Microscopic morphology of the antennule and antenna of the marine isopod Cirolana harfordi

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Abstract

The use of scanning electron microscopy on the cuticle outgrowths called setae (that contain sensilla) in crustaceans is useful for, understanding how crustacean species sense their environment and for taxonomic studies. So far, most of the setal morphology studies have been performed on decapod crustaceans such as lobsters and shrimp and there has been a comparative lack of such research on marine isopods, from which terrestrial isopods such as woodlice or slaters have evolved. In the current study, electron microscopy was used to study the antennal setae of the marine isopod *Cirolana harfordi* using magnification powers of up to 40, 000. Some antennae samples were glued in line to the ends of toothpicks and then mounted at right angles to the flat surface of sample mounts so that they could be rotated and studied from different aspects. *C. harfordi* displayed a number of setal types including setae that had a sub-terminal pore that contained a cupule shaped structure and plumose setae which are pennate in structure and have two rows of setules along the setal shaft in a fashion analogous to a palm leaf. These plumose setae may be useful to the animal in detecting water currents. Differences in the structure and placement of setae on the antennae of *C. harfordi* as compared to two other marine isopods, *Bathynomus pelor* and *Natatolana borealis* (that have had a comprehensive study of antennae setal morphology performed on them in previous studies) indicate that scanning electron microscopy of isopod setae may be useful in taxonomic studies of isopods.

Introduction

Research that employs microscopy on crustacean antennae and their cuticular outgrowths called setae, that contain sensilla, has been performed for a number of reasons including that the information it reveals is helpful in understanding how crustacean species detect food and mates (Hallberg and Skog 2010, Zhu et al. 2011). Furthermore, setal morphology is useful for showing differences and similarities between species in taxonomic studies (Al-Zahaby et al. 2001, Fleminger 1973, Thomson 2013, Watling 1989). The term, antennae, is often used collectively in crustaceans to name the larger pair of ventrally placed antennae as well as the smaller dorsally placed pair of antennulae. Microscopy of crustacean setae, has been predominately been performed on decapods such as crabs and shrimp (Garm 2004, Wortham et al. 2014) but there are two studies that have used scanning electron microscopy performed to investigate the entire setal collections on the antennae and antennulae of deep-sea isopods (Kaïm-Malka et al. 1999, Thomson et al. 2009).

The intertidal zone isopod *Cirolana harfordi* (LOCKINGTON 1877) is a gregarious animal and one of the many marine isopods, where little was known about its antennal setae prior to this study (Salma and Thomson 2016, Thomson, Robertson and Pile 2009). Specimens of *C. harfordi* are common inhabitants of sandy beaches and intertidal environments in several global areas (Johnson 1976). These animals are found in temperate regions of the world including Japan, Malaysia (Bruce and Jones 1981), Australia (Bruce 1986) and the West coast of America from British Columbia to California (Richardson 1905, Ricketts and Calvin 1968). There are differences, however, between the different *C. harfordi* populations in the Pacific Ocean. The Japanese animals are occasionally described as a subspecies, *Cirolana harfordi japonica* due to morphological differences including that they are smaller than specimens from America and Australia and there are structural differences in the spoon shaped outer mouthpart, named the maxilliped (Bruce and Jones 1981). *C. harfordi* in Australia is described as an invader in some publications with a suggested invasion time in the 1970's, but these publications do not provide any actual evidence that *C. harfordi* invaded Australia (Currie et al. 1998, Hewitt et al. 2004). Indeed, there is evidence that the Australian animals did not invade Australia in the 1970s, they have 9-10 spines on the pleotenson which differs from American and Japanese specimens that have 30-36 pleotenson spines (Bruce 1986). It is unlikely that such a dramatic morphological feature evolved in the last half-century and the Australian animals may be a subspecies of *C. harfordi* (Bruce 1986) or distinctly different species (Thomson 2014). The possibility that the Australian *C. harfordi* is a distinct species from the American animals is further supported by the finding that the Australian animals give live birth (Thomson 2014) whereas the American animal is described as following the usual isopod reproductive strategy of laying eggs into a marsupial pouch (Johnson 1976). Whether there are differences in the microscopic anatomy of setae on the antennae and mouthparts of the Australian, American and Asian populations of *C. harfordi* is unknown. Prior microscopy research on the setae of the Australian *C. harfordi* specimens has been on the mouthparts only, not the antennae (Thomson 2013). There have been no electron microscopy studies on the setae of American and Asian *C. harfordi* specimens and information from such studies in the future could help determine whether the Australian *C. harfordi* is a separate species. Indeed, the proposal to use setal morphology and site-specific setal placement in phylogenetic studies of isopods (Al-Zahaby, El-Aal and El-Bar 2001, Menzies 1956, Thomson 2013) could be extended to the Cirilanidae family to which *C. harfordi* belongs, because there is a perceived need for more anatomical and cladistic analysis to maintain or modify the present categorization of the Cirolanidae into a trio of subfamilies (Riseman and Brusca 2002).

Microscopy of isopod antennal setae has revealed interesting structures that may be helping these animals sense their environment. For example, some setae on the antenna of the deep sea scavenging marine isopod *Natatolana borealis* (LILLJEBORG 1851) have a pore close to the distal end that contains a structure that resembles a satellite dish or cup, and is approximately 700 nm in diameter. This structure has been named a cupule but it is not known whether it is functioning in chemoreception or mechanosensory processes (Kaïm-Malka , Maebe, Macquart-Moulin and Bezac 1999). Whether cupules are also present isopods that inhabit shallow water was not known until 2013 when they were shown to be present on the mouthpart setae of the shallow water dwelling *C. harfordi* (Thomson 2013) Prior to this study, however, it was not known whether cupules were present in the terminal pores of setae on the antennae of *C. harfordi*. Additionally, the deep sea isopod *Bathynomus pelor* (BRUCE 1986) has a furrow on the ventral side of the antennule bordered by cone-shaped projections and tubular setae involved in chemoreception called aesthetascs lye in this furrow (Thomson, Robertson and Pile 2009). This feature in *B. pelor* may regulate the flow of water over the aesthetascs and help the animal sense the direction of distant food (Thomson, Robertson and Pile 2009).

The present study was conducted to test the following hypotheses, a) different types of setae are positioned in a site-specific manner on the antenna and antennules of *C. harfordi*, b) aesthetascs located in a furrow on the antennules ventral surface is a feature that will not be present on a shallow water (where food is not as scarce as in the deep-sea) dwelling isopod, c) the terminal pore of some setae on the antennae of *C. harfordi* will contain cupules.

Material and Methods

Specimen collection

Animals were captured at Sirius Cove, Sydney, NSW, Australia in baited traps similar to those previously described (Keable 1995), a diagram and photo of the modified trap has been published (Thomson 2013, Umme and Thomson 2018). Briefly, the body of the trap was constructed by a 30 cm long piece of PVC pipe of 8cm diameter. A pipe cap with a rainwater mosquito stainless steel 0.9 mm space mesh insert was fitted at one end of the pipe to allow water, but not isopods, to escape. At the other end of the pipe, a pipe cap was fitted, this had inserted an inverted funnel that has a 10 mm diameter inlet. A stainless-steel eye-bolt was bolted onto the pipe and attached to a 5 kg diver's weight to weigh down the traps. Traps were baited with fish cat food and left for 24 hours. Animals were transferred to seawater and transported back to the lab and held in filtered artificial sea-water until preservation the next day.

Light microscopy of living specimens

Stereo light microscopy was performed using a Leica EZ4 microscope. To obtain a ventral view of the animal a specimen was placed in chilled artificial sea-water on ice to temporarily anaesthetise it and rolled onto its back.

