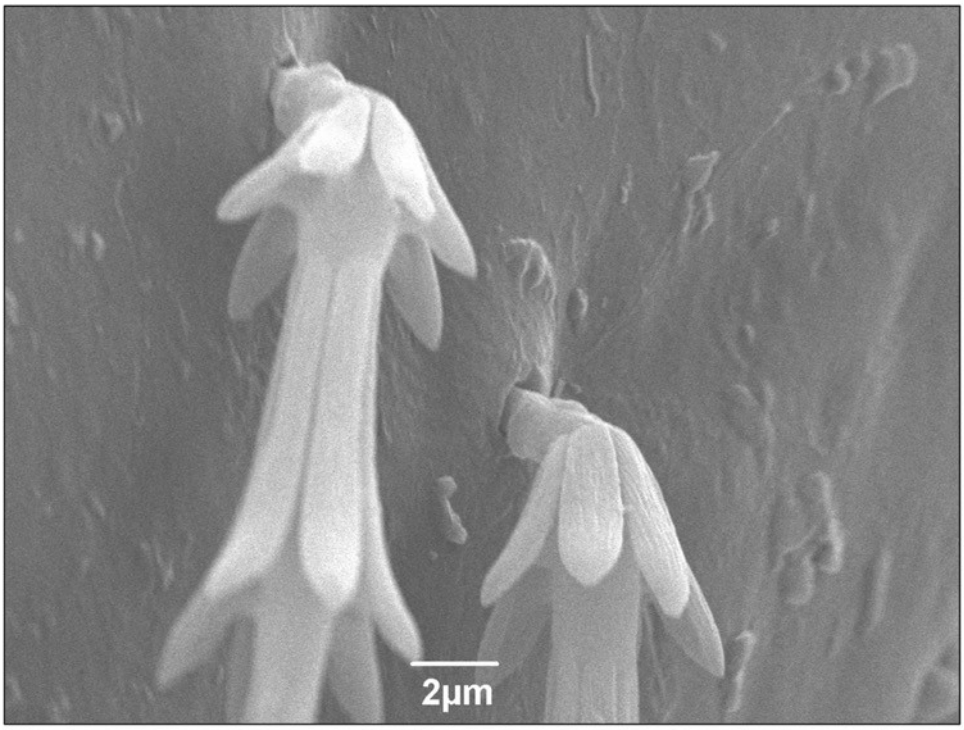

Fig. D. 6000x. Detail of the insertion of the hastisetae on the cuticle of the tergites.

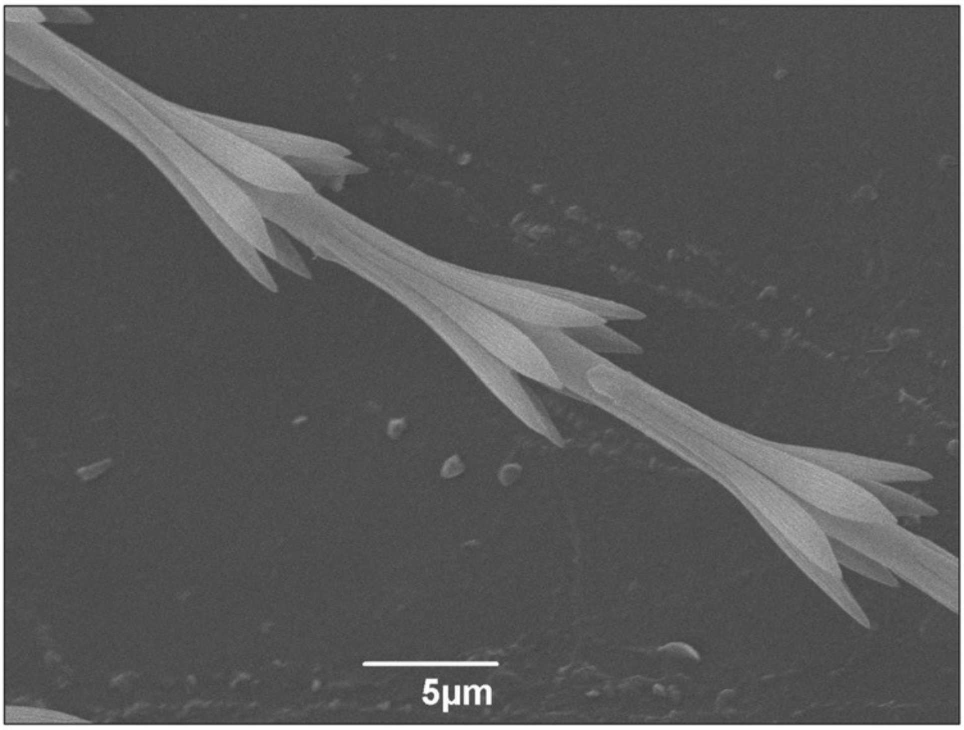

Fig. E. 550x. Detail of the rosette constituting the central part of the stalk.

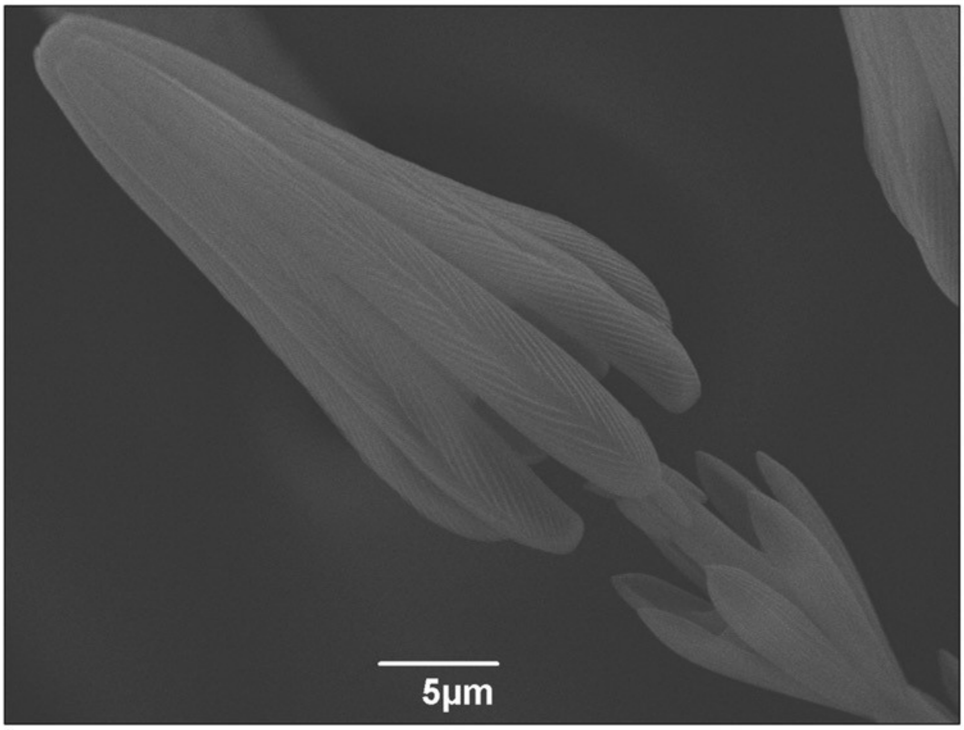

Fig. F. 3300x. Apical part of the hastiseta, highlighting the longitudinal furrows on the processes of the head of the hastiseta.

Trogoderma granarium Everts, 1898

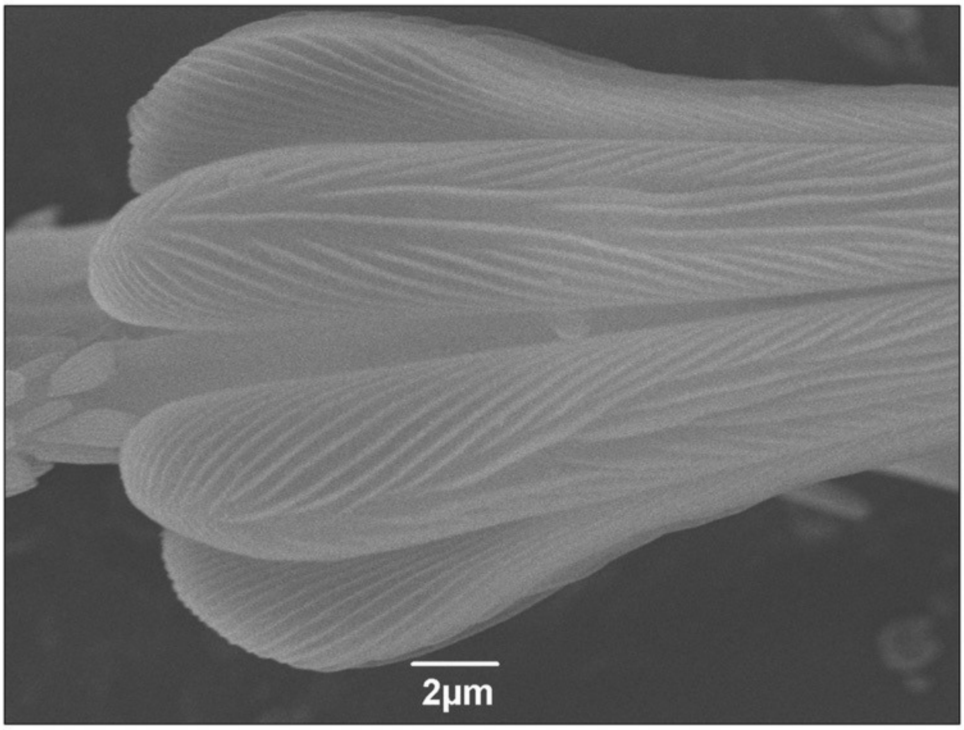

Fig. G. 6000x. Detail of the apical part of the longitudinal processes of the head of the hastiseta showing the arrangement of the knurls.

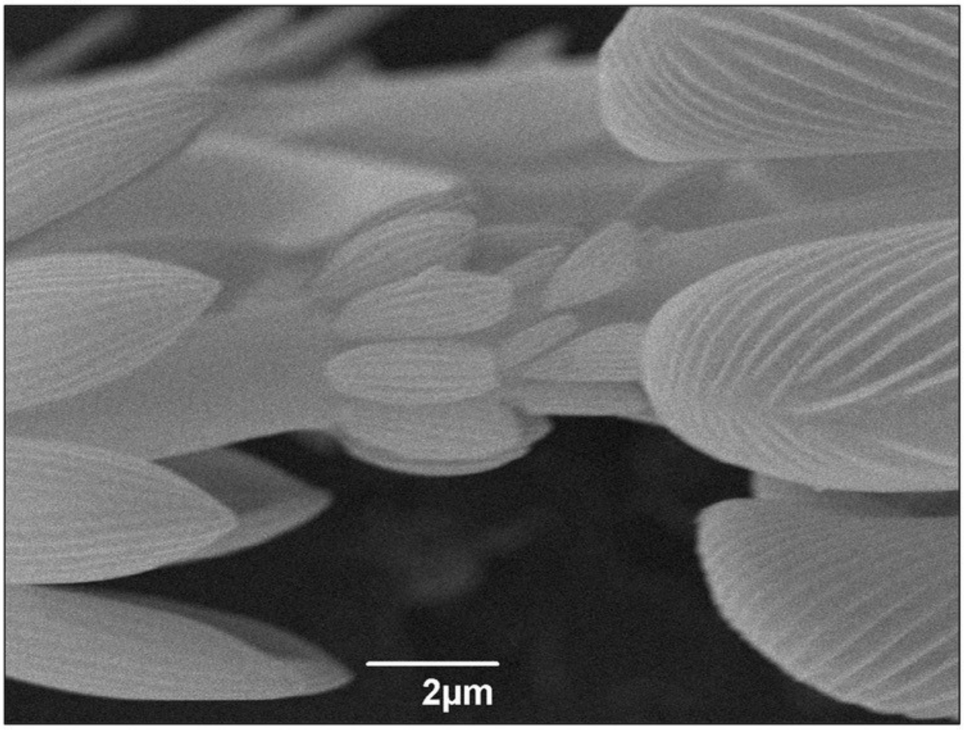

Fig. H. 9000x. Detail of the stalk between the ultimate rosette and the head highlighting the irregular set of micro scales.

Supplementary material 14

Trogoderma simplex Jayne, 1882

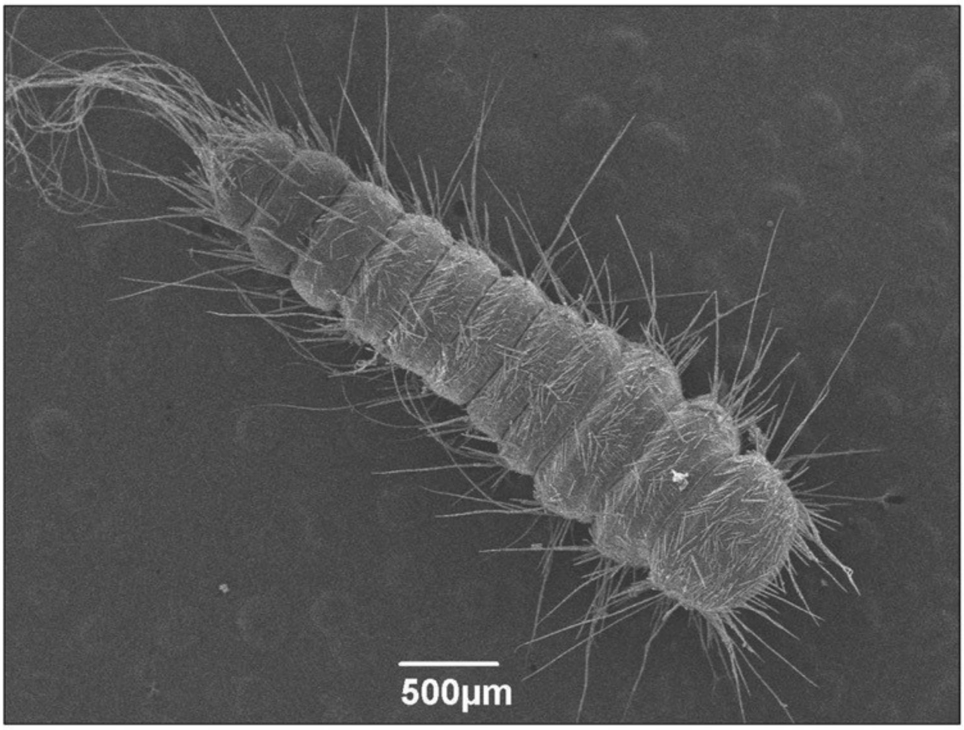

Fig. A. 27x. Mature larva, dorsal view.

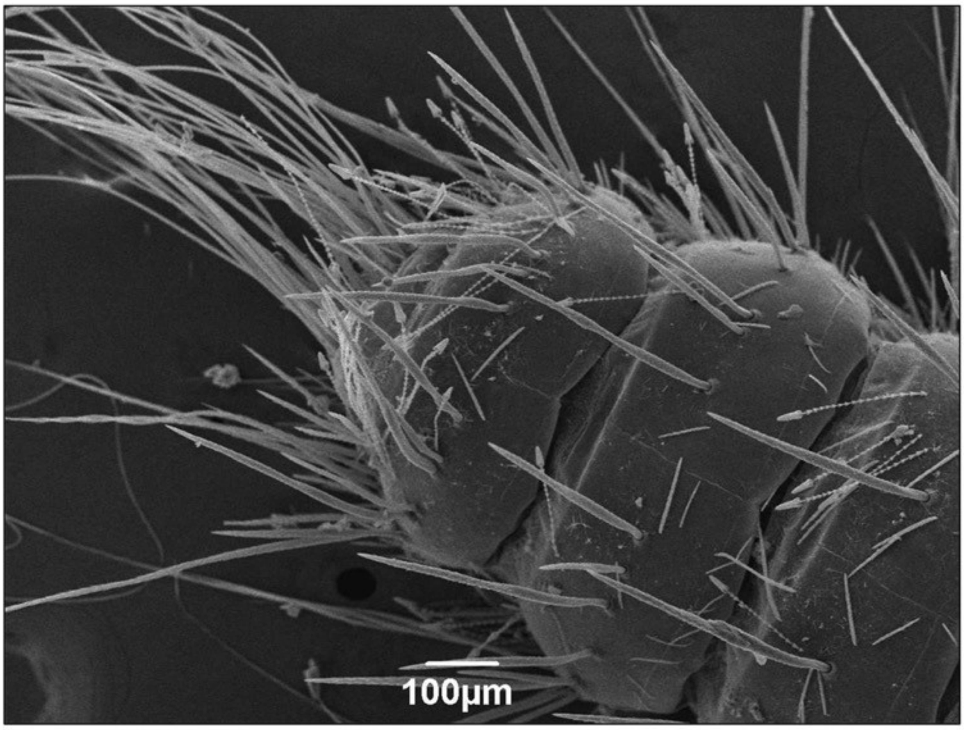

Fig. B. 100x. Detail of the last four abdominal segments, dorsal view.

Trogoderma simplex Jayne, 1882

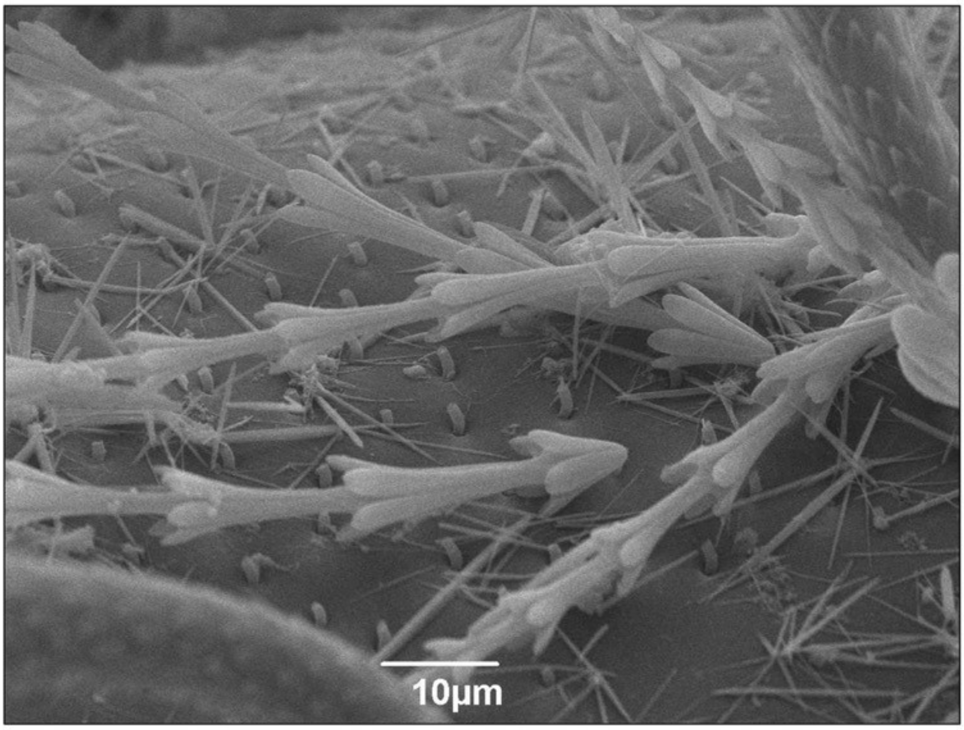

Fig. C. 1600x. Detail of the 4th abdominal tergite illustrating a series of broken pedicels, result of hastisetae detachment.

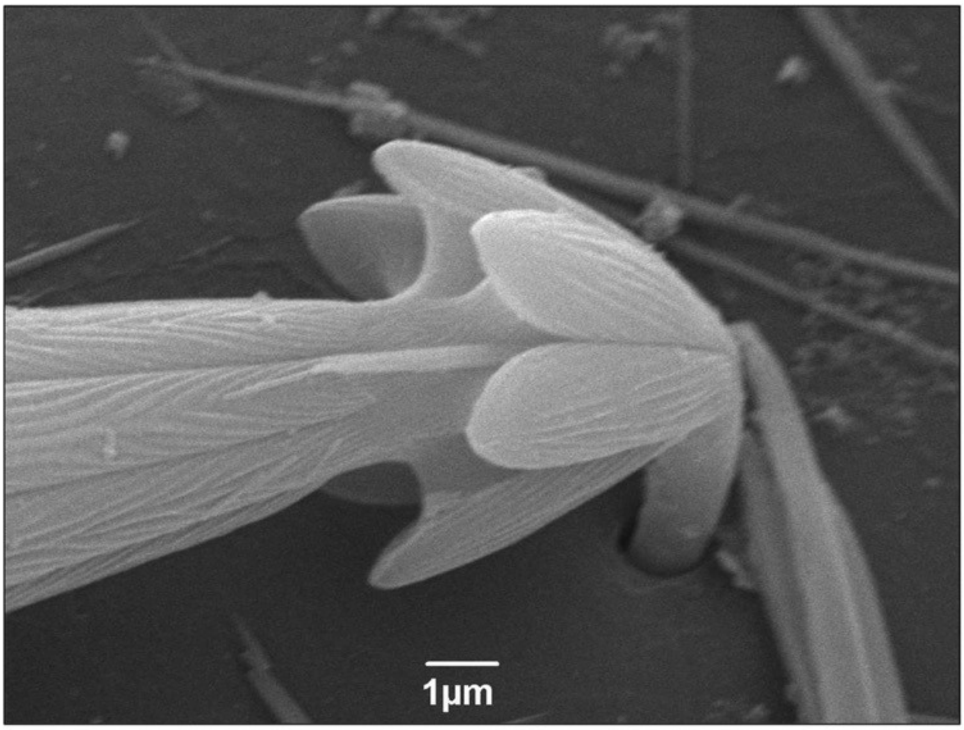

Fig. D. 10000x. Detail of the first rosette illustrating the angle between the pedicel and the stalk main axe.

Trogoderma simplex Jayne, 1882

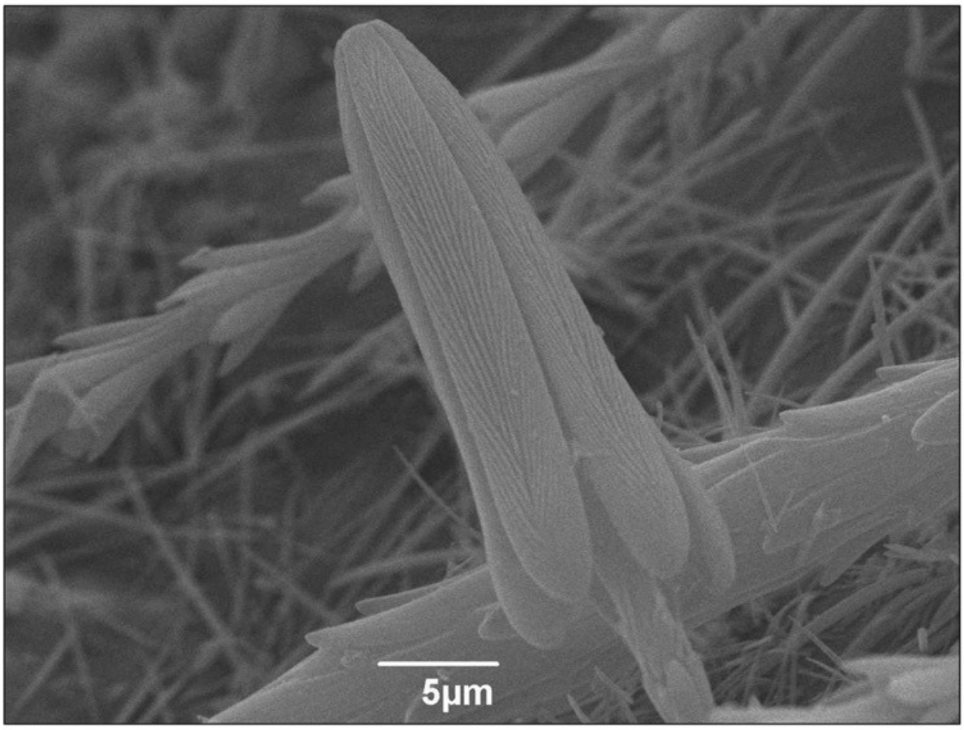

Fig. E. 3300x. Head of the hastiseta on the abdominal segments.

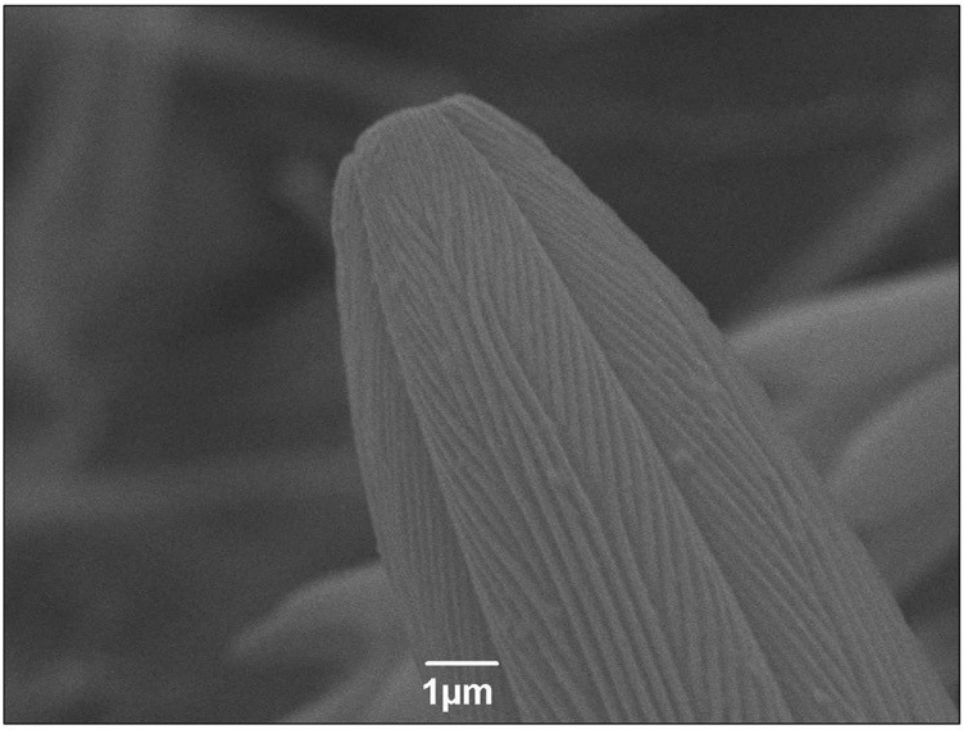

Fig. F. 10000x. Apex of the head of the hastiseta, lateral view.

Trogoderma simplex Jayne, 1882

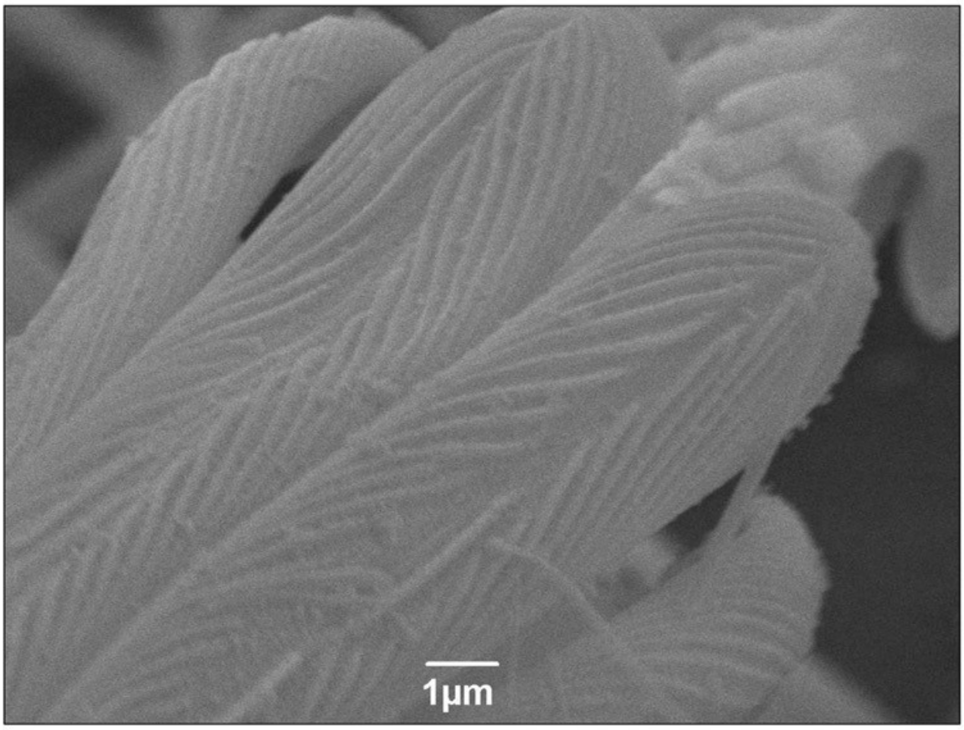

Fig. G. 10000x. Detail of the longitudinal processes of the head of the hastiseta showing the knurls and the longitudinal furrow.

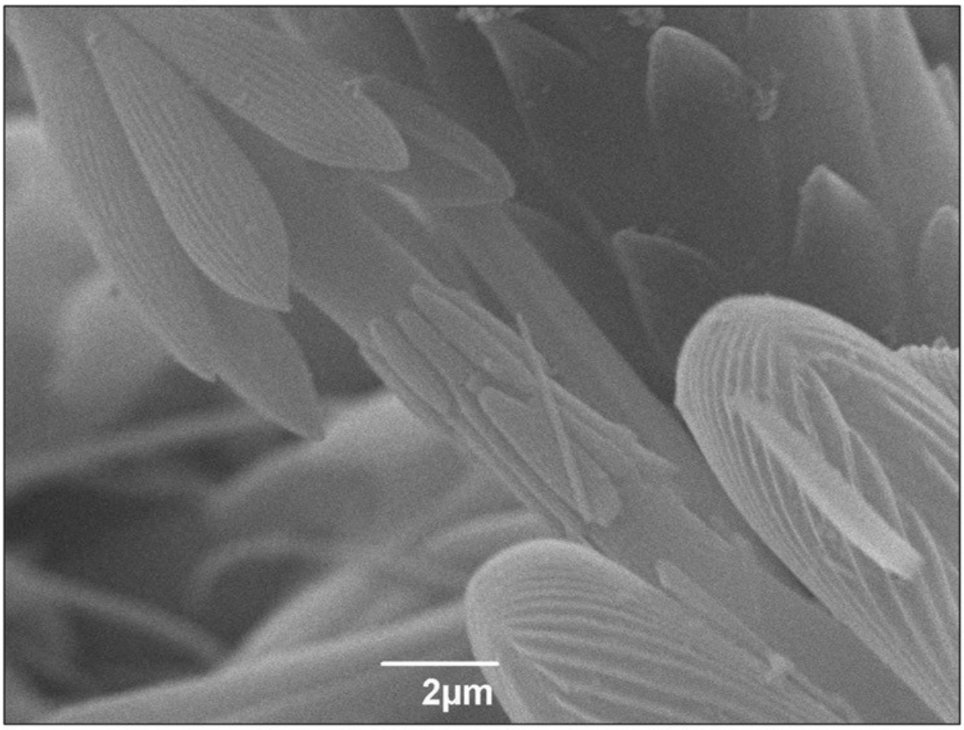

Fig. H. 8000x. Detail of the set of irregular scales on the stalk situate just before the head of the hastiseta.

Supplementary material 15

Trogoderma variabile Ballion, 1878

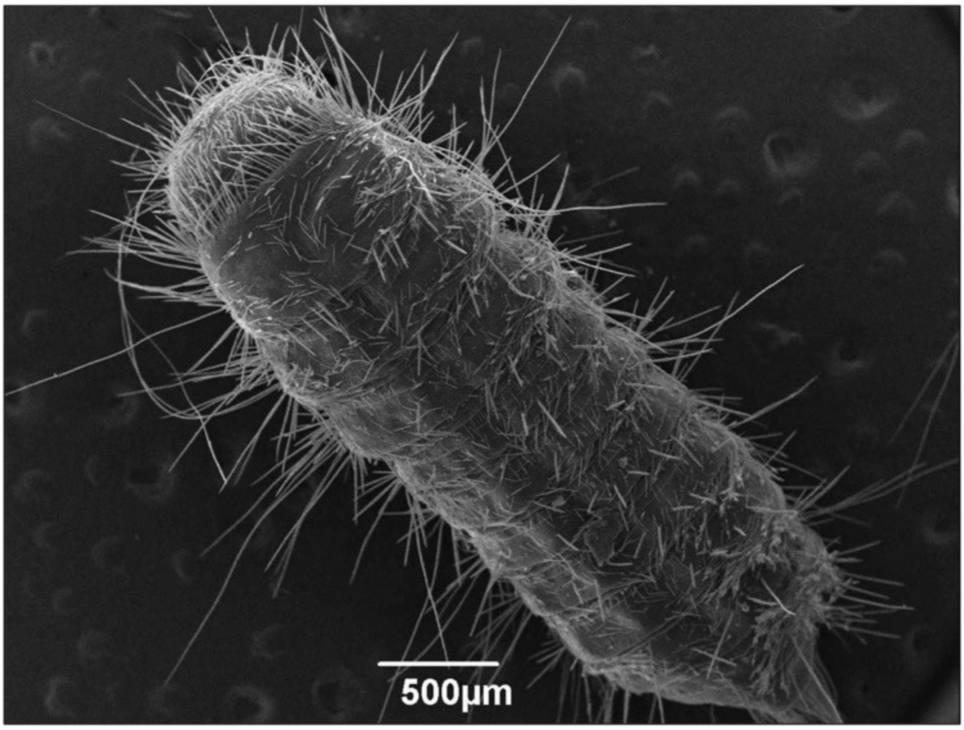

Fig. A. 33x. Mature larva, dorsal view.

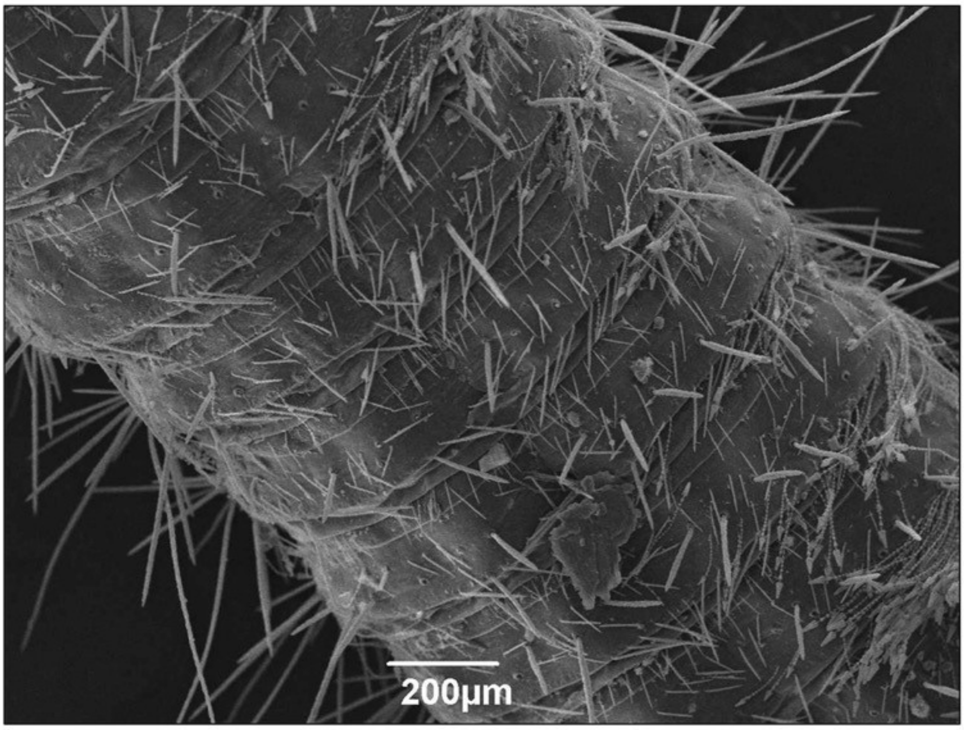

Fig. B. 75x. Dorsal view of the first abdominal tergites.