Sample preparation and scanning electron microscopy

Samples were fixed for 18 hours in 2.5 % glutaraldehyde in 100 mM sodium phosphate buffer, pH 7.2 (buffer A), washed 3 times for 5 minutes each in buffer A, then postfixed in 0.1% (v/v) osmium tetroxide. After 3 washes in buffer A each for 5 minutes, the samples were dehydrated in the following percentages of ethanol 30, 50, 70, 90, 95, 100 each step was a 15 minute incubation. The samples were then prepared using critical point drying (Hegna 2010). The samples were then mounted on stubs coated with carbon and aluminium tape and sputter coated with gold/palladium. Scanning electron microscopy was performed using a Zeiss Ultra Plus and a Zeiss EVO 50 microscope. The secondary electron detector was used for most of the images, with the exception of those labelled as obtained using the backscatter detector. Some antennae and antennules were glued to the tip of the end of a toothpick that was then attached standing straight up on a sample holder so that the appendage could be rotated in the microscope and studied from different sides.

Terminology

Aesthetasc: Long thin-walled tubular seta found on the antennulae thought to be involved in olfaction

Annulus: A depressed indentation that circumscribes the shaft of the seta.

Biramous; Describes a crustacean appendage with two rami.

Companion setae: Setae that are closely positioned on either side of the aesthetascs.

Cupule: A hemispherical structure within a pore.

Cuticle extension: An extension that is neither a seta nor spine and has no setules or denticles, often found on the apices of scales, can range in length from short to long and ribbon-like.

Flagella: The tapering multi-article distal portion of the antennula or antenna attached to the peduncle.

Finial: A pointed piece of tissue located at the apex of a seta.

Peduncle: The basal articles of the antennula or antenna.

Plumose seta: Two rows of long (length over 10 times that of the width at the base) setules on either side of the setal shaft, forming a pennate structure.

Pore: Opening in the terminal region of a seta.

Rami: A branch of an appendage connected to the peduncle.

Scale: A flattened, shingle-like component of the cuticle. The term scale has also been used to describe the vestigial remnant of a second flagella lost that has been extensively lost through evolution.

Seta: A projection on the cuticle that contains sensilla.

Setule: A projection on a seta (analogous to a leaflet on a palm leaf), often flexible.

Simple seta: A seta without setules, may only have very small protuberances (less than 1 µm long) present.

Subterminal pore: A pore that is usually located on the side of a seta close to the tip.

Uniramous: Describes a crustacean appendage with only one ramus.

Results

Position of the antennae on the animal

Figure 1 shows a photograph of the dorsal aspect of a specimen taken with a stereo light microscope in which the animal's distinctive and high contrast patterning on its dorsal surface can be seen. When *Cirolana harfordi* specimens were active they were often observed to move their antennae around rapidly often pointing them forwards. Figure 1 shows a resting animal with its antenna pointing posteriorly at about 45^{o} to the centre line of its body. The inset line drawing in Figure 1 shows the anatomical position that puts each antenna in a linear arrangement and 90 degrees to the animal's body midline. The front of the antennae in this position can be referred to at the anterior aspect, and the back of the antenna as posterior. Living specimens were observed to often hold their antennae and antennulae well under their eyes (Figure 2A). The antennulae are positioned dorsally, and slightly anterior, to the antennae (Figure 2B, C).

The antennule

The antennule consists of a peduncle of 4 articles and a flagellum of 11-15 articles (Figure 3, 9) that reaches almost to the distal end of the antenna peduncle (Figure 1, 2B). The peduncle articles are numbered 1 to 4 beginning at the most proximal article. The posterior region of the peduncle articles 1, 2 and 3 are each concave along the length of the article (Figure 3A, 9). No evidence of a vestigial rami of a second flagellum (sometimes called a 'scale') was observed on the peduncle the antennule which is completely uniramous (Figure 3).

The antennae

The antenna is composed of a five-article peduncle, and like the antennule, the articles are numbered beginning at the proximal article. The peduncle houses a flagellum of 29-38 articles (Figure 4, 10).

The tegument

Most of the antennae and antennules are covered with scales that make up the outer layer of the cuticle and in some regions, the distal portions of the shingles have needle-like cuticle extensions (Figure 5A, B).

Cuticle pits and pit setae

The first, second and third peduncle articles of the antennule are decorated with concave pits that are approximately 20 µm in diameter (Figure 3A, 9). The third, fourth and fifth articles of the antennae peduncle are decorated with similar pits (Figure 4, 10). Each pit has a simple pit seta in the middle. The pit seta, rests against a thin flap of cuticle that extends out at approximately 90^o to the setal shaft, and lies along approximately half the length of the pit seta (Fig 5E, 8). At the tip of the pit seta, there is a large oval shaped pore, the lengthwise diameter of the pore is approximately equal to the thickness of the setal shaft. Around the pore, there appears to be a ring of tissue shaped like a toilet seat. At the most distal end of the pit seta, there is a cone of tissue, or finial that sits atop the apex of the seta. Inside the pore, there is a cup-shaped piece of tissue termed a cupule (Figure 5E, F).

Simple setae

Simple setae are so named because they do not have prominent setules (Figure 8). There are simple setae positioned on the peduncles and flagella of both the antennulae and antenna. The first, second and third articles of the antennule peduncle have simple setae (Figure 3, 9). Some of the antennule flagella have simple setae positioned singly or in pairs on the dorsal aspect (Figure 9). The antenna peduncle contains simple setae at points close to the distal ends of the second, third, fourth and fifth articles and the fourth and fifth articles have clusters of simple seta with adjacent origins (Figure 4, 10). The antenna is extensively decorated on the articles of the flagellum with simple setae and the origins of the simple setae are situated close to the distal region of the articles (Figures 4, 6D, E, 10). Some simple setae on the antenna flagellum are positioned singly (Figure 6A) while others are in clumps (Figure 6B, 10). Some simple setae are somewhat flattened on either side and have a wider or bulbous proximal end (Figures 6A, B, 8). Most of the simple setae have a terminal distal end that contains a pore with a similar structure to the pit setae except the finial is either much reduced or absent (Figure 8). Simple setae with a pore have a ring of tissue like pit setae and contain a cupule within the pore (Figures 6C, 8). Simple setae that did not have a sub-terminal pore were the companion setae found on the anterior side of the aesthetasc row Figure 7.

Plumose Setae

Plumose setae are pinnate in structure, they are characterized by two rows of setules on either side of the setal shaft in a fashion analogous to a feather (Watling 1989). The second, third and fourth articles of the antennule peduncle house plumose setae (Figure 3, 9). The fourth and fifth articles of the antenna peduncle contain plumose setae (Fig 10). The flagellum articles of the antennule and antennae were not found to house plumose setae. The distal half of the plumose setae on *C. harfordi* antennules and antennae somewhat resembles a palm leaf, in that they have two rows of tapered ribbon-like setules on either side of the setal shaft. The difference is that the setules on the plumose setae have the flattened aspects facing the top and bottom of the shaft whereas the flat surfaces of a palm leaf's leaflets are parallel to the shaft or rachis of the leaf (Figure 6A, 8). Plumose setae were often found housed in a socket in the cuticle (Figure 6A).

Aesthetascs

Aesthetascs are long hollow tube-shaped setae that are only found on the flagellum of the antennule and not on the antenna of isopods and other crustaceans (Watling 1989). A line of aesthetascs is positioned on the posterior/ventral aspect of the flagellum of *C. harfordi*, each article of the flagellum has aesthetascs that have their origin at the distal end of the article. The aesthetascs have an annulus where they are joined to the cuticle at the distal end of each flagella article of the antennulae (Figure 7A, B). After drying, the base of the aesthetasc for approximately 25 μm of its length, was round cylinder shaped with a smooth surface whereas the rest of the aesthetasc displays a crinkled surface and is flattened resembling a collapsed tube (Figure 7C).