Trogoderma variabile Ballion, 1878

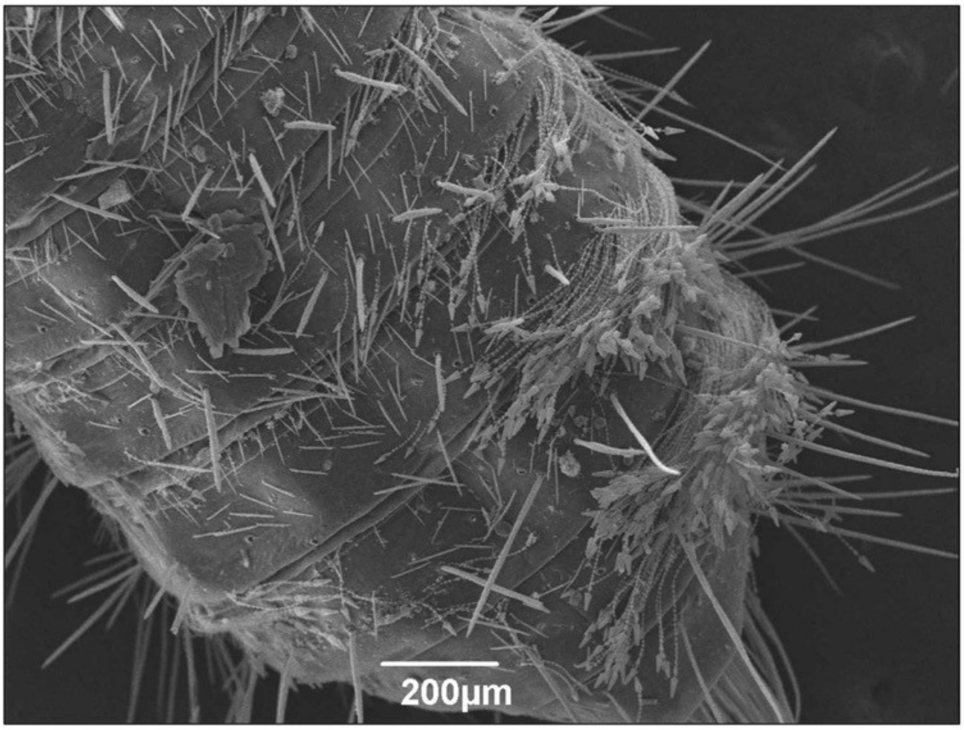

Fig. C. 80 x. Dorsal view of the last four abdominal tergites.

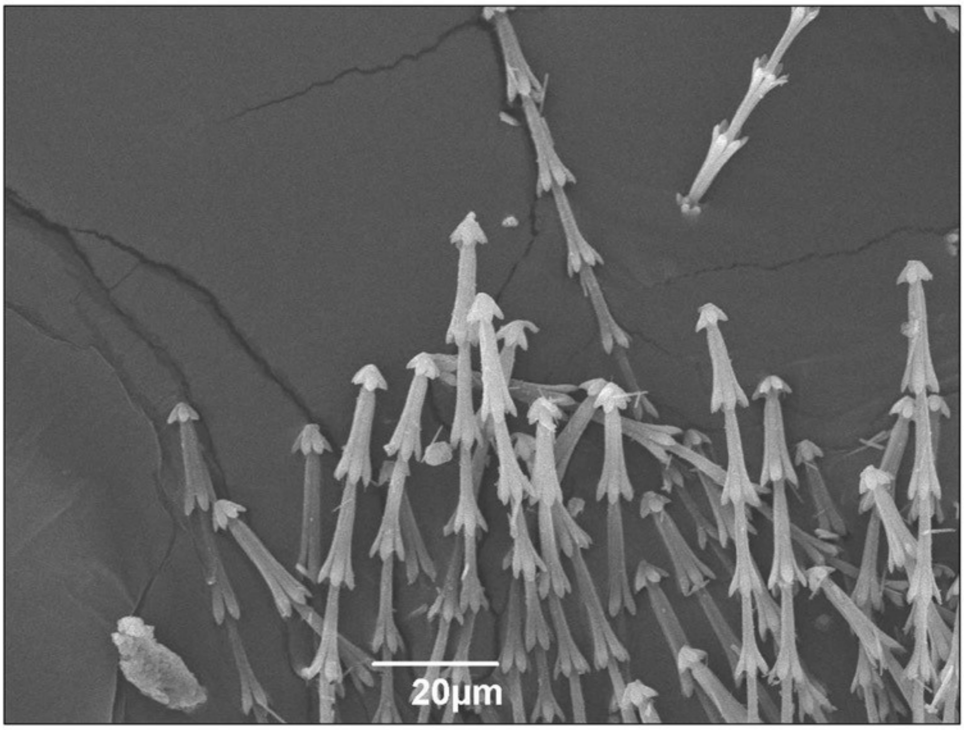

Fig. D. 850x. Detached hastisetae all broken at the level of the pedicel.

Trogoderma variabile Ballion, 1878

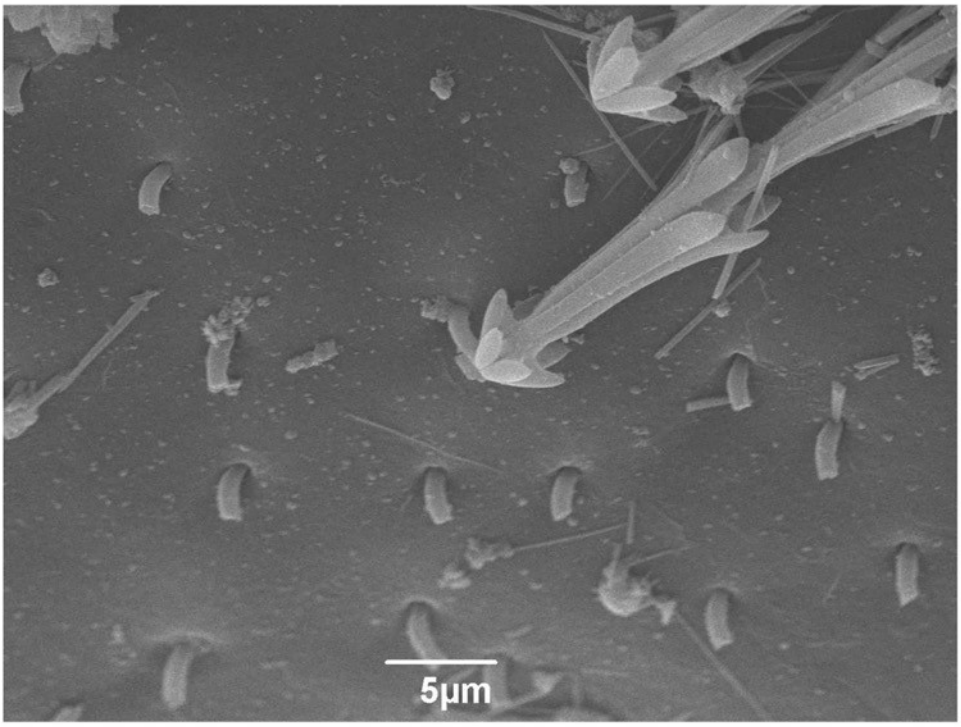

Fig. E. 3000x. Series of broken pedicels and one hastiseta still in place.

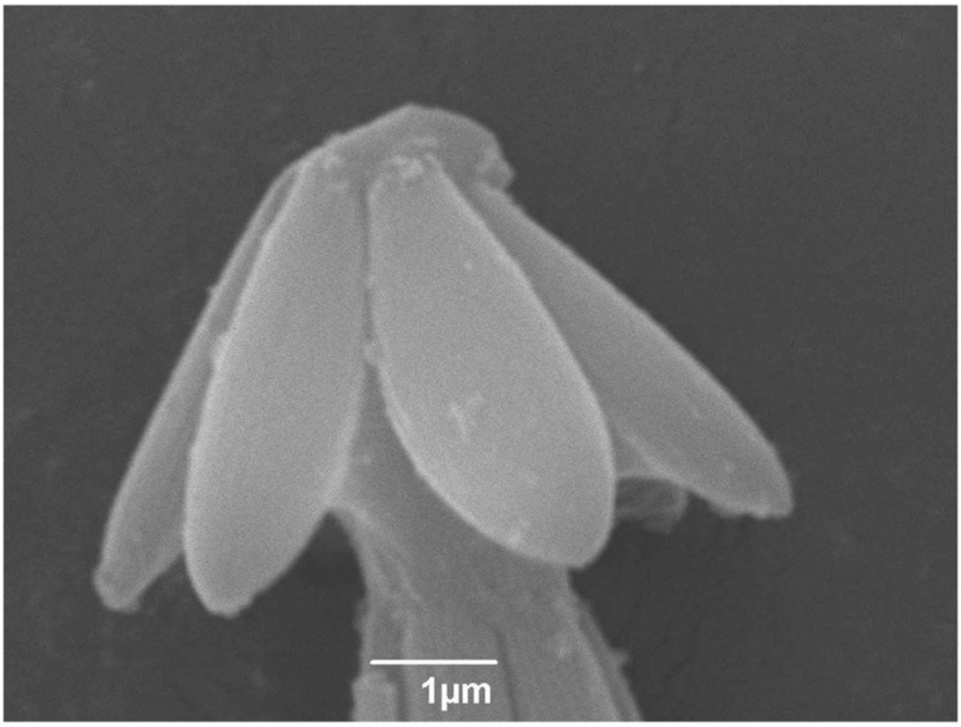

Fig. F. 17000x. Detail of the first rosette showing the breaking point of the pedicel, lateral view.

Trogoderma variabile Ballion, 1878

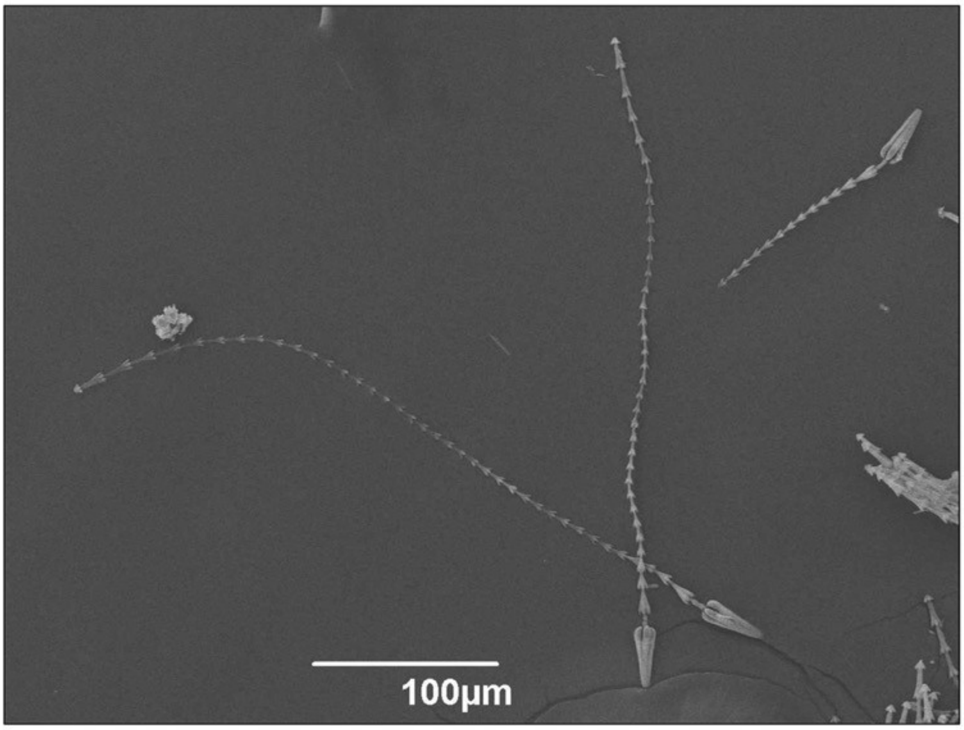

Fig. G. 250x. Hastisetae belonging to the tufts on the last abdominal tergites.

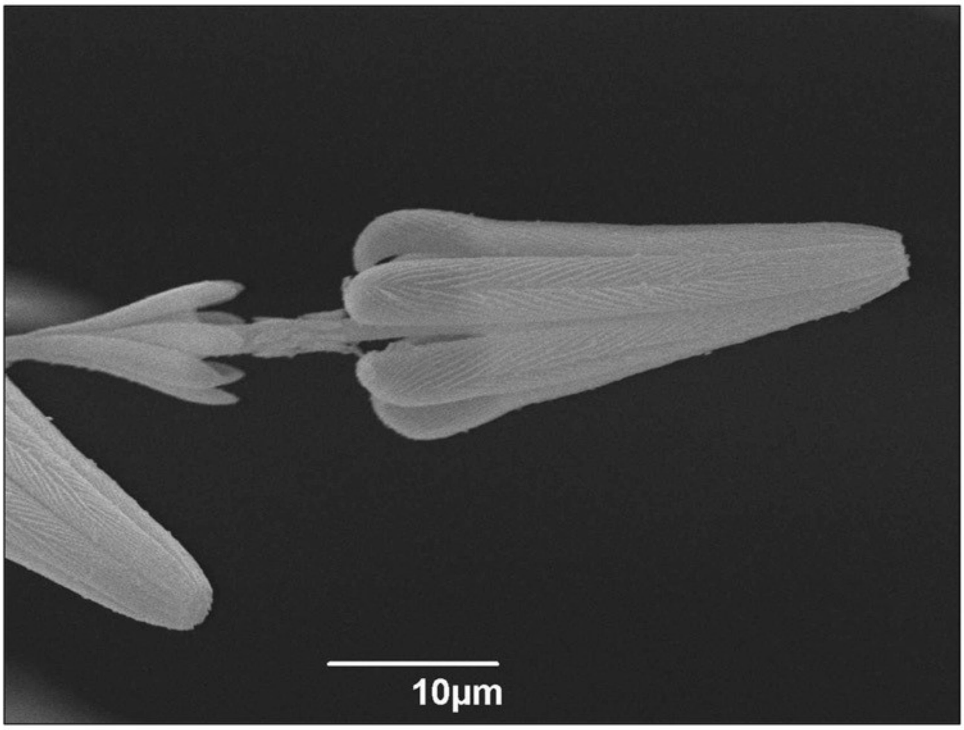

Fig. H. 2300x. Detail of the apical part of the hastiseta, lateral view.

Trogoderma variabile Ballion, 1878

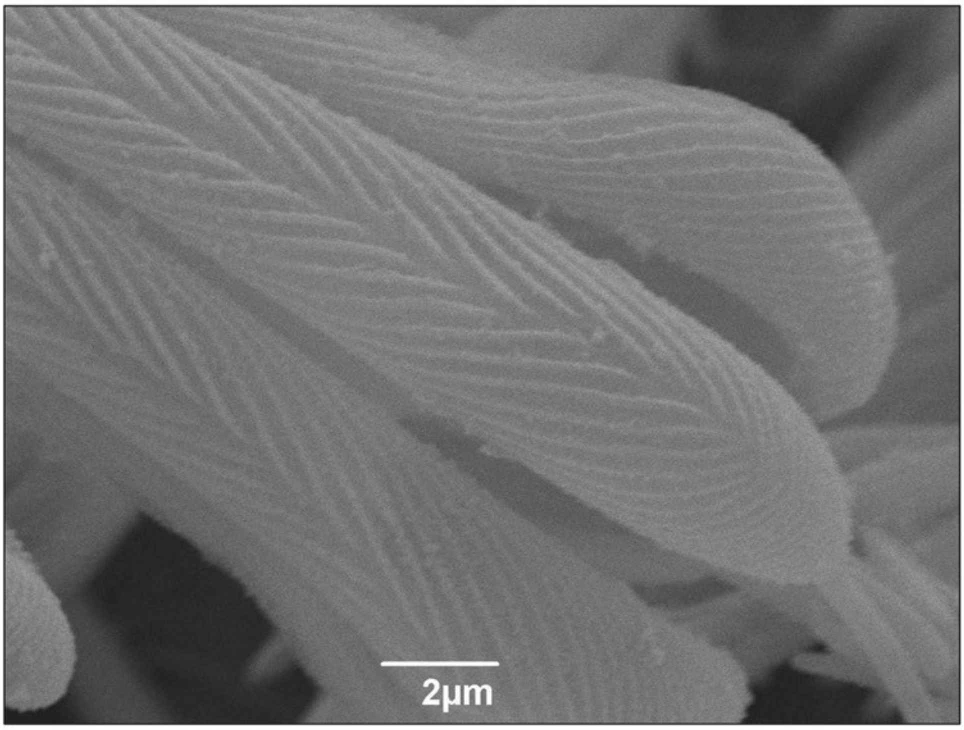

Fig. I. 8000x. detail of the longitudinal processed of the head of the hastiseta illustrating the knurls and the longitudinal furrow.

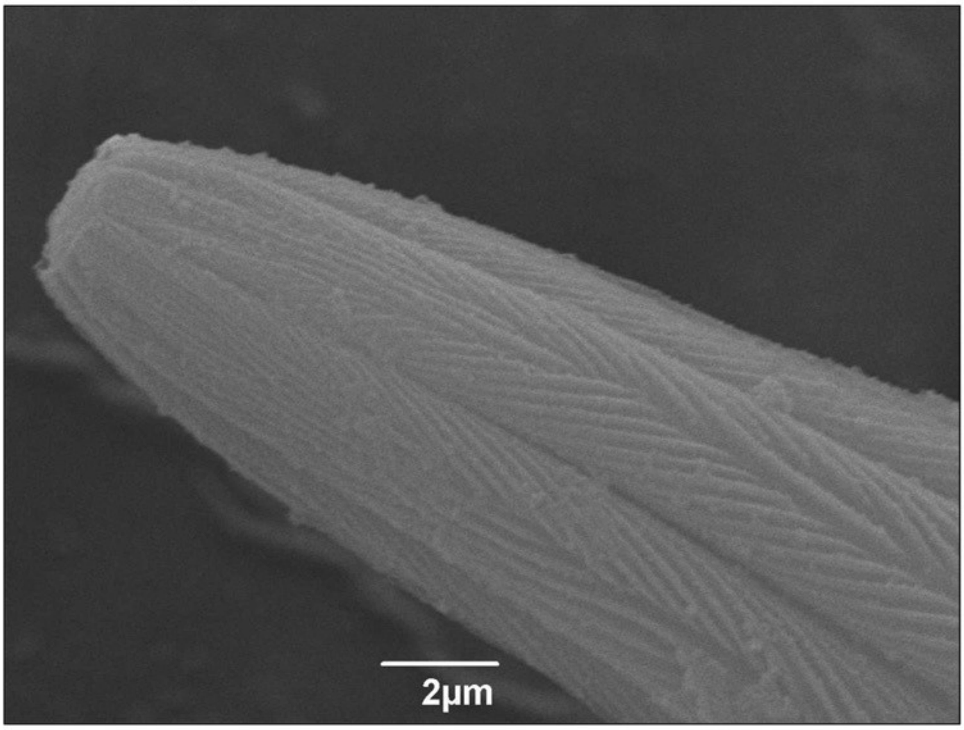

Fig. J. 8000x. Apex of the head of the hastiseta, lateral view.