Companion setae

Companion setae are non-aesthetasc setae that are located on either side of a row of aesthetascs (Steullet et al. 2000). In *C. harfordi* the companion setae on the anterior side of the aesthetasc row on some of the antennule flagella articles were simple seta with a small terminal pore at the tip of the seta (Figure 7, 8). Some antennule flagella articles have a single simple companion seta the posterior side of the aesthetasc row (Figure 7B) that has a sub-terminal pore positioned on the side of the terminal portion of the shaft similar to that shown in Figure 6C.

Discussion

Light and electron microscopy on decapod crustaceans, mainly shrimp, prawns and lobsters, has revealed information on the different types of setae these animals possess on their antennae (as well as elsewhere on the body) and this research has led to an increased understanding of how these animals gather chemical and mechanical information from their environment and provided useful information for taxonomic purposes (Garm 2004). The two species of marine isopod that have had their entire collection of antennae setae studied are *Natatolana borealis* and *Bathynomus pelor*, both are deep-sea isopods and belong to the Cirolanidae family (Kaïm-Malka , Maebe, Macquart-Moulin and Bezac 1999, Thomson, Robertson and Pile 2009). Additionally, microscopy has been performed on the simple setae found at the tip of the antenna of *Ligia oceana* (LINNAEUS 1767), an amphibious isopod that lives on rocky shores (Alexander 1977).

Triangular scales, often each with a raised apex on the surface of the cuticle as seen in this study are a common structural feature of crustaceans, for example, similar triangular scales are present on amphipods (Wong and Williams 2009). Like the deep-sea isopod *B. pelor*, *C. harfordi* has cuticle extensions at the apex of some its scales and these are elongated on some regions of the first peduncle of the antennule. It has been proposed that these cuticle extensions could roll over each other and help the antennulae peduncle article move against close surfaces such as on the antenna with reduced friction (Thomson, Robertson and Pile 2009). The concave shape on the posterior surface of the antennule peduncle articles of *C. harfordi* may serve to allow the antennule to fit snuggly against the antenna.

In the eighties, there was some debate as to whether the fourth article of the isopod antennule peduncle belonged to the flagellum or the peduncle. Wagele referred to the fourth article of the peduncle of the isopod antennule as the first article of the flagellum (Wägele 1983), however Bruce later pointed out that the absence of aesthetascs and the thickness of the cuticle defined this article as the fourth article of the peduncle and not belonging to the flagellum (Bruce 1986). In *B. pelor*, the presence of pits with setae on all the antennule peduncle articles including the fourth, and the absence of pits on flagella articles also suggested that the fourth article of this species belonged to the peduncle (Thomson, Robertson and Pile 2009). Unlike *B. pelor*, *C. harfordi* does not display pits on the fourth article of the antennule, it does, however, posses plumose setae on the fourth as well as the first to third articles of the peduncle and plumose setae are absent from the flagellum articles. A similar pattern is seen in some species of the genus *Politolana* a marine isopod genus that lives at depths of often over 300 metres (Riseman and Brusca 2002). The finding that all *C. harfordi* antennule flagellum articles have aesthetascs and the fourth article of the peduncle does not, indicates that the fourth article of the peduncle is not part of the flagellum and provides a clear distinction between the peduncle and flagella of the antennule. Isopoda is a monophyletic order, and in contrast to *B. pelor*, the antennule of *C. harfordi* is completely uniramous and has no vestigial second rami or flagella and this suggests that the putative ancestral second flagellum has been completely lost through evolution in *C. harfordi* (Thomson, Robertson and Pile 2009, Wägele 1983).

The antennae of *C. harfordi* has the common isopod configuration of five peduncle articles with a multi-article flagellum (Wägele 1983). *C. harfordi* is similar to *B. pelor* in that the antennae and antennule peduncles of both species are decorated with depressed pits, each containing a single and centrally located simple pit seta (Thomson, Robertson and Pile 2009). Peduncle pits are not found in all crustaceans for example, the amphipods *Hyalella* *castroi* (GONZALEZ, BOND-BUCKUP & ARAUJO 2006) and *Hyalella pleoacuta* (GONZALEZ, BOND-BUCKUP & ARAUJO 2006) do not possess them (Zimmer et al. 2009). Interestingly while the pits in *B. pelor* and *C. harfordi* look very similar when observed by light microscopy, scanning electron microscopy shows clear differences in morphology between the pit setae in the two species. The pit setae in *B. pelor* are tapered along the distal half of their length and do not have a subterminal pore (Thomson, Robertson and Pile 2009), whereas in *C. harfordi* the shaft of the pit setae is non-tapered and has a sub-terminal notched pore. Additionally, the thin flap of cuticle that the setal shaft rests against (and has been proposed to keep the pit setae upright) is wrapped around the seta in *B. pelor* (Thomson, Robertson and Pile 2009) but completely unattached to the seta in *C. harfordi*.

The aesthetascs of *C. harfordi* display the morphology of a long thin-walled tube with a thickened base, which is common among crustaceans of different orders including decapods within the class Malacostraca (Steullet, Cate, Michel and Derby 2000, Zhu, Zhang and Lin 2011). Aesthetascs are unique to the antennule and widely thought to function as chemoreceptors and the number and the length of these setae can vary between different genera (Hallberg et al. 1992, Hallberg and Skog 2010, Steullet, Cate, Michel and Derby 2000). The aesthetascs of *C. harfordi* do not lie in a long channel formed by lines of cone-shaped projections on either side of the aesthetascs as observed in *B. pelor*. It has been proposed that this arrangement of the aesthetascs in *B. pelor* could direct water flow over the aesthetascs and function to give *B. pelor* a superior sensing of the direction of molecules (Thomson, Robertson and Pile 2009). Alternately or additionally the arrangement could serve to physically protect the delicate aesthetascs. Some other crustacean animals such as lobsters protect their aesthetascs using long stiffened setae named guard seta positioned on either side of the aesthetasc line (Laverack 1964). The aesthetascs of *C. harfordi* are not flanked by inflexible guard setae but are flanked by simple companion setae which are unlikely to afford any physical protection of the aesthetascs (Laverack 1964). In lobsters, the companion setae are thought to be involved in both chemosensory and mechanosensory functions (Laverack 1964). It would appear that *C. harfordi* are not in so much need of physical protection for their aesthetascs, as some other crustaceans and may use their companion setae for olfactory and mechanosensory functions.

It has been proposed that plumose setae with their two rows of long setules could function to detect water currents (Phillips and Macmillan 1987). The plumose setae of *C. harfordi* are similar to the pennate tufted setae found on the antennae of *N. borealis* except that the *C. harfordi* plumose setae have setules that appear more flattened and ribbon-like (Kaïm-Malka , Maebe, Macquart-Moulin and Bezac 1999). The socket that *C. harfordi* plumose setae are often housed in may limit the circumduction movement of the plumose seta shaft.