Trogoderma variabile Ballion, 1878

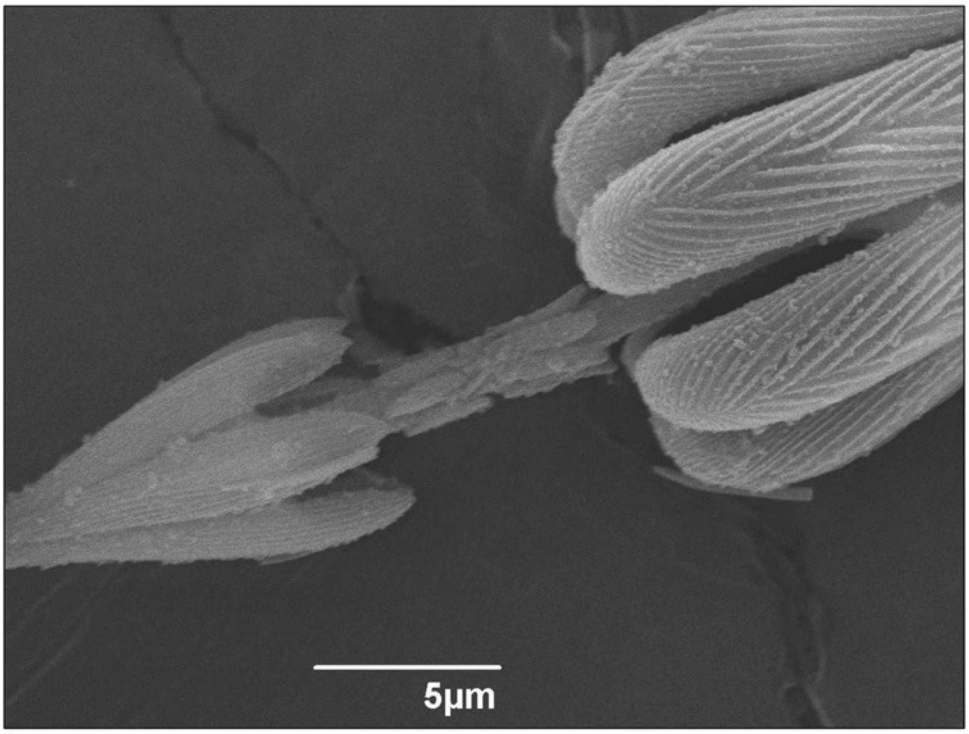

Fig. K. 5000x. Lateral view of the last rosette, the irregular scales on the shaft and the base of the head of the hastiseta.

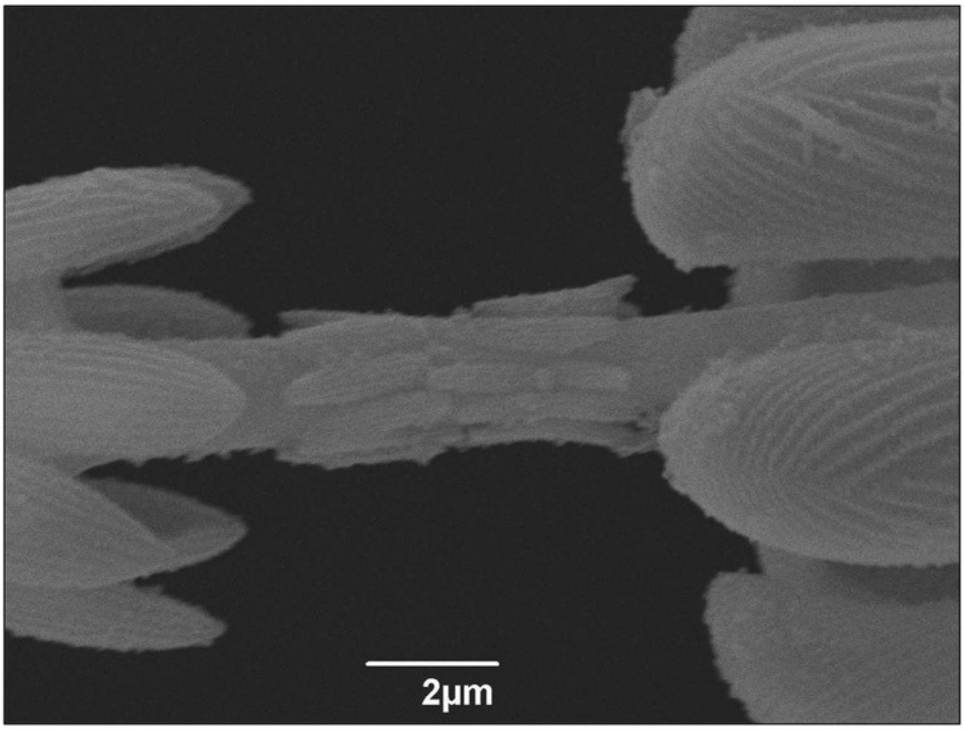

Fig. L. 9000x. Detail of the irregular scales on the shaft.

Supplementary material 16

Trogoderma versicolor (Creutzer, 1799)

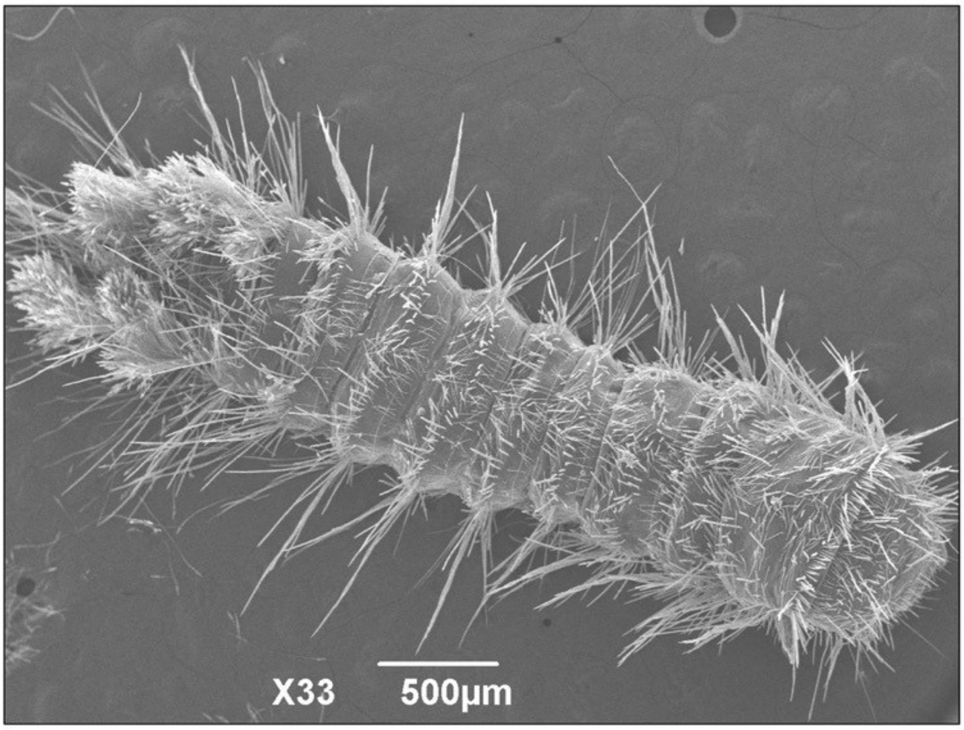

Fig. A. 33x. Mature larva, dorsal view.

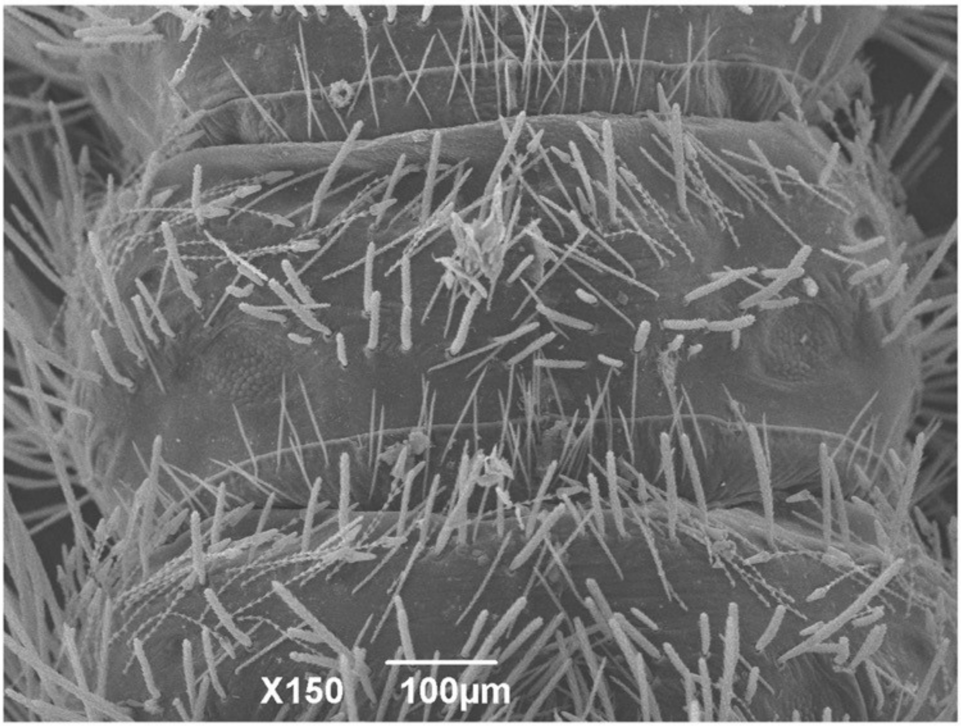

Fig. B. 150x. Detail of the thirs thoracic tergite, dorsal view.

Trogoderma versicolor (Creutzer, 1799)

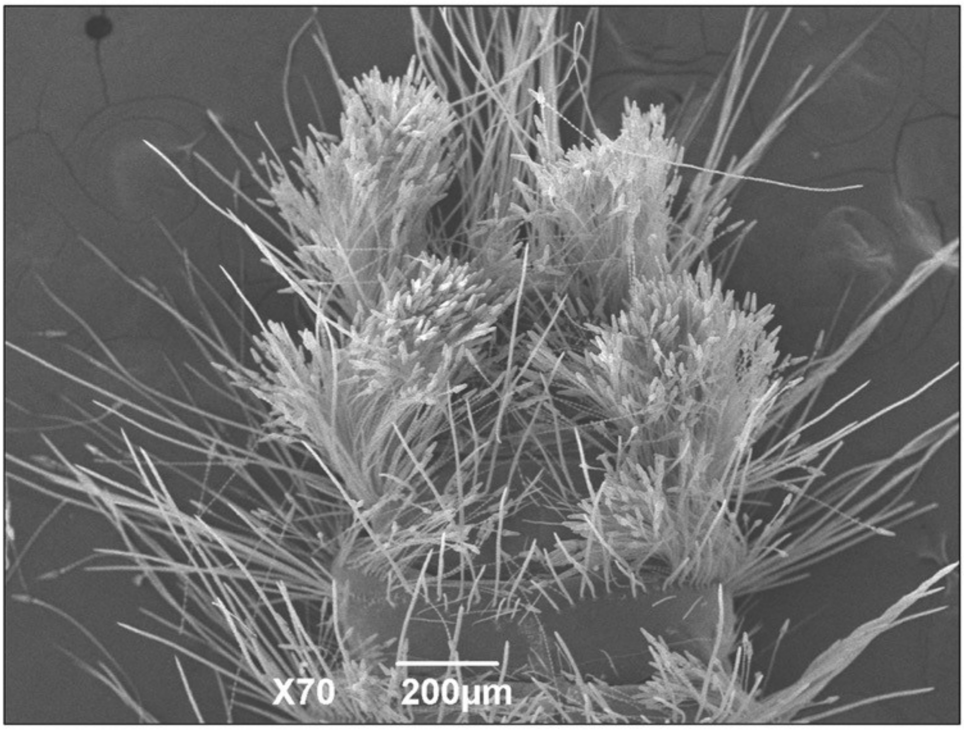

Fig. C. 70x. Tufts of hastisetae on the last abdominal tergites, dorsal view.

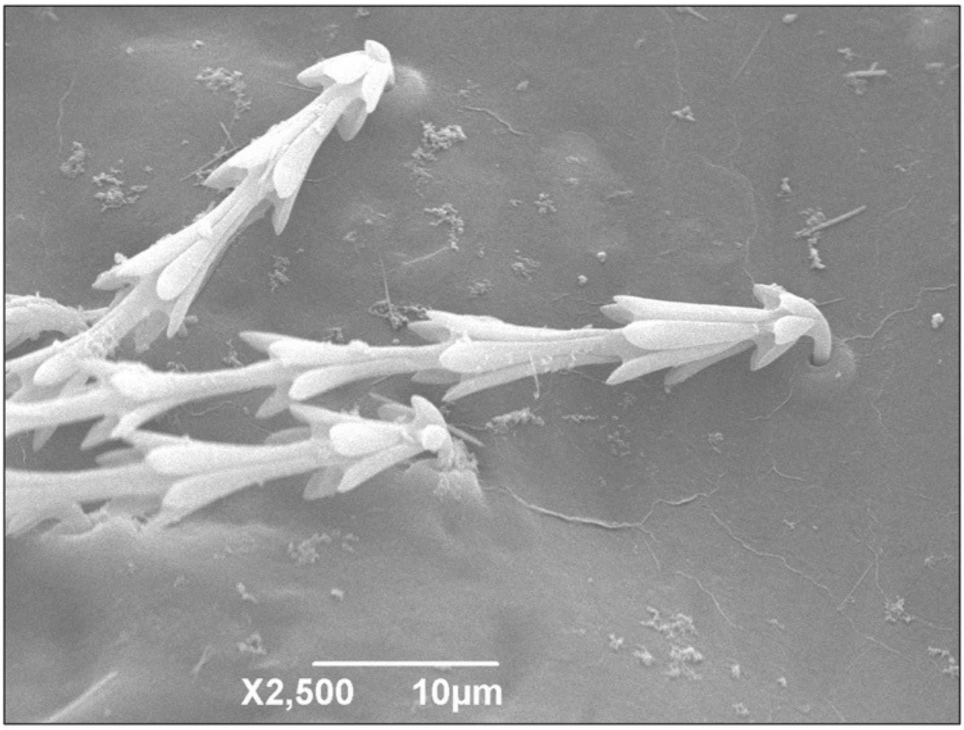

Fig. D. 2500x. Detail of the insertion of the hastisetae on the integument of the tergite.

Trogoderma versicolor (Creutzer, 1799)

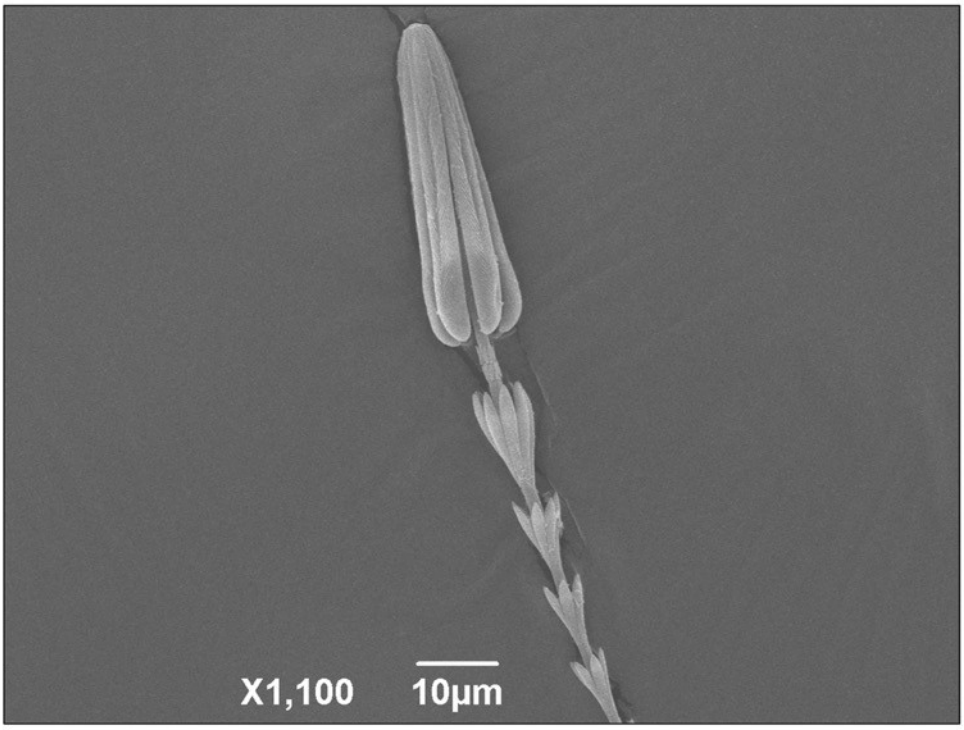

Fig. E. 1100x. Apical part of the hastiseta, lateral view.

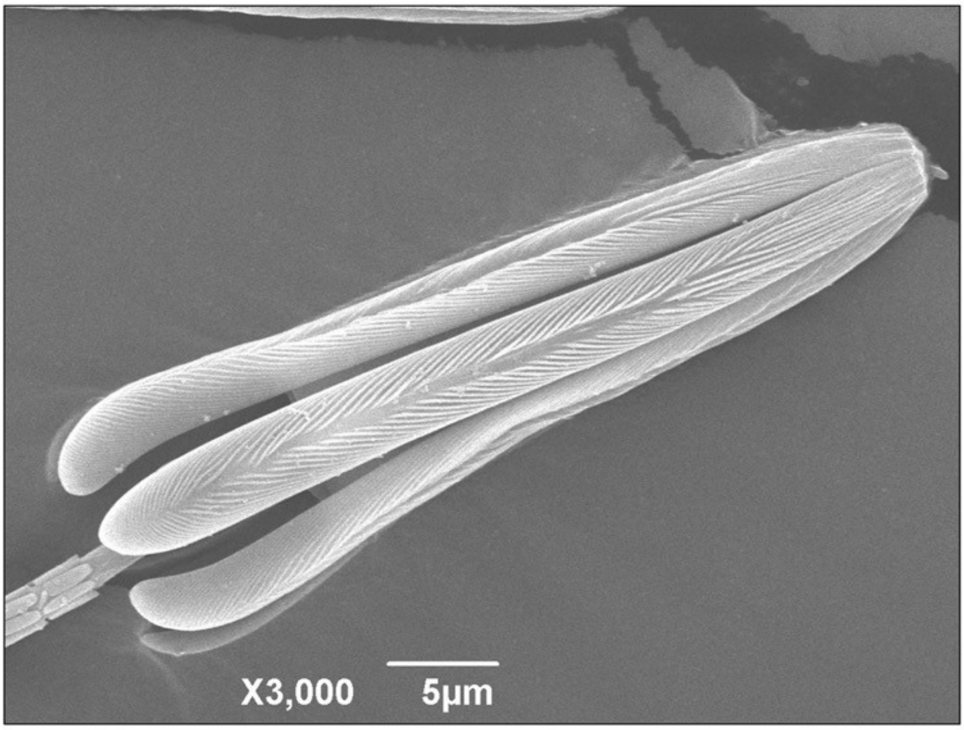

Fig. F. 130x. Detail of the head of the hastiseta, lateral view.

Trogoderma versicolor (Creutzer, 1799)

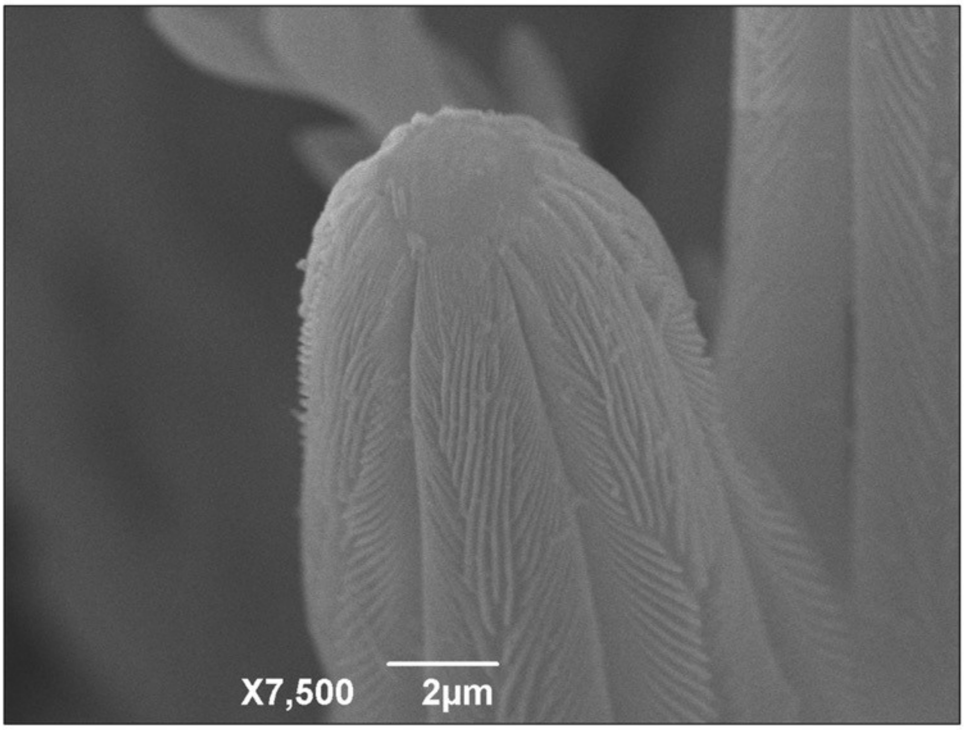

Fig. G. 7500x. Head of the hastiseta presenting the apical circular depression.

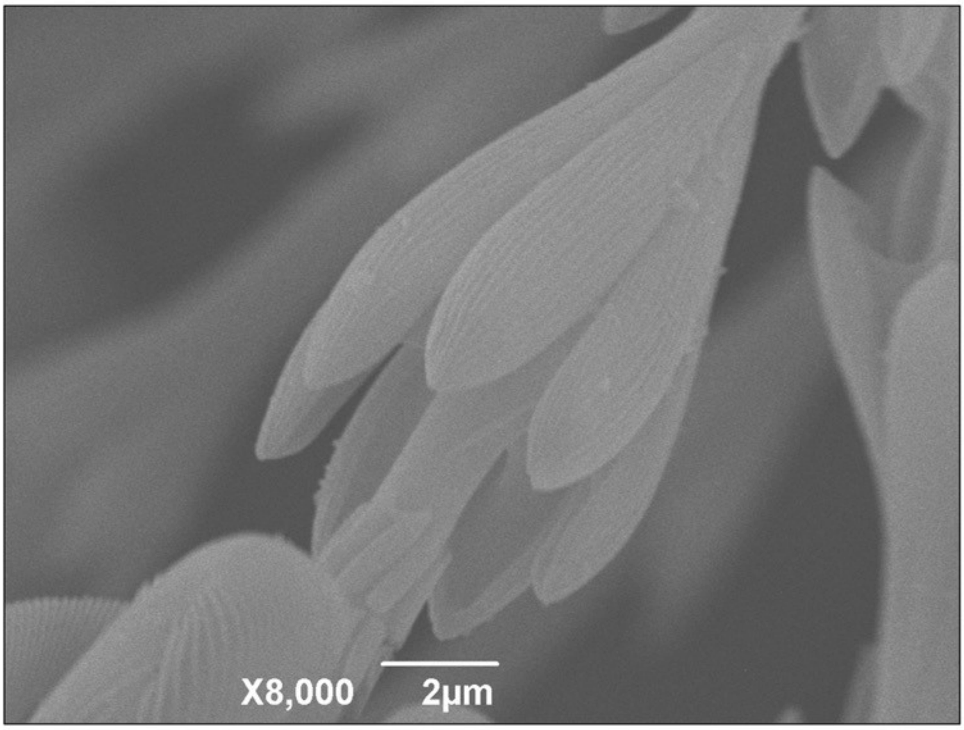

Fig. H. 8000x. Detail of the ultimate rosette, antero-lateral view.

Trogoderma versicolor (Creutzer, 1799)

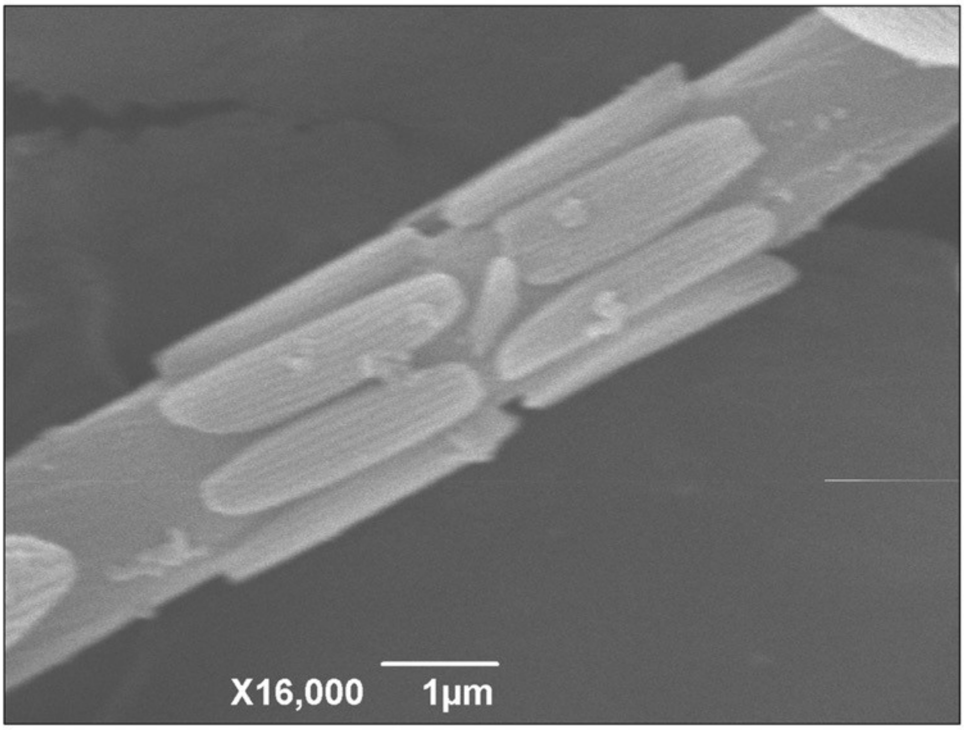

Fig. I. 16000x. Detail of the irregular scales on the shaft between the last rosette and the head of the hastiseta.

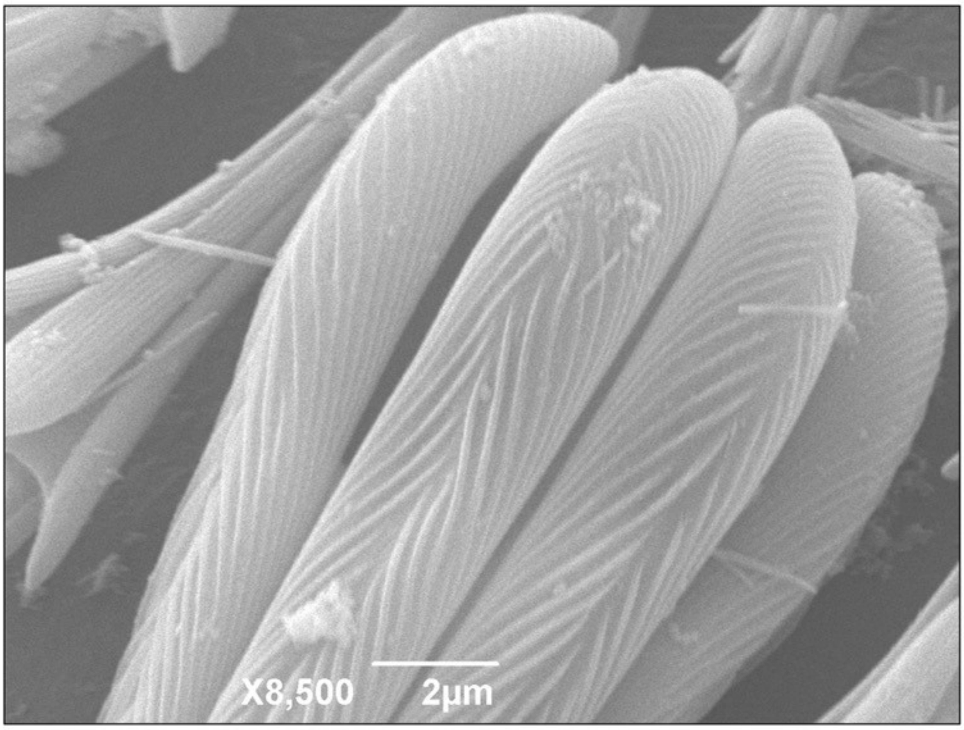

Fig. J. 8500x. Detail of the longitudinal processes of the head of the hastiseta illustrating the knurls and the longitudinal furrow.

Supplementary material 17

Trogoderma spp. 1 (Italy)

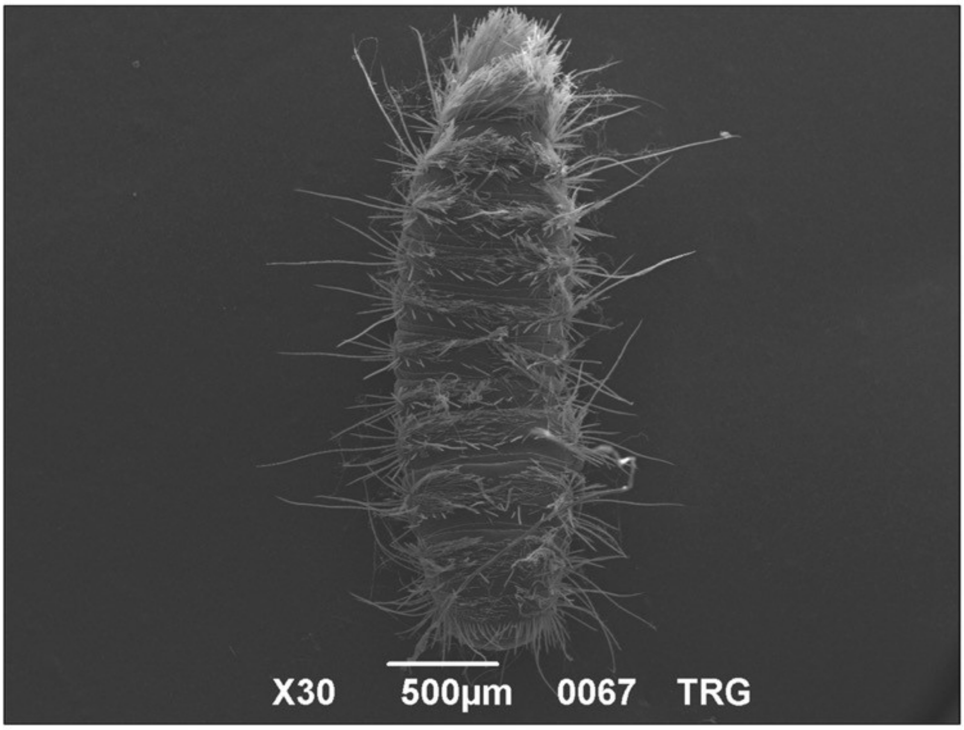

Fig. A. 30x. Mature larva, dorsal view.

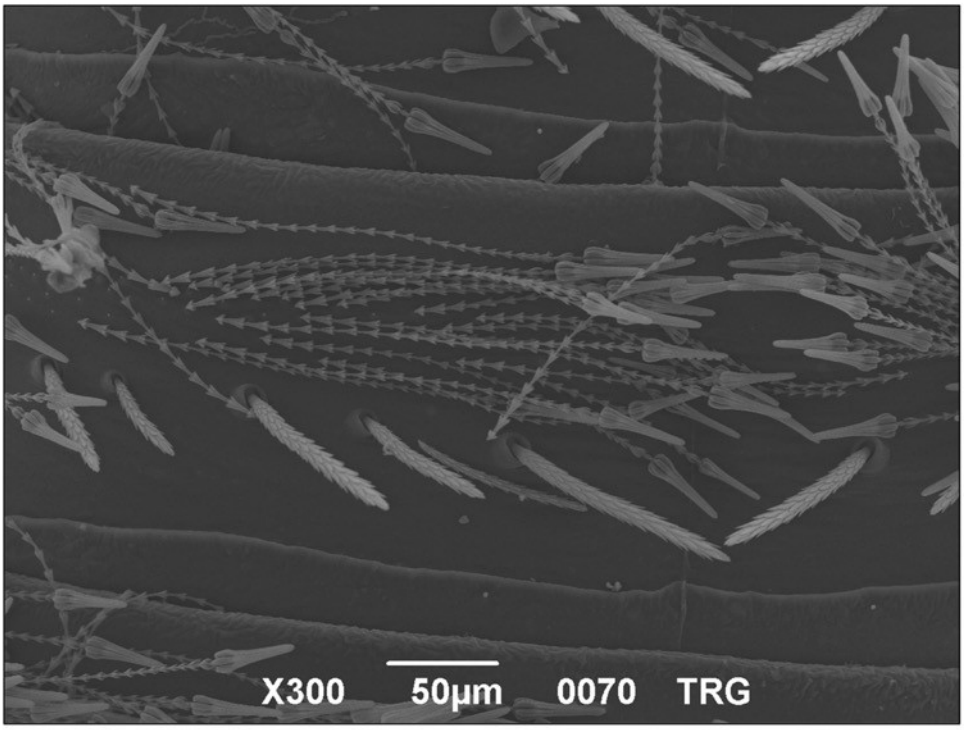

Fig. B. 300x. Recumbent hastisetae on the 3rd abdominal tergite.

Trogoderma spp. 1 (Italy)

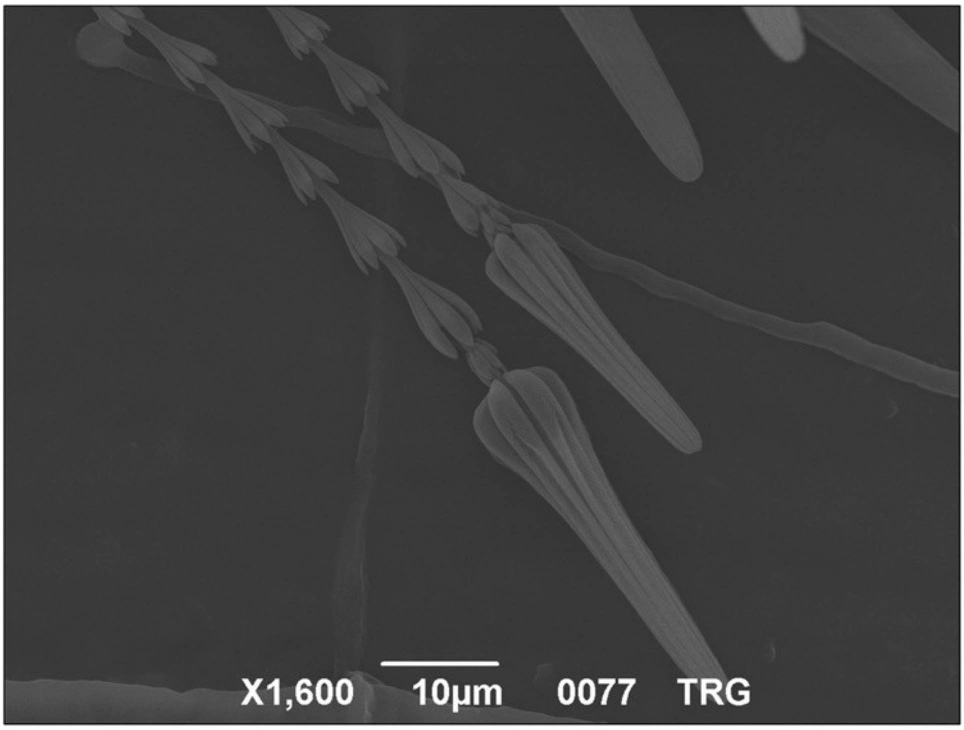

Fig. C. 1600x. Hastisetae on thoracic and first abdominal tergites, detail of the apical part.