The present study demonstrated that there can be some variation in the morphology of simple setae, for example, some simple setae were somewhat flattened from side to side and had a bulge in the plane 90^{o} to the flattened aspect. The simple setae on *C. harfordi* antennules and antennae (except filament setae and companion setae on the anterior side of the aesthetascs) have a subterminal pore with a cupule inside and a similar structural configuration has been observed on simple setae in *N. borealis* but not *B. pelor* who's simple setae often have a bifurcated or branched tip with no pore. A similar ring structure around the sub-terminal pore has also been observed in simple setae of the freshwater shrimp *Atya innocuous* (HERBST 1792)(Felgenhauer 1992) and this ring structure may be made of stiffened tissue that keeps the pore open (Thomson 2013). Whether or not the pore allows odour molecules to reach sensilla in the lumen of the seta as proposed by several authors, is not known at present (Kaïm-Malka , Maebe, Macquart-Moulin and Bezac 1999, Lombardo et al. 2006, Nishida and Kittaka 1992, Schmidt and Gnatzy 1984). What role the cupule plays is unknown but it has been proposed that it may be mediating chemosensory and/or mechanosensory information (Kaïm-Malka , Maebe, Macquart-Moulin and Bezac 1999). Cupules are not present in all crustaceans that have seta with a pore. Indeed the simple setae on the antennal tip in the marine and somewhat amphibious isopod *L. oceana* are very different to that of *C. harfordi*, they have a rounded bulbous tip, a relatively smaller pore and tissue that slightly protrudes through the pore, with no apparent cupule (Alexander 1977). In this laboratory, live specimens of *C. harfordi* are often observed dragging their antenna over the terminal article of the palp (unpublished results), the three-article appendage on the mandible that has numerous serrated setae that each resemble a comb (Thomson 2013). This action may serve to clean the numerous simple setae that *C. harfordi* has on its antennae.

In conclusion, prior to this study only rudimentary line drawings of the antennulae and antennae of *C. harfordi* existed (Bruce 1986, Holmes 1904), however, scanning electron microscopy has now been used to create both photographic images that depict these organs and their setae, as well as detailed line drawings that map the locations of their often site-specific setal collections. Comparison of the morphology of the antennulae and antenna and their setae between *C. harfordi* and the two other marine isopods that have been studied in detail (*B. pelor* and *N. borealis*) have revealed many areas of homology but also numerous differences. These results lend weight to the repeated proposals in the literature that the use of scanning electron microscopy to show the detail of cuticular structures including setae could be increasingly used in taxonomic studies and revisions, in crustaceans (Al-Zahaby, El-Aal and El-Bar 2001, Calazans and Ingle 1998, Thomson 2013, Zimmer, Araujo and Bond-Buckup 2009). Scanning electron microscopy of cirolanid isopod setae may be helpful in future studies to develop the taxonomy of the Cirolanidae family (Bruce 1986).

Figures

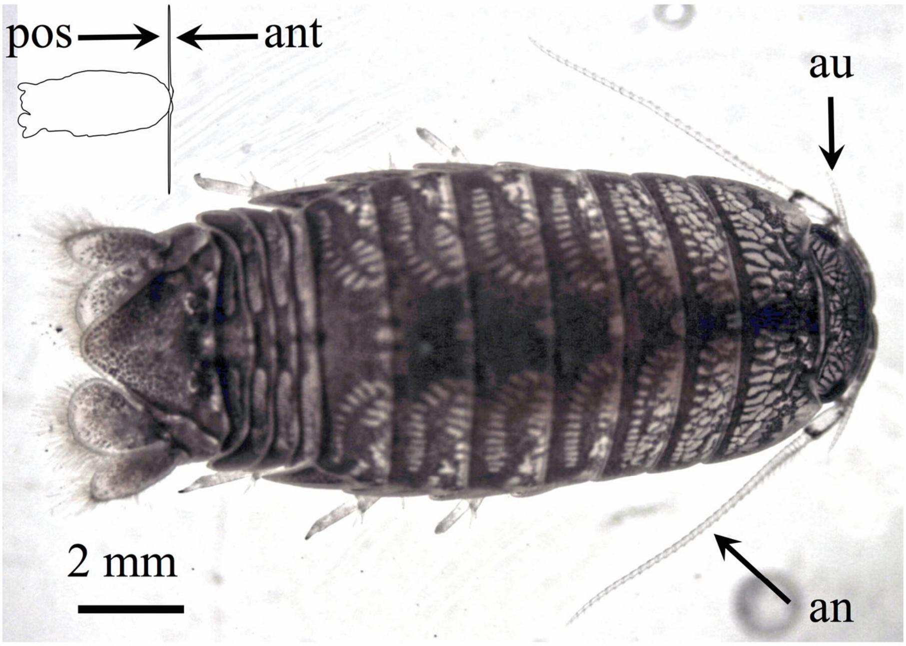

Figure 1 Dorsal aspect of a living *Cirolana harfordi* specimen (light microscopy). Inset line drawing shows animal with antennae in the anatomical position at right angles to body midline.

an, antenna; ant, anterior aspect of the antenna; au, antennule; pos, posterior aspect of the antenna.

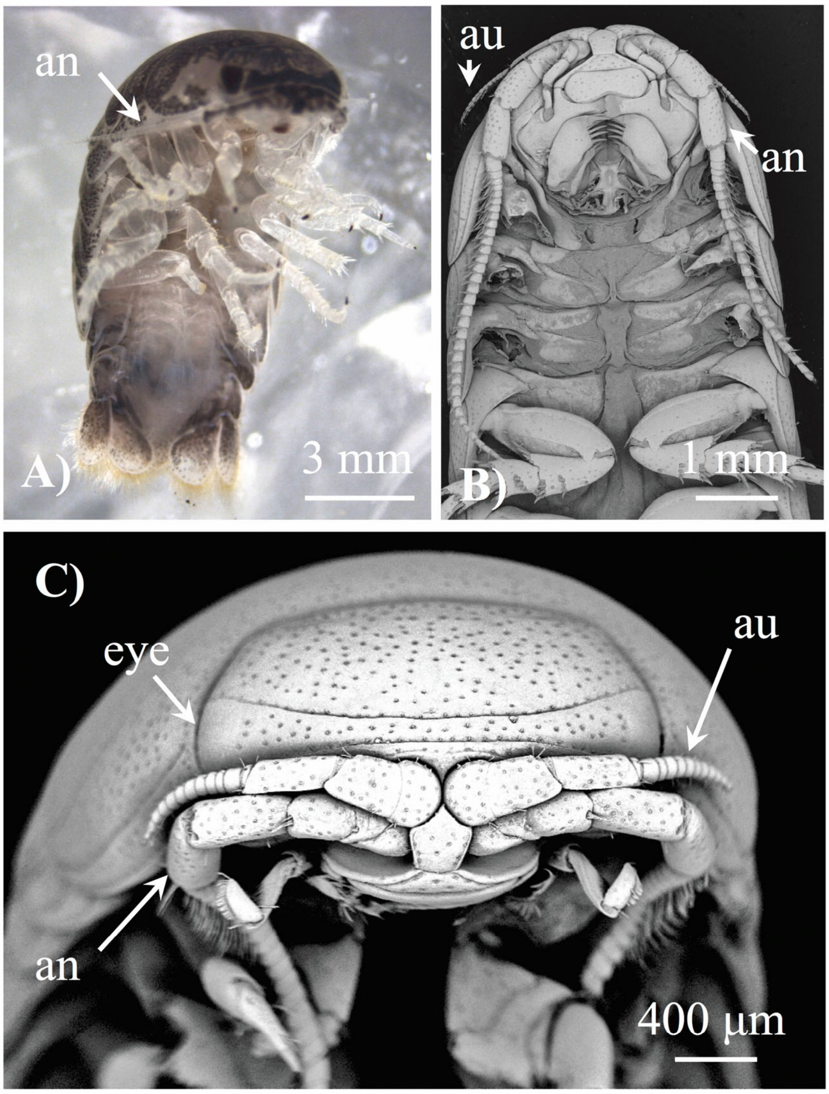

Figure 2. The antennae and antennulae *in situ*. A) mainly ventral aspect of a living animal that is displaying some flexion of its pereon and cephalon. The antenna is characteristically held underneath the eye (light microscopy). B) Ventral view of the pereon (thorax) and cephalon (head). The maxillipeds (spoon-shaped outer mouthparts) and the three most anterior pairs of pereopods (walking legs) have been removed (backscatter detector used). C) Anterior view of the cephalon and its antennulae and antennae (backscatter detector used).

an, antenna; au, antennule.

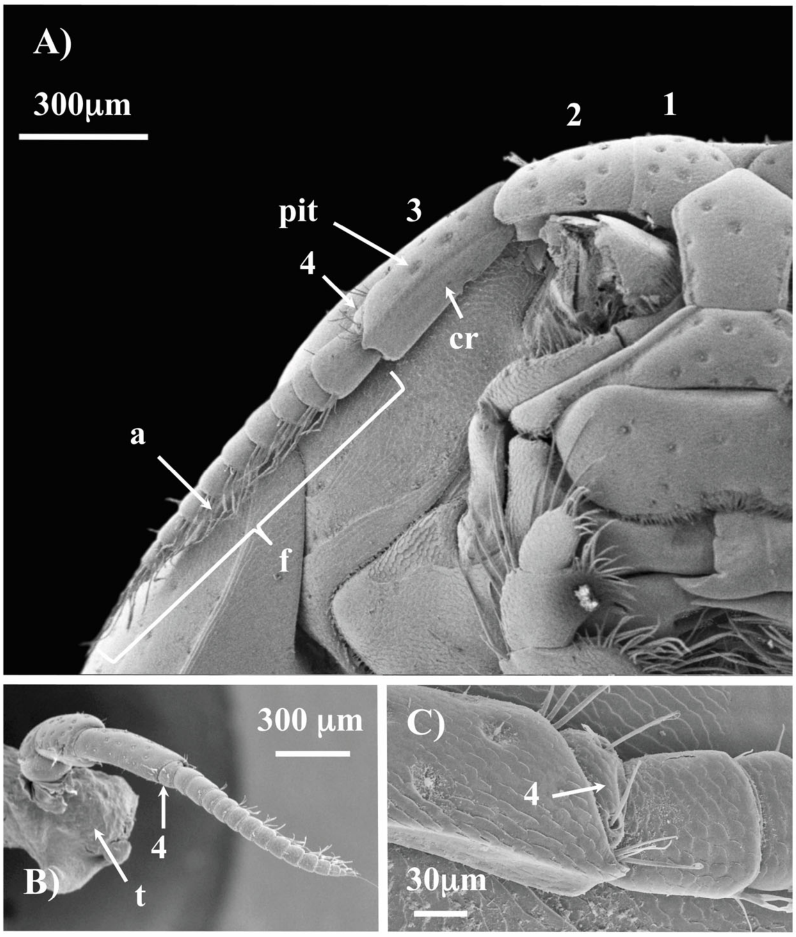

Figure 3 The Antennule. A) The right antennule *in situ*, ventral aspect, the antenna has been dissected away, peduncle articles are numbered, the flagellum is indicated with a bracket; B) An excised antennule mounted on the end of a toothpick, dorsal aspect with the flagella angled towards the foreground showing the fourth article of the peduncle; C) Ventral aspect of the fourth article of the peduncle on the left antennule, the third article of the peduncle is seen to the left and the first article of the flagellum is seen to the right.

a, aesthetasc; cr, concave region; f, flagellum; p, pit; t, toothpick.

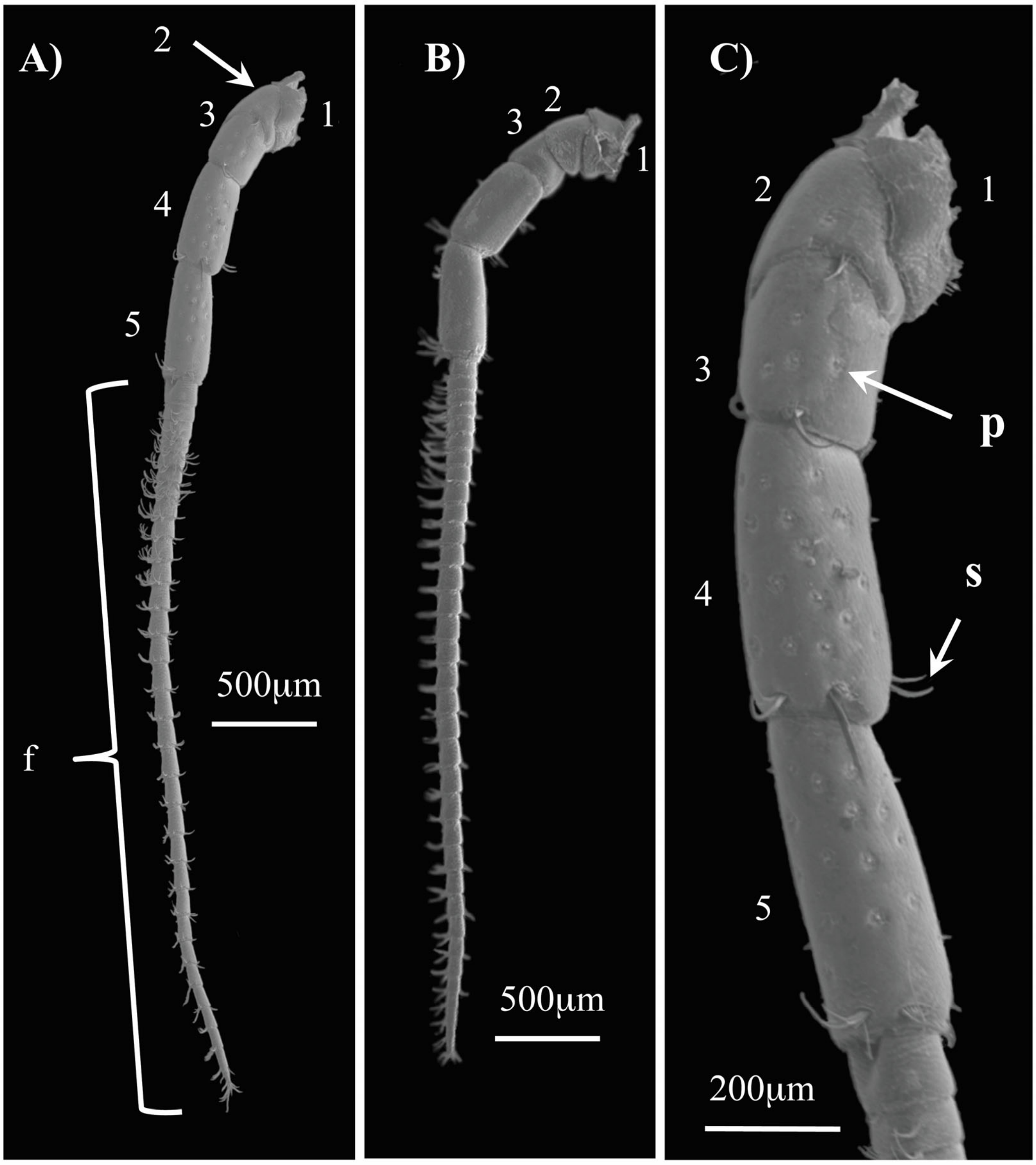

Figure 4 The Antenna. A) the right antenna, ventral aspect, peduncle articles are numbered; B) the left antenna, dorsal aspect; C) the peduncle of the right antenna, ventral aspect.