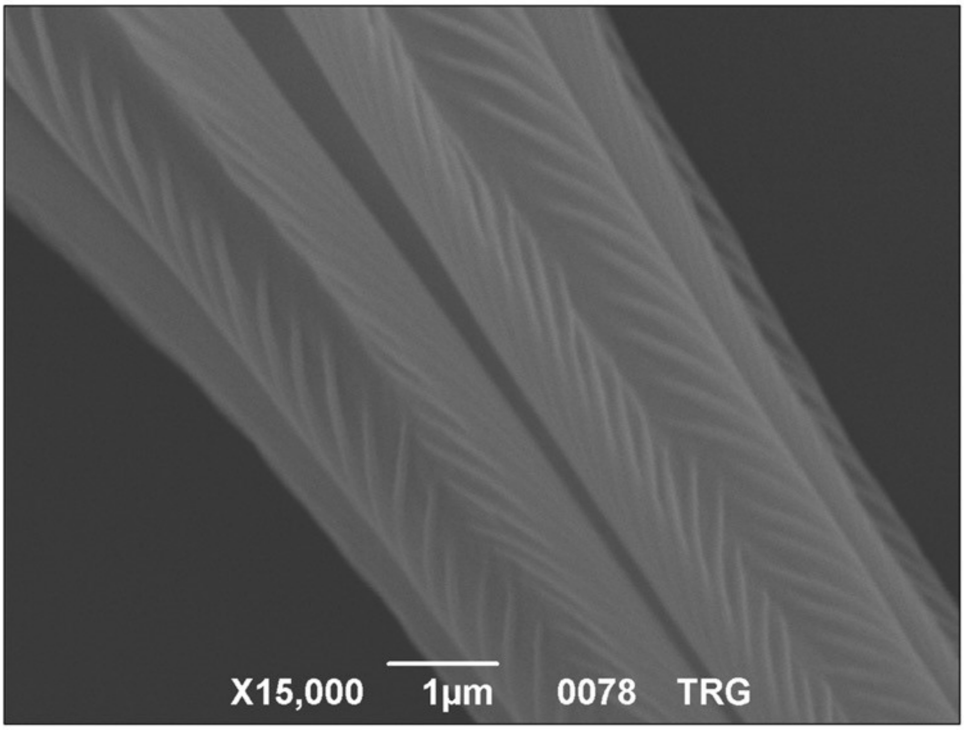

Fig. D. 300x. Detail of the longitudinal processed of the head of the hastiseta illustrating the knurls and the longitudinal furrow.

Trogoderma spp. 1 (Italy)

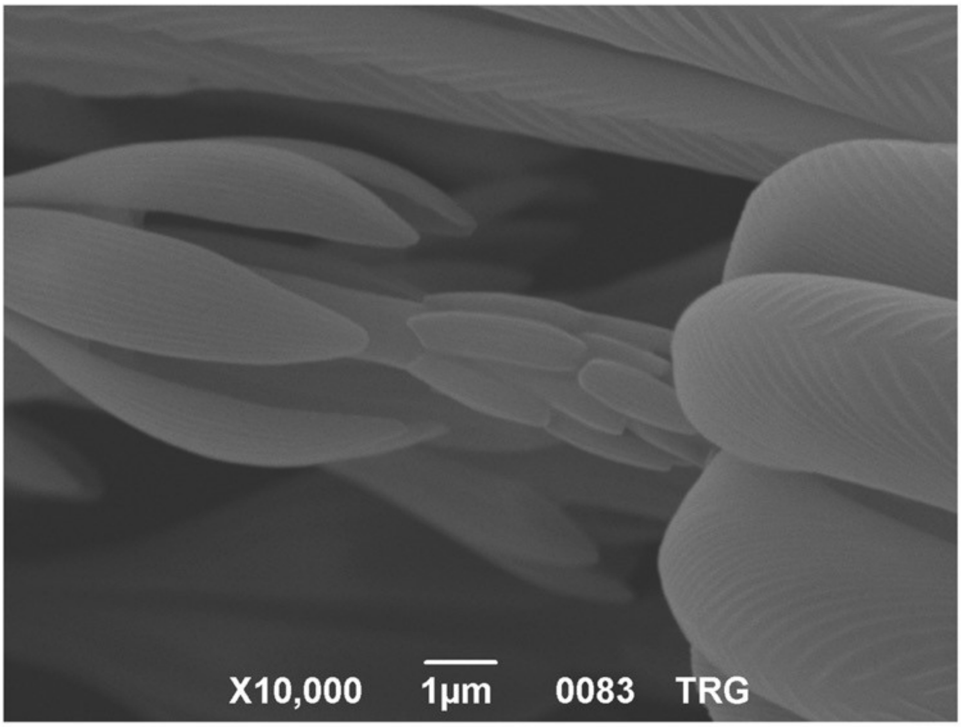

Fig. E. 1000x. Detail of the last rosette and the irregular scales on the stalk.

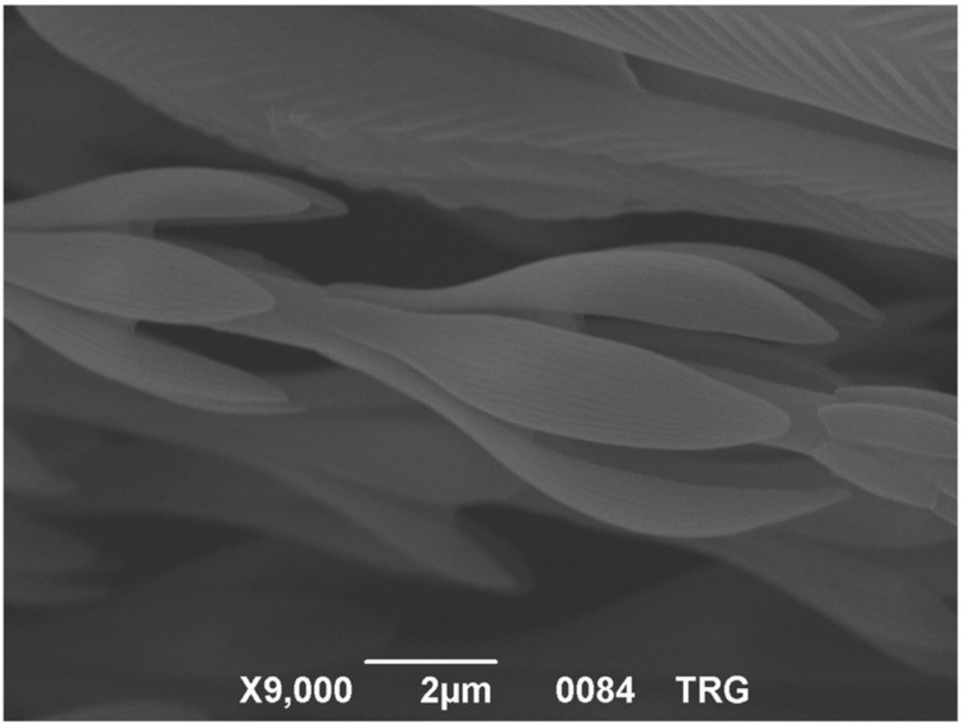

Fig. F. 9000x. Size and shape comparison between the penultimate and the ultimate rosette.

Supplementary material 18

Trogoderma spp. 2 (Romania)

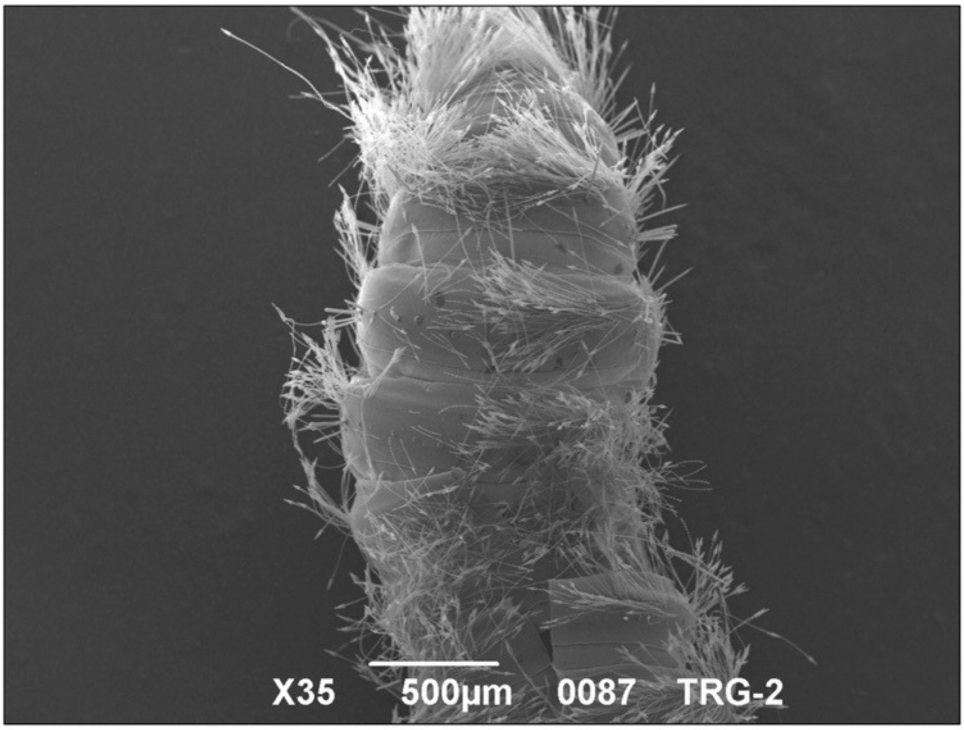

Fig. A. 35x. Mature larva, dorsal view of the abdominal tergite showing the tufts of hastisetae.

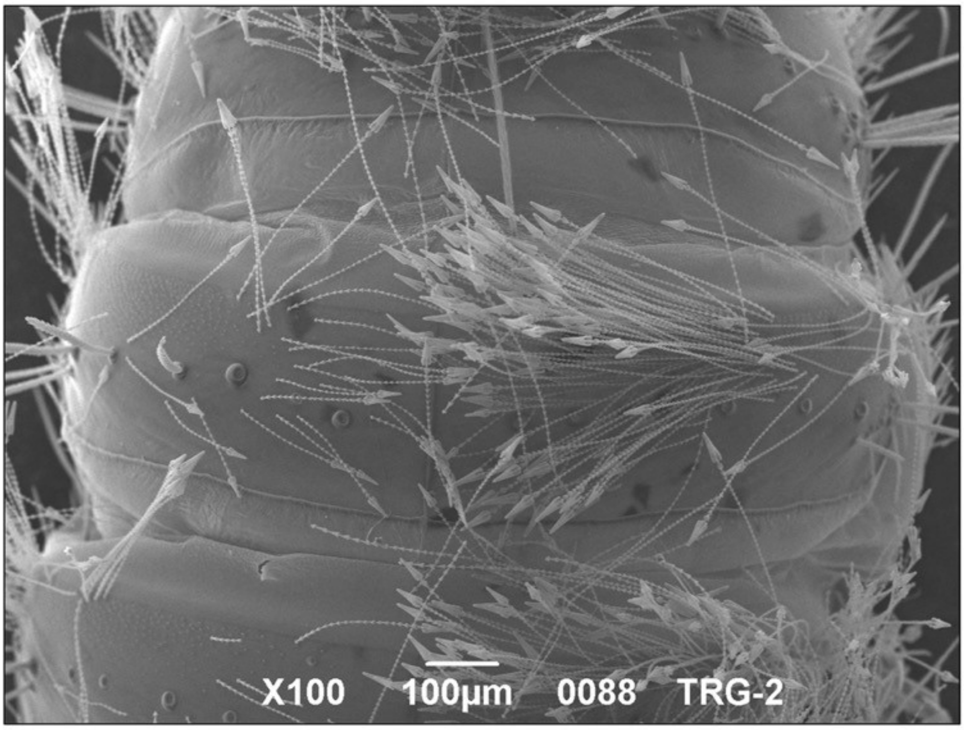

Fig. B. 100x. 4th abdominal tergite, dorsal view. Tufts of hastisetae on the left almost completely missing.

Trogoderma spp. 2 (Romania)

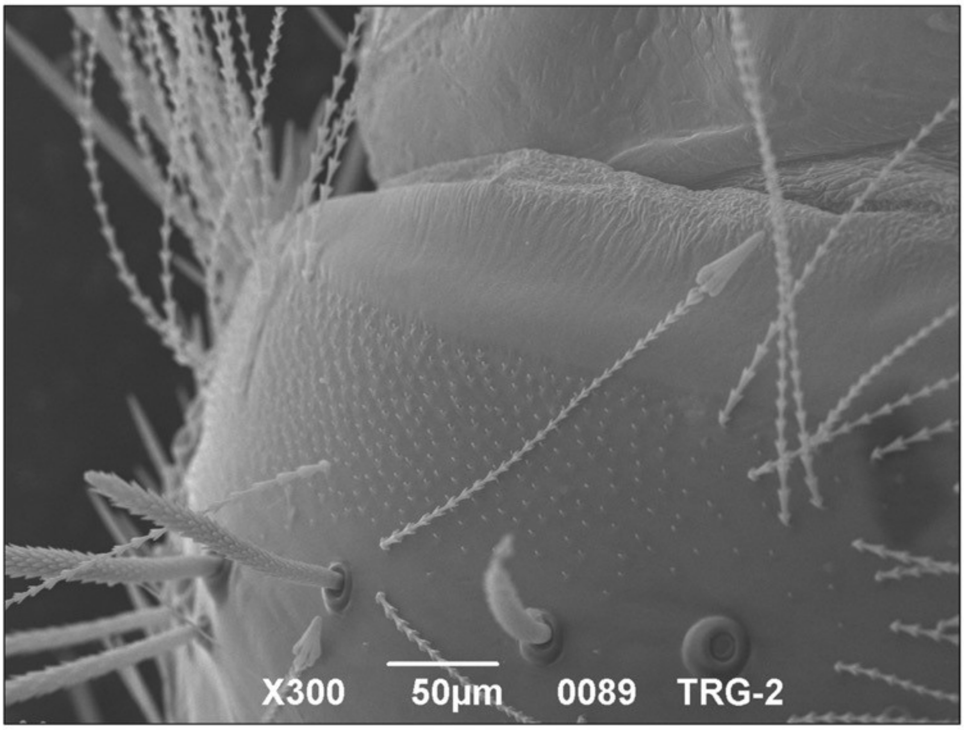

Fig. C. 300x. Detail of the 4th abdominal tergite illustrating the location of the hastiseta on the lateral side of the thergite.

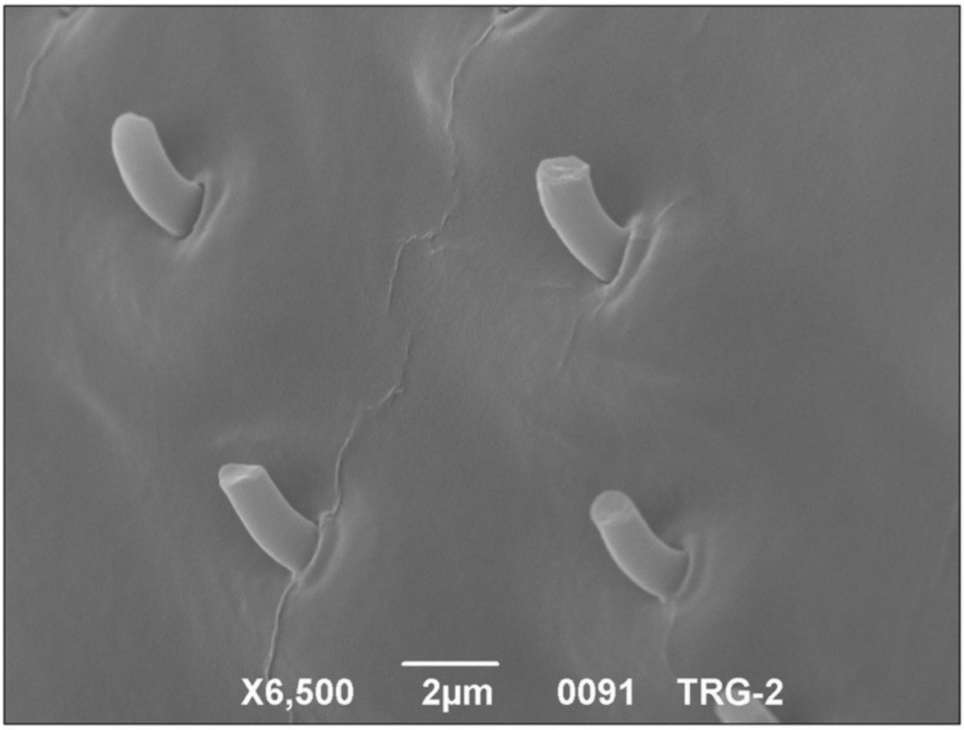

Fig. D. 6500x. Detail of a series of broken pedicels illustrating orientation and curvature of the structure.