f, flagellum; p, pit; s, seta

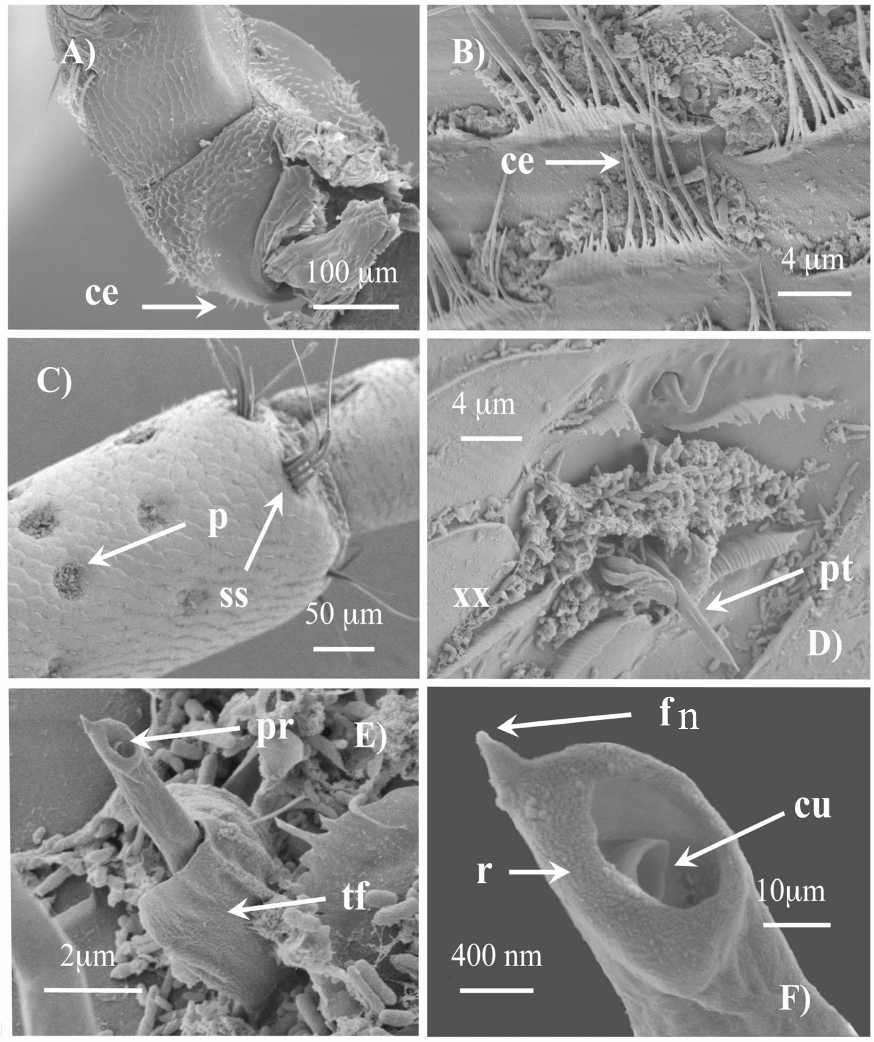

Figure 5 Scales and pits of the integument. A) posterior aspect of the first article of the peduncle of the right antennule showing scales that make up most of the integument surface, cuticle extensions on scales can be seen in some regions, B) close up of the edge of scales that have cuticle extensions; C) left antenna, fifth article of the peduncle, ventral aspect; D) a pit with central pit seta; E) pit seta showing the sub-terminal pore; F) the pore of a pit seta with cupule inside.

ce, cuticle extensions; cu, cupule; fn, finial; p, pit; pr, pore; pt, pit seta; r, ring of tissue around the pore; ss, simple seta; tf, thin flap of cuticle.

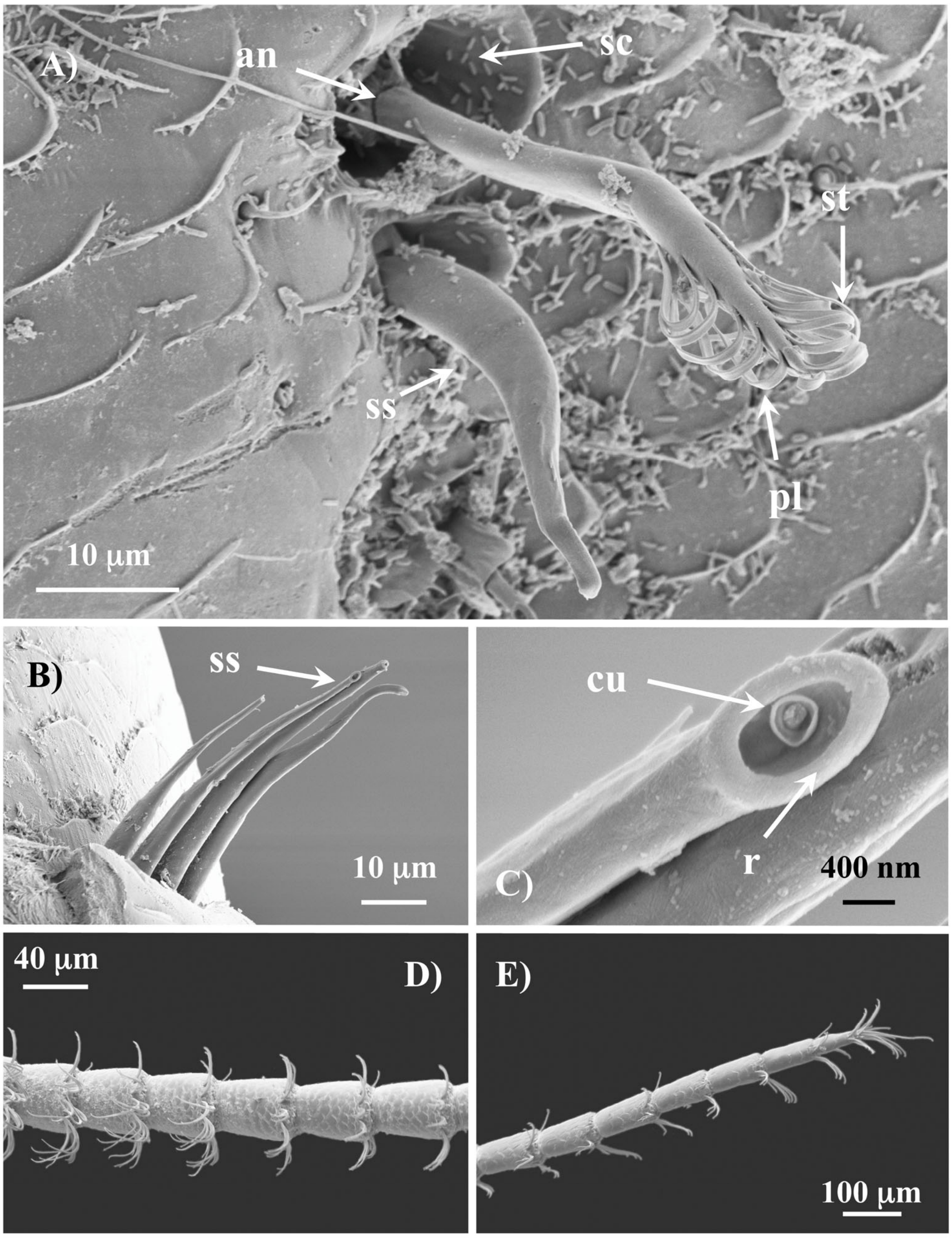

Figure 6 Simple and plumose setae. A) a plumose and simple setae on the peduncle of the antennule (second article of the antennule peduncle, anterior view); B) simple setae cluster (distal region of the second article of the antenna peduncle, anterior view); C) close up of a setal pore from B; D) Simple setae on antenna flagella articles; E) antenna tip.

an, annulus; cu, cupule; pl, plumose seta; r, ring of tissue around the pore; ss, simple seta;

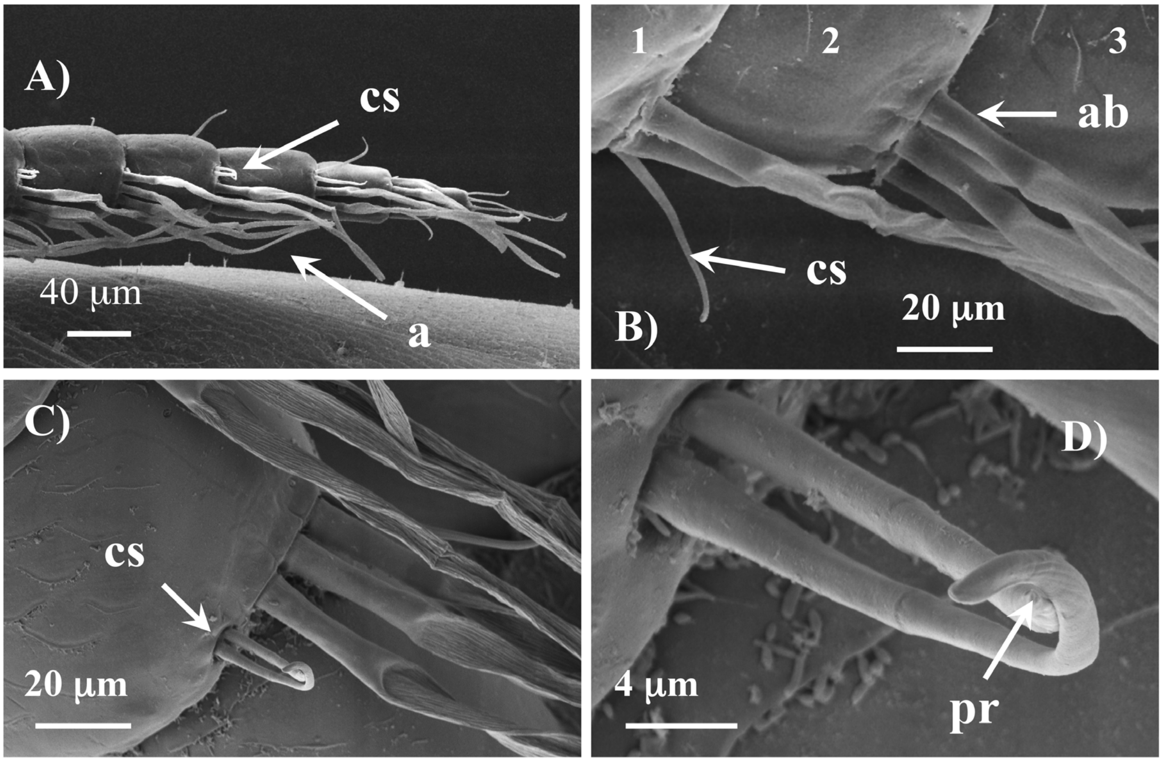

Figure 7 Aesthetascs and companion setae (all ventral aspect of the antennule). A) tip of the left antennule; B) proximal articles (numbered) of the flagella, left antennule, C) companion setae on the third article of the flagella, right antennule; D) close up of the companion setae from C.

a, aesthetasc, ab, aesthetasc base, cs; companion setae; pr, pore

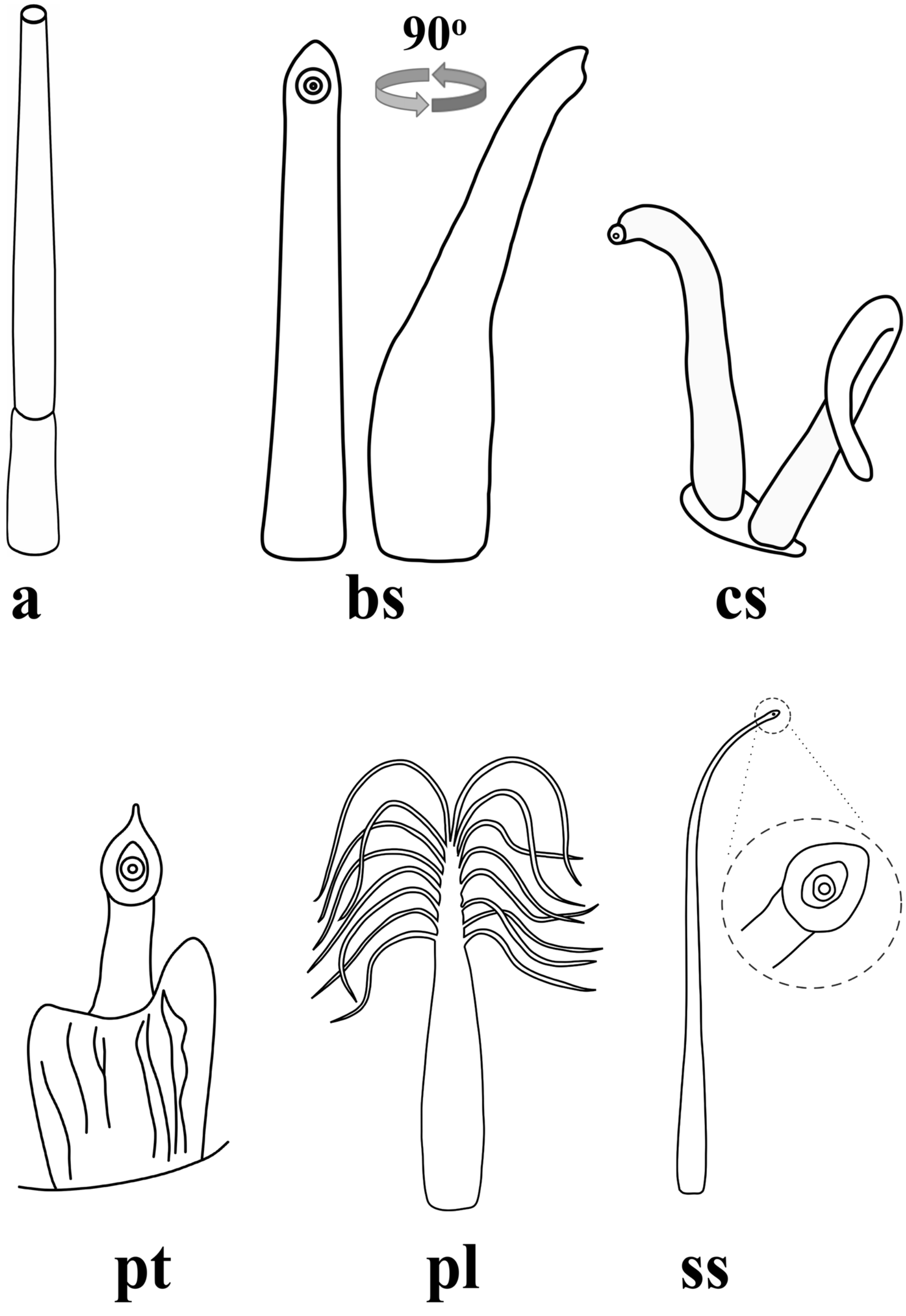

Figure 8 Setae types.

a, aesthetasc; bs, bulbous simple seta (the two diagrams are rotated 90º in relation to each other); cs; companion setae; pt, pit seta; pl, plumose seta; sf, filamentous simple seta; ss, simple seta