Trogoderma spp. 2 (Romania)

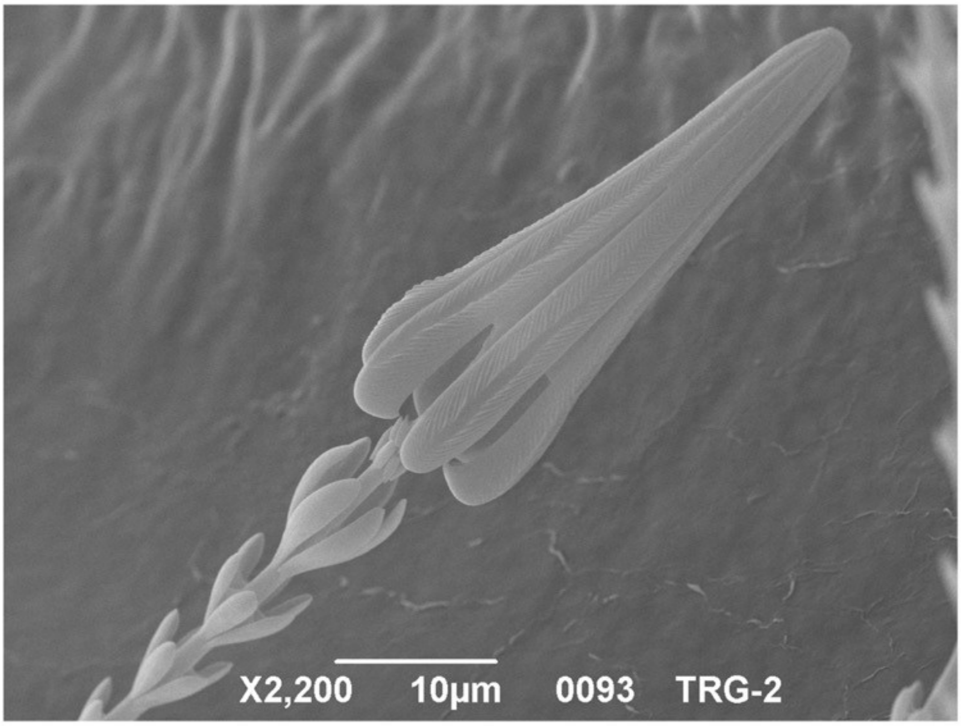

Fig. C. 300x. Lateral view of the apical part of the hastiseta.

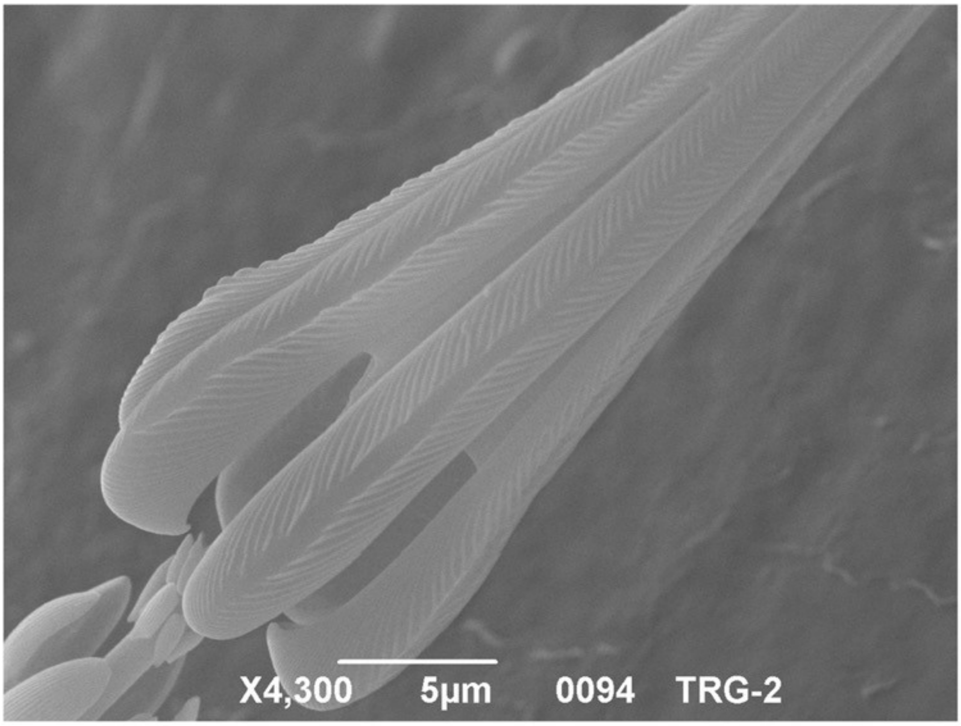

Fig. D. 4500x. Detail of the head of the hastiseta illustrating the knurls and the longitudinal furrow on the processes of the head.

Supplementary material 19

Trogoderma spp. 3 (Italy)

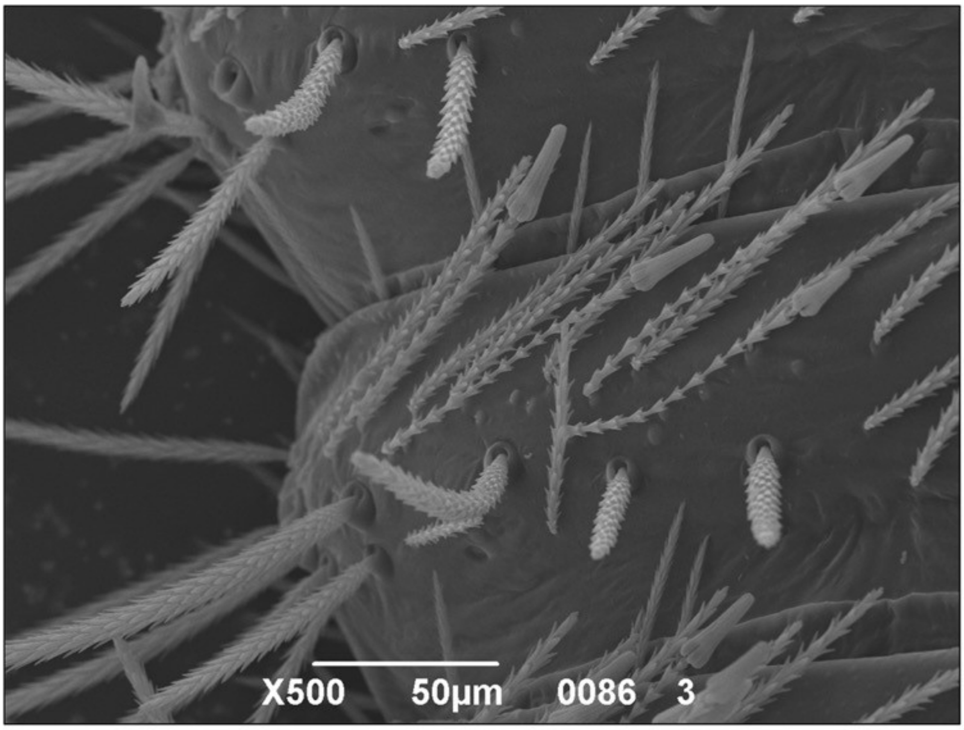

Fig. A. 500x. Tuft of hastisetae on an abdominal tergite.

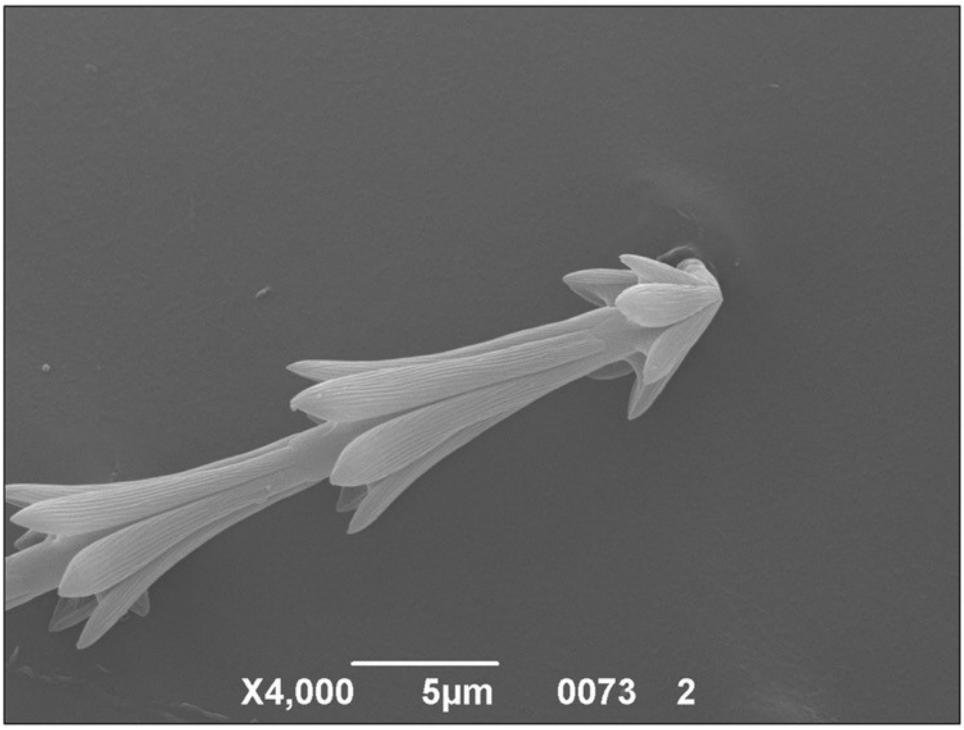

Fig. B. 4000x. Detail of the insertion of the hastiseta on the integument of the tergite.

Trogoderma spp. 3 (Italy)

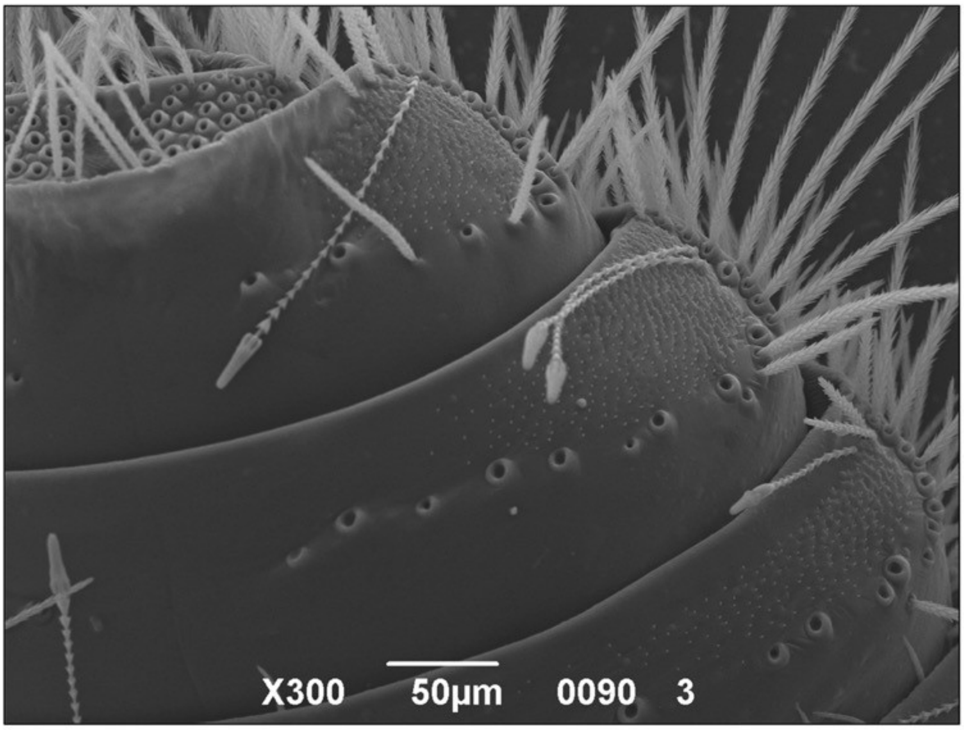

Fig. C. 300x. Dorso-lateral view of abdominal tergites 6th to 8th showing the missing tufts of hastisetae.

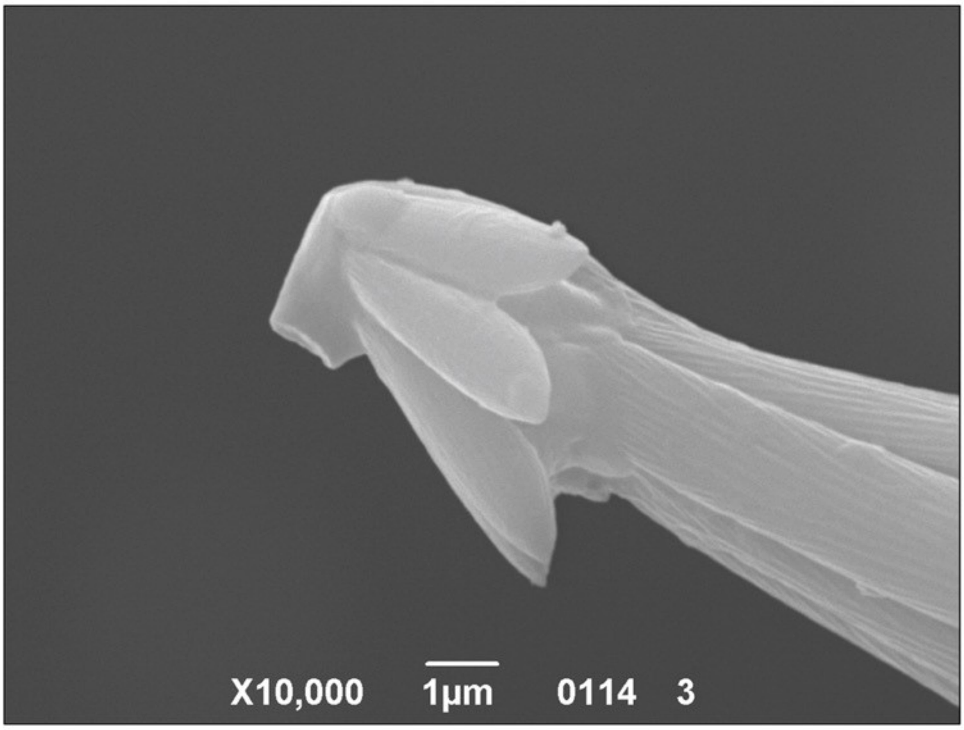

Fig. D. 10000x. Detail of the first rosette and broken pedicle on a detached hastiseta, lateral view.

Trogoderma spp. 3 (Italy)

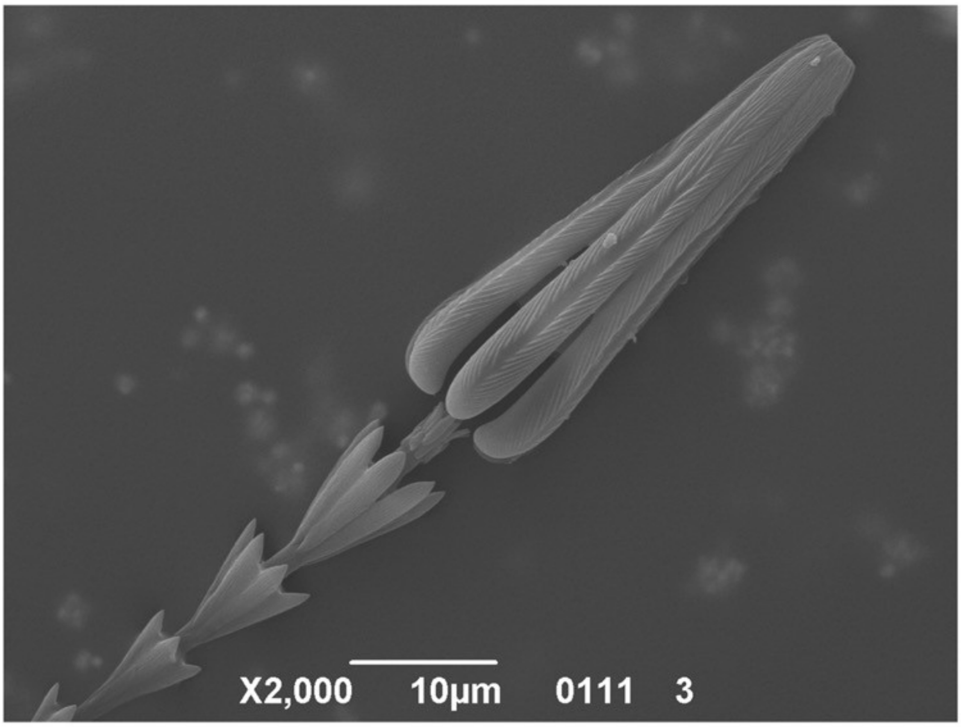

Fig. E. 2000x. Lateral view of the apical part of the hastiseta.

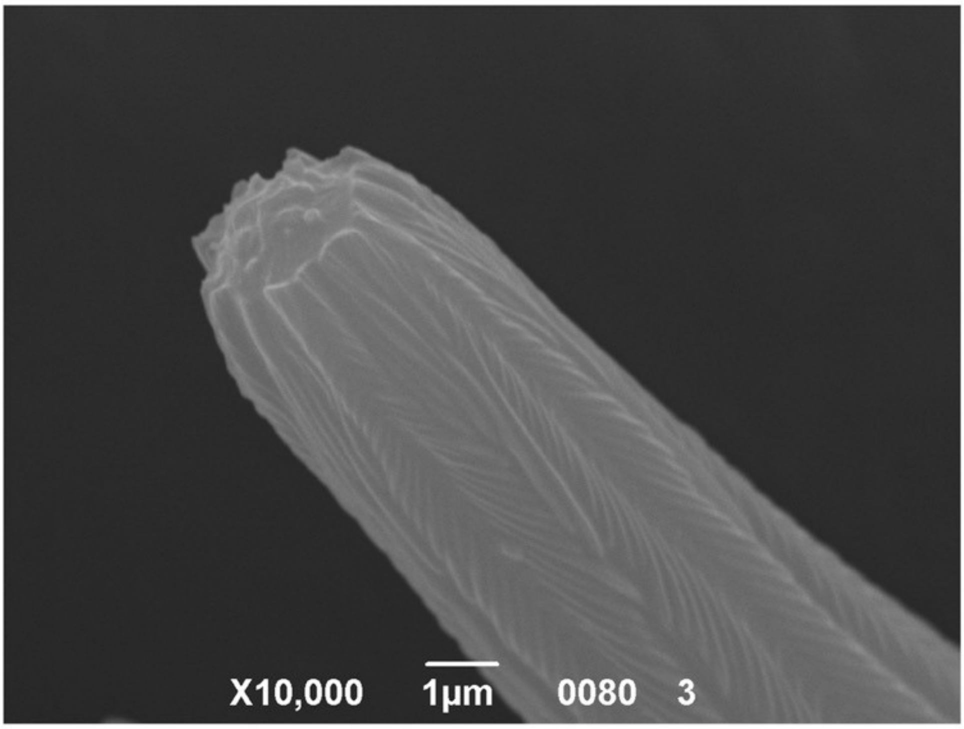

Fig. F. 10000x. Detail of the apex of the head of the hastiseta, lateral view.

Trogoderma spp. 3 (Italy)

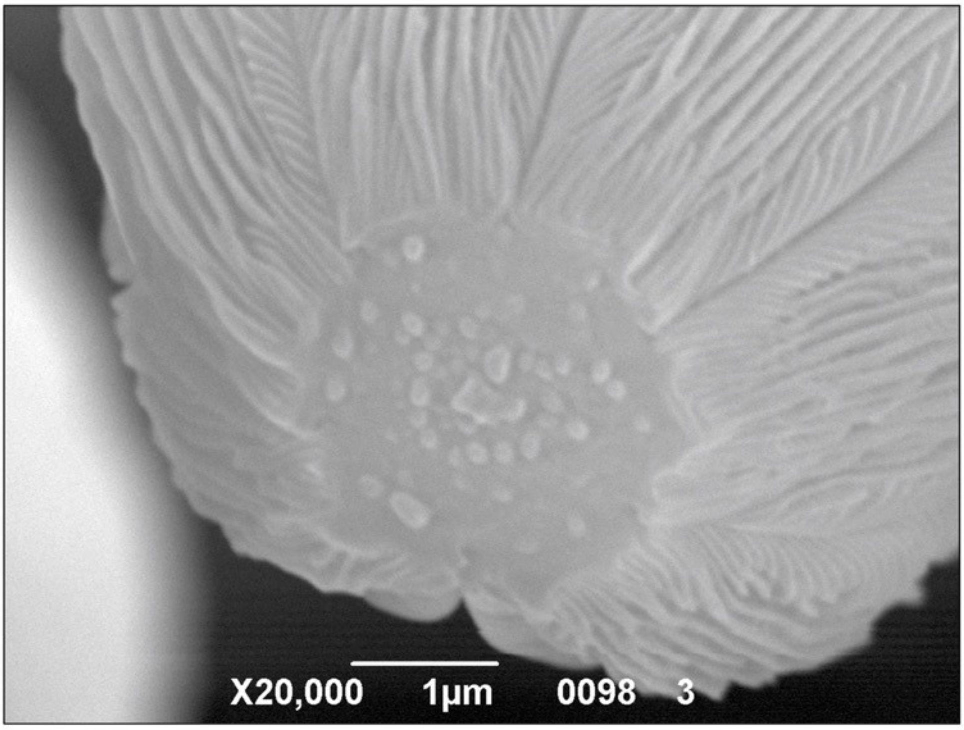

Fig. G. 20000x. Detail of the circular depression on the head of the hastiseta, vertical view.

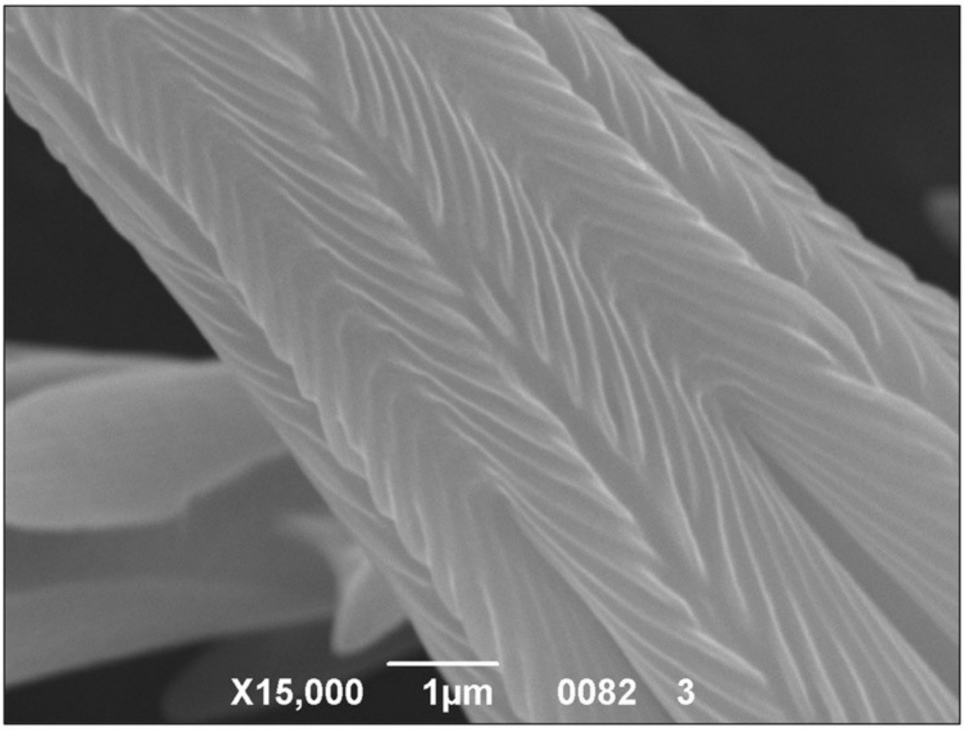

Fig. H. 15000x. Detail of the head of the hastiseta illustrating the knurls and the longitudinal furrow on the head processes.

Trogoderma spp. 3 (Italy)

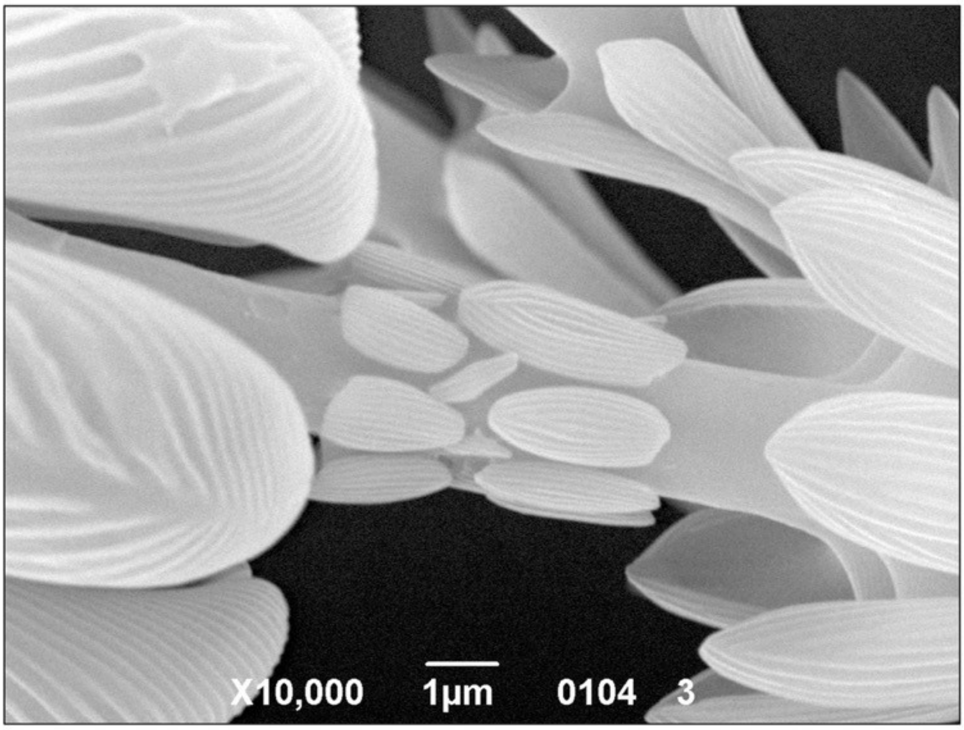

Fig. I. 10000x. Detail of the irregular scales on the stalk between the last rosette and the head of the hastiseta.

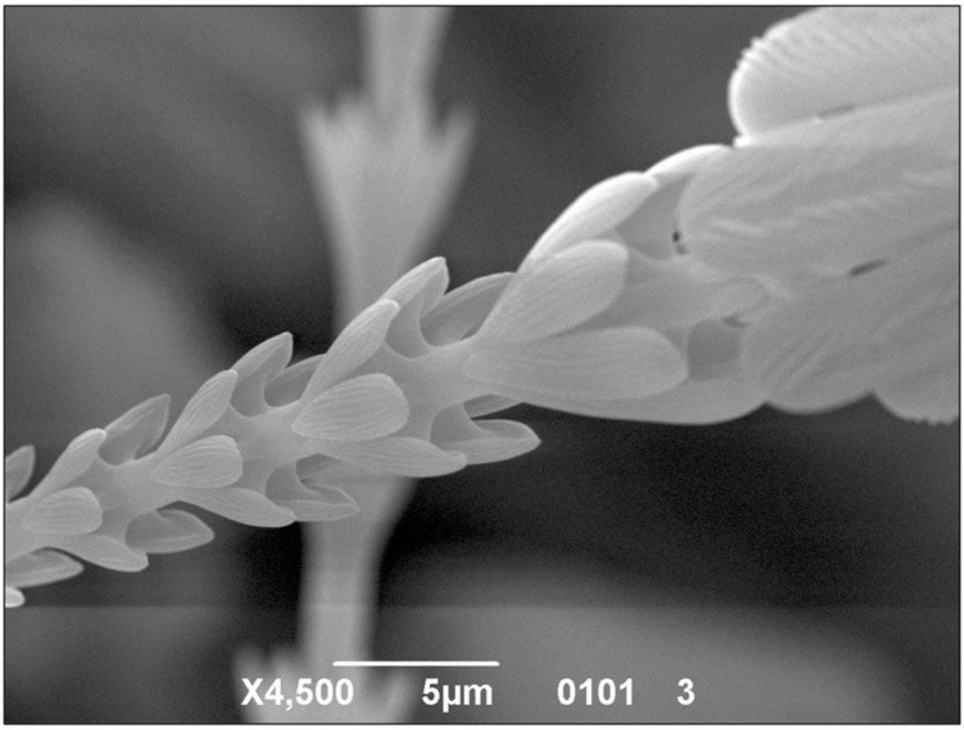

Fig. J. 4500x. antero-lateral view of the last rosettes illustrating the general morphology of the rosette and the chitin bridge supporting the scales.

Trogoderma spp. 3 (Italy)

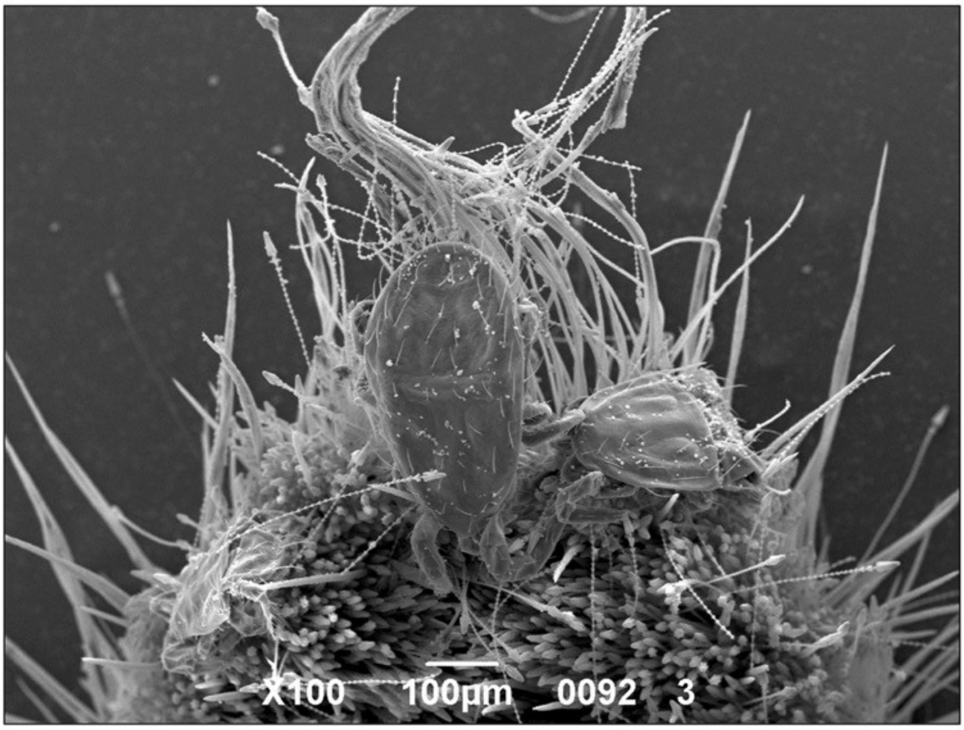

Fig. K. 100x. Two Mesostigmata mites entangled on the hastisetae on the last abdominal segments.

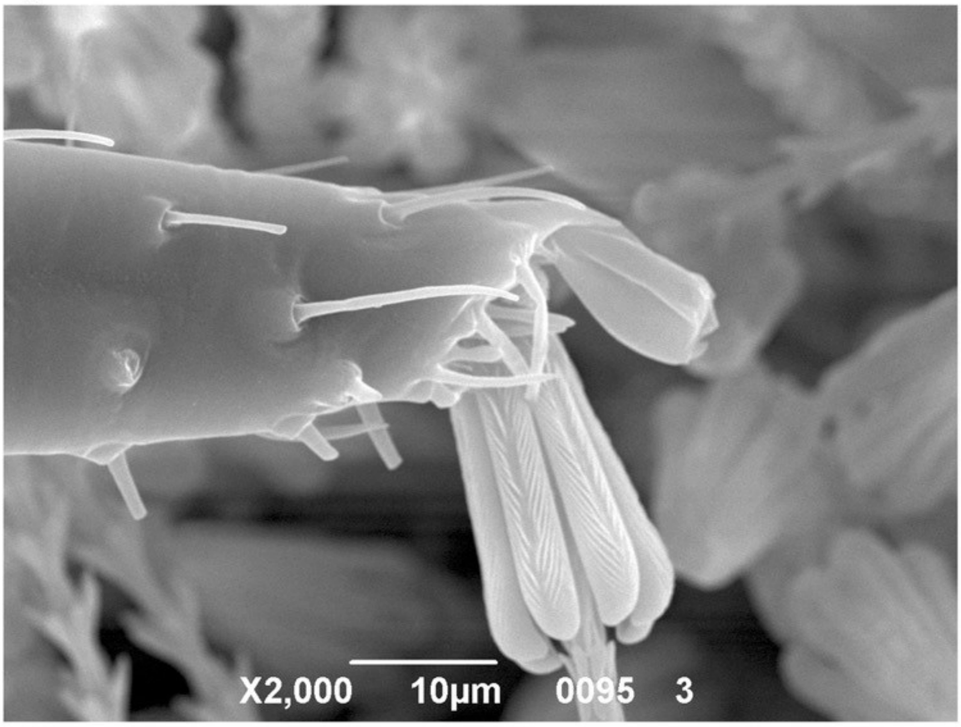

Fig. L. 2200x. Mite tarsus entangled by an hastiseta; the hairs are trapped between the longitudinal processed of the head of the hastiseta.

Supplementary material 20

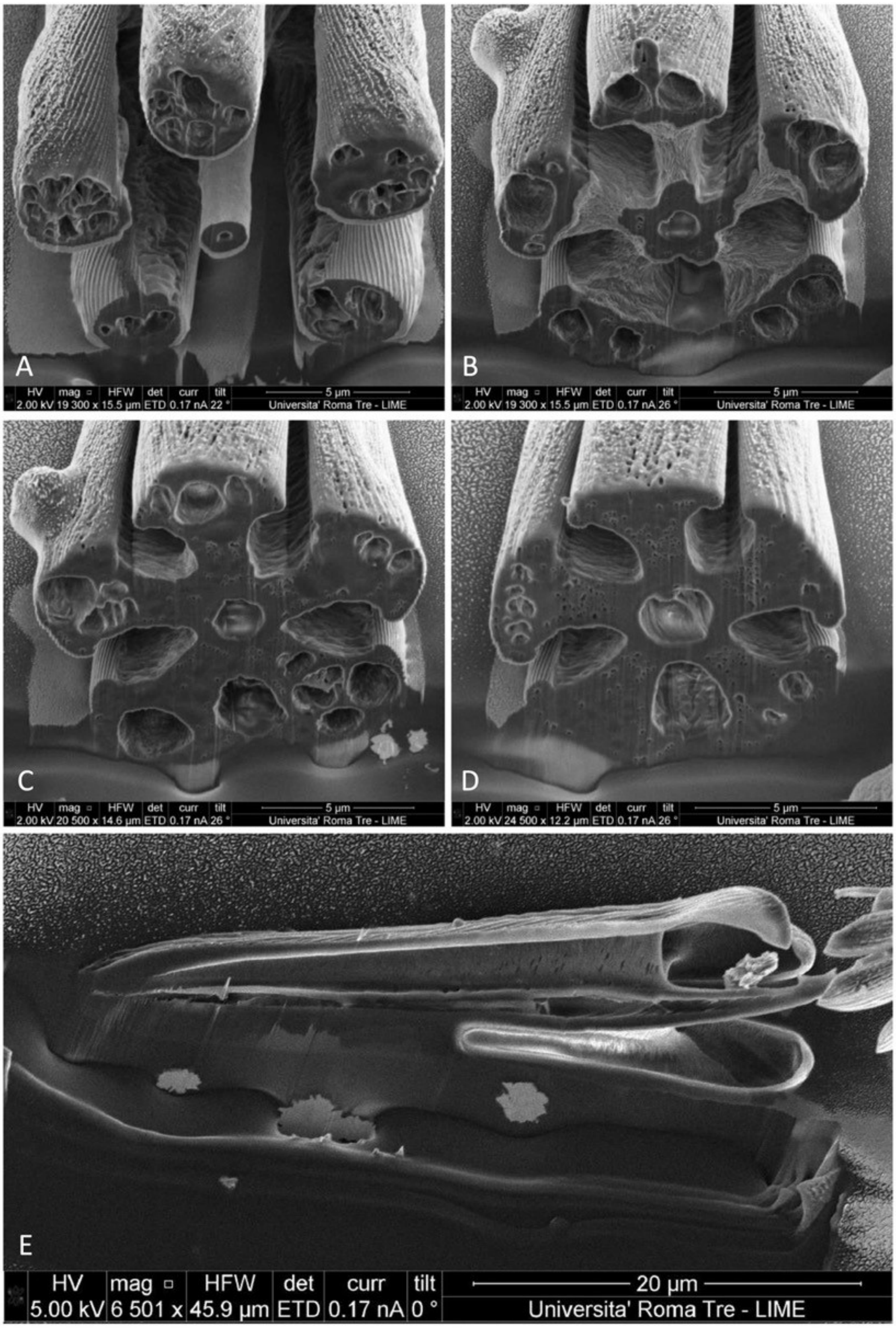

Fig. A-D. Multiple transversal section of the head of the hastiseta of *Anthrenus* spp. 2; sections starting from the base and moving toward the apex of the head. E. Sagittal section of the head of the hastiseta of *Trogoderma variabile* showing the hollow core of the stalk.