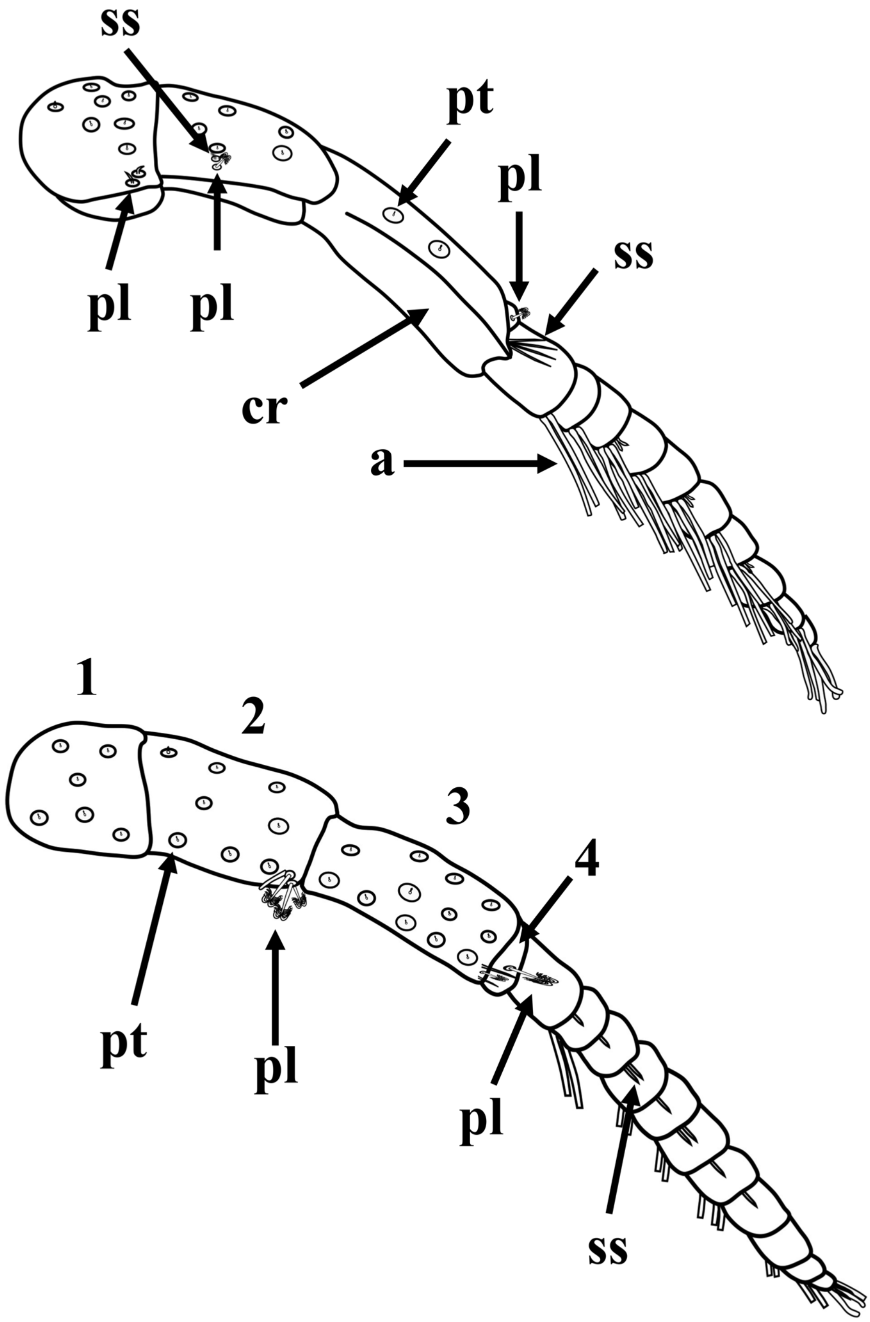

Figure 9 Diagram of the antennule. A) left antennule, ventral aspect; B) right antennule, dorsal aspect.

a, aesthetasc; cs; companion setae; pt, pit seta; pl, plumose seta; ss, simple seta

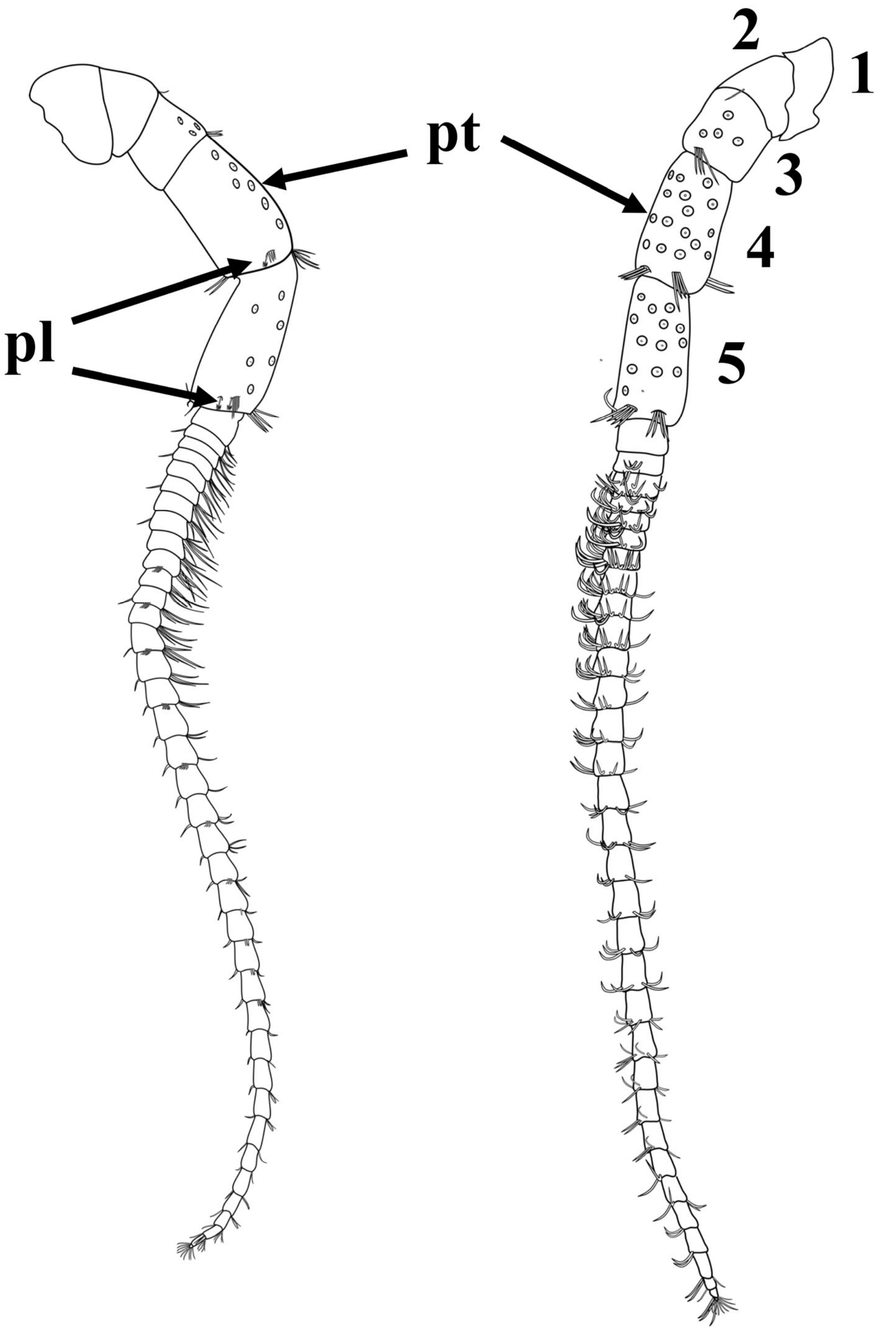

Figure 10 Diagram of the antenna. A) right antenna, dorsal aspect; B) right antenna, ventral aspect. Plumose setae and pit setae are indicated, all other non-arrowed setae are simple.

pl, plumose setae, pt, pit setae

